How *clone* can you go? Seedbank density and a multiscale assessment of genotypic diversity in the seagrass *Halodule wrightii*

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Abstract

Seagrass conservation and management plans are placing increasing emphasis on the collection of data related to seagrass bed "condition" such as vegetative characteristics, seed production and genetic diversity. One important aspect of genetic diversity in species that reproduce both sexually and asexually is *genotypic* diversity. Genotypic (clonal) diversity is usually described as the proportion of unique genets within a population (*richness*), but it can also be characterized in other ways such as the degree to which individual ramets are distributed among genets (*evenness*) or the spatial arrangement of genets relative to one another (*architecture*). Genotypic richness, evenness, and architecture have the potential to influence sexual reproduction by affecting the proximity of genets, a key feature in dioecious species where pollen dispersal is limited and clones can vary greatly in size. They may also differ substantially according to the scale at which they are measured. This study examined genotypic richness, evenness and architecture across multiple spatial scales in the seagrass *Halodule wrightii*, and its association with seedbank density from three sites in the northwest Gulf of Mexico. While the magnitude of diversity estimates differed, the overall patterns remained consistent across scales. Seedbank density ranged from 19 +/- 9 to 188 +/- 30 seeds m^{-2}, following a gradient from north to south. The highest and most consistent production of seeds occurred at a site where richness (*R*: 0.24 -

0.42) and evenness (*ED*: 0.67 - 0.93) were uniformly high across spatial scales, and clonal architecture (A_c: 0.20 – 0.36) was represented by a high degree of intermingling.

Keywords: Seagrass, *Halodule wrightii*, Microsatellites, Genetic Diversity, Clonal Growth

Highlights

- Seedbank density was higher at locations exhibiting greater genotypic richness, evenness and intermingling across spatial scales
- Metrics of genotypic diversity were most efficiently captured at the largest (2 m) scale of sampling
- Spatial genetic structure and pairwise genotypic relatedness was low, suggesting *H. wrightii* has an efficient mechanism to avoid inbreeding

1. Introduction

Seagrass communities have been in decline in many parts of the world, including portions of the Gulf of Mexico (Handley et al., 2007; Waycott et al., 2009). Managers have many objectives concerning seagrass conservation, including: the monitoring of existing beds, reduction of harmful impacts from residential, commercial and agricultural practices, and restoration of habitat where losses have occurred (Pulich and Calnan, 1999). Monitoring has traditionally considered the extent and status (e.g. declining or expanding, patchy or contiguous) of seagrass beds but it is evolving towards the collection of additional parameters to better characterize bed condition. These attributes include percent cover, shoot density, genetic information and data on the rates of sexual reproduction and seedbank density (Texas Parks & Wildlife Department, personal communication). Rapid assessment protocols have been developed to measure vegetation characteristics (Neckles et al., 2012) but a variety of sampling schemes and scales have been used to collect genetic data, which can make comparisons difficult (Arnaud-Haond et al., 2007).

Genetic attributes such as heterozygosity and allelic diversity have been associated with a number of population fitness parameters in plants (Leimu et al., 2006). Because seagrasses reproduce both sexually and asexually care must also be taken to assess *genotypic* diversity in genetic surveys. Usually this takes the form of genotypic *richness* (*R*), which describes the proportion of sampled ramets that belong to unique genotypes, or *genets* (Ellstrand and Roose, 1987). Richness is used as a proxy for estimating the relative levels of sexual vs asexual reproduction. It can vary widely across seagrass species, geographical regions, and even populations within the same region (Olsen et al., 2004; Sinclair et al., 2014; van Dijk et al., 2018). Values can range from zero, where every sample belongs to the same clone, to one, where every sample represents a unique genotype (Arnaud-Haond et al., 2007). Genotypic richness has been correlated with a number of population processes across taxa such as nutrient utilization, flowering, and biomass production (Engelhardt et al., 2014; Salo and Gustafsson, 2016). Richness has also been positively correlated with resistance to environmental stress, including grazing, heat waves, and macroalgal blooms (Hughes and Stachowicz, 2004; Ehlers et al., 2008; Hughes and Stachowicz, 2011).

Richness, however, may not be the only feature of genotypic diversity that has a positive influence on population processes. For sexual reproduction, the *evenness* with which ramets are distributed among genets, or the *architecture* of their arrangement may also play a role (Arnaud-Haond et al., 2007). Evenness has its roots in ecology, where estimates such as the Simpson index were developed to characterize species diversity and distribution (Simpson, 1949). From a clonal plant perspective, a more even distribution of genotypic proportions could result in a more equitable division of male and female flowers in dioecious species. Sixty percent of seagrass taxa are dioecious (Les, 1988; den Hartog and Kuo, 2006). Clonal architecture describes the spatial arrangement of genets at a location. A more intermingled arrangement of genets is expected to lead to more successful pollination events, as the proximity of male and female flowers increases (Eckert et al., 2016). The richness (variety), evenness (relative proportions) and architecture (proximity) of genets in clonal populations would therefore be expected to have a significant impact on processes such as fertilization and seed production. These processes are gaining prominence as indicators of seagrass bed condition. Seedbanks have been associated with more rapid recovery after disturbance and can serve as important sources or donor material for restoration projects (Orth et al., 2006).

With few exceptions most studies characterizing genotypic diversity in seagrass populations have restricted their analyses to richness, at relatively large (≥ 1m) scales (but see Ruggiero et al., 2005). A larger sampling scale covers more area per sampling event, and increases the probability of collecting more unique genotypes but the richness, evenness, and architecture at one scale may not extend to another in clonal plant populations. Differences in clone size, seedling and vegetative recruitment strategies may alter these features, especially at smaller scales. Repeated seedling recruitment (RSR, Eriksson, 1993), for example, is characterized by a pattern of small clones that may only be detected at smaller sampling scales (Eriksson, 1993). *Guerilla* forms of vegetative recruitment, in which the ramets extend rapidly in a linear or branched form into the surrounding space, are associated with more a more intermingled genet architecture that may be overlooked at larger scales (Lovett-Doust, 1981).

The aim of this study was to assess genotypic richness, evenness and architecture across multiple spatial scales in populations of the seagrass *Halodule wrightii* L. Ascherson (Cymodoceaceae) from the northwestern Gulf of Mexico. We wished to determine how these features differed depending on the scale at which they were measured. We also wanted to assess whether they were associated with differences in seedbank density and spatial genetic structure, proxies for sexual reproduction and dispersal as well as important indicators of seagrass bed condition. This is important because the benefits of genotypic diversity may depend on factors besides the number of unique genets present. It's also important to know from a management perspective which scale of sampling (meter, decimeter, centimeter) most efficiently characterizes the different aspects of genotypic diversity, including situations where it would be desirable to avoid collecting clonal replicates.

2. Methods

2.1 Study area and sample collection

H. wrightii is the predominant seagrass species in the northwestern Gulf of Mexico and highly productive under a variety of light, nutrient, and salinity conditions (Dunton, 1996). A colonizing, dioecious species, *H. wrightii* has a fast rhizome elongation rate (ca. 2 m per year, Marba and Duarte, 1998) and produces negatively buoyant seeds that have a low dispersal capacity (Darnell et al., 2015). Samples were collected from sites in the three, major

seagrass producing basins on the Texas (USA) coast: the Coastal Bend (CB), Upper Laguna Madre (ULM), and Lower Laguna Madre (LLM) (Figure 1). Sampling occurred at three different scales: large grid (2 m apart), medium grid (20 cm apart) and small core (samples within a 10 cm diameter). Large scale sampling occurred in the summer of 2008 as part of a larger study to assess genetic diversity, structure, and connectivity in this species from the northwestern Gulf of Mexico (Larkin et al., 2017). It consisted of a 6 x 30 m grid, formed from 4 parallel transects 2 m apart. GPS coordinates and a single rhizome were collected at 2 m intervals along each transect (12 per) for a total of 48 samples per site. Medium scale sampling occurred at three sites in 2008. A 60 x 80 cm grid, blocked off into 20 (4 x 5) sampling points, was laid down at a random position within the large-scale grid. GPS coordinates were recorded and a single rhizome was collected from each grid point. The same grid was laid down at a position adjacent to the coordinates in 2010. Twenty additional samples were collected as before for a total of 40 medium scale samples from each site.

Small-scale sampling was also performed in the summer of 2010. A 10 cm diameter coring device was used to collect 15 cm deep cores from five random positions within the large grid coordinates for each site. The plant tissue from each core was carefully disentangled to avoid breaking rhizome connections and six to eight individual ramets were collected from each core for a total of 38 to 40 samples per site. All samples were stored in seawater and kept on ice until return to the laboratory, where they were freeze dried and stored with silica gel desiccant (28 to 200 mesh) prior to analysis.

Seedbank density sampling was also performed in the summer of 2010. Forty eight cores (10 cm diameter x 15 cm deep) were taken using the GPS sample coordinates from the large scale grids in 2008. Cores were transferred to 0.5 mm mesh nylon bags and washed free of sediment while in the field. Seeds were counted and converted to density estimates (seeds m^{-2}) by dividing the number of seeds in a core by its area (0.00785 m^2). These values were then averaged over the total number of cores per site.

2.2 Genetic Analysis

Approximately 20 mg of freeze dried rhizome tissue from each sample was homogenized in a FastPrep 24® instrument using Lysing Matrix A® (MP Biomedicals). Genomic DNA was extracted from the homogenate using the Plant Dneasy® kit from Qiagen. DNA was

quantified using the QuanIT® double stranded DNA assay kit and a Qubit® fluorometer (Invitrogen). Each sample was genotyped with eight, previously described loci (Larkin et al., 2017) using the Type it® microsatellite PCR kit from Qiagen according to the manufacturer's instructions. Thermal cycling was performed on a BioRad S1000 thermal cycler and PCR products were separated on a CEQ 8000 Genetic Analyzer (Beckman Coulter). Microsatellite alleles were scored using the CEQ 8000 Fragment Analysis System Software (v 9.0) and a 400 base pair standard. 10 % of samples were run in duplicate to confirm alleles. The scoring error rate was confirmed to be less than 1%.

2.3 Data Analysis

Unique, multi locus genotypes (MLGs) were identified from the allelic scores using GenClone 2.0 (Arnaud-Haond and Belkhir, 2007). This program calculates the probability of finding identical MLGs that have arisen from independent sexual events (p_{sex}). Identical MLGs with p_{sex} re-encounter values < 0.01 were considered to belong to the same clone. Slightly distinct MLGs (small number of allelic differences) were re-assayed at the variable loci to determine if the differences were the result of scoring errors or somatic mutations. Those with differences determined not to be due to errors had their p_{sex} values re-calculated without the variable loci. If the resulting p_{sex} value was < 0.01 the MLGs were still considered to belong to the same clone (multi-locus lineage, MLL).

Genotypic richness, evenness, mean clone size, clonal subrange, and pairwise relatedness among unique genotypes were estimated using GenClone 2.0 and GenAlEx 6.5 (Peakall and Smouse, 2012). Here richness (*R*) represents the proportion of samples within a dataset that are comprised of unique genets. It is calculated after Dorken & Eckert (2001) as (*G*-1)/(*N*-1) where *G* represents the number of unique genotypes and *N* is the sample size. Evenness describes the equitability of clonal membership, and is estimated using Simpson's evenness index (*ED*), the slope of the Pareto distribution (β), and the frequency distribution of clone sizes (Arnaud-Haond et al., 2007). The Simpson index ranges from 0 to 1, where higher values represent more equitable distributions. Similarly, the slope of the Pareto distribution increases as genet distributions become more equitable. Clonal subrange (*CR*) describes the spatial scale at which the probability of clonal identity approaches zero. It represents the maximum size of genets in a sample set. Pairwise relatedness among unique genotypes at

each scale of sampling was determined using the estimator of Queller and Goodnight (Queller and Goodnight, 1989). The estimator ranges between -1 and 1, where 1 represents complete allelic identity (clones), positive values reflect varying degrees of relatedness, and values of zero or less represent unrelated individuals. Differences among means for clone size, pairwise relatedness and seed density among locations and sampling scales were tested using single factor ANOVA followed by Games-Howell post-hoc analysis.

Clonal architecture (intermingled, clumped) was quantified for the large scale grids, and independently for the medium scale grids, using the *Aggregation index* (A_c) (Arnaud-Haond et al., 2007). The index measures the probability that nearest neighbors are identical clones. A_c values vary between 0 and 1, with lower values reflecting greater intermingling and higher values greater clumping.

Spatial genetic structure was estimated for both large and medium sampling scales using Loiselle's coefficient of coancestry F_{ij} (Loiselle et al., 1995). It represents the likelihood that a pair of ramets a certain distance apart is more (positive value) or less (negative value) similar than a pair selected at random. Both the aggregation indexes and coancestry coefficients were estimated using GenClone 2.0.

3. Results

3.1 Genotypic richness

Genotypic richness was highest at the largest (2 m) scale of sampling (R: 0.06 – 0.48) and, where present, considerably lower at the medium (0 - 0.21) and small (0 – 0.24) scales (Table 1). It was nearly absent from the Coastal Bend site, which was dominated by a single clone at all three sampling scales (Figure 2). Indeed, the medium and small core scales from this site showed no diversity whatsoever, containing only ramets of the dominant clone It was considerably higher at the Laguna Madre sites, where a multitude of clones were found at all three sampling scales . While richness at the large scale was highest at the ULM, the LLM possessed the highest number of unique genotypes at the medium (5 genets) and small scales (4 genets). In contrast, no unique genotypes were found at the medium scale for the ULM site, although three unique genotypes were found at the small scale.

3.2 Evenness

As with richness, genotypic evenness was highest at the largest scale of sampling (*ED* : 0.12 – 0.93), followed by the small (null to 0.90) and medium scales (null to 0.67) (Table 1). Evenness could not be calculated for the CB site at the medium and small scales because all samples were members of the same genet. For the Laguna Madre sites, evenness was highest at the LLM for the large and medium scales, and tied with the ULM at the smallest scale. Greater evenness was also reflected in the frequency distribution of clone sizes (Figure 3). The LLM exhibited a broader and more even distribution compared to the other sites. It also showed a higher mean clone size and larger clonal subrange compared to its ULM counterpart.

3.3 Architecture

Aggegation index (A_c values) were lower (more intermingled) at the largest scale of scale of sampling and higher (more clumped) at the medium scale. While A_c values were not calculated at the small scale, due to the discontinuous nature of the sampling, several cores possessed multiple genotypes indicating intermingling at this level. Clonal architecture across sites was mixed. The pattern of clonal growth at the CB was dominated by a single genet, interrupted at points by other genotypes. The Aggregation Index (A_c) value was rather low but insignificant (Table 1). This pattern of occasional disruption did not carry over to smaller scales, where all samples were members of the dominant genotype. The pattern for both Laguna Madre sites was more intermingled. Both the ULM and LLM exhibited low A_c values and a pattern of intermingling. Even the small scale samples from these sites were intermingled, with up to 4 different genotypes within a single 10 cm diameter core. This was highest at the LLM, where 3 of the 5 cores contained multiple genets.

3.4 Spatial Genetic Structure and Pairwise Relatedness

Spatial genetic structure across scales and locations was either non-significant or weak for both ramets and genets. At the large scale, positive spatial autocorrelation was only significant among ramets at the shortest distance class of 2 – 4 m, with F_{ij} values ranging from 0.03 (CB, $p < 0.01$) to 0.07 (LLM, $p < 0.01$). F_{ij} values among unique genets never exceeded 0.013 for any distance class (2 – 22 m) across all locations. At the medium sampling scale, F_{ij} values among ramets were only significant at the smallest distance class

of 20 – 30 cm for one location (LLM), with a value of 0.15 ($p < 0.01$). F_{ij} values among genets never exceeded 0.07 for any distance class (20 – 90 cm) across all locations.

Mean pairwise relatedness among unique genotypes was also low across scales and locations. They were generally higher at the largest scale of sampling, with mean +/- SE values of -0.33 +/- 0.19 in the CB and -0.05 +/- 0.02 for both the ULM and LLM sites. They were generally lower for both the medium and small core scales of sampling, ranging from -0.5 +/- 0.20 to -0.14 +/- 0.07 (Table 1). Differences in mean pairwise relatedness between the small and both medium and large scales were significant ($p < 0.01$ for both), but not between the medium and large scales ($p = 0.14$).

3.5 Seedbank Density

Seedbank density followed a gradient from north to south. It was lowest in the Coastal Bend (mean +/- SE of 19 +/- 9 seed m^{-2}), substantially higher in the ULM (133 +/- 43 seeds m^{-2}), and highest in the LLM (188 +/- 30 seeds m^{-2}). Differences in mean seedbank density between the CB and both ULM and LLM sites were significant ($p = 0.03$, < 0.01, respectively), but not between the ULM and LLM ($p = 0.54$). The distribution of seeds at each site also varied considerably. Only 5 of 48 cores (10.4%) from the CB contained seeds. At the ULM site 14 of 48 cores (29.2 %) contained seeds, while the LLM had seeds in 33 of 48 cores (68.8%).

4. Discussion

Ellstrand and Roose (1987) were among the first to show that clonal plant populations are typically comprised of a variety of genotypes. They found that most (terrestrial) populations showed intermediate levels of genotypic diversity and evenness. Standard schemes for the sampling of clonal populations were later developed by Arnaud-Haond et al (2007). They recommended strategies based on a knowledge of clonal growth rate and lifespan of the species of interest. Pilot studies need to be performed to determine the most efficient scale of sampling, depending on the goal of the investigation. A larger sampling scale, for instance, can result in the collection of more unique genotypes, and thus provide a better estimate of genotypic richness but it may lack the resolution to properly assess evenness, architecture,

vegetative or sexual recruitment. While a smaller scale might increase resolution of these features it could also strain time and resource budgets if the meadow to be analyzed is larger than a few square meters.

We investigated genotypic diversity (richness, evenness, architecture) at three different spatial scales in the seagrass *Halodule wrightii* for a typical number of population samples (40-50). Although the patterns of genotypic richness, evenness, and architecture remained fairly consistent across scales, the values of *R, ED*, β and A_c for a sample size of 40-50 individuals were most informative at the largest scale of sampling. While one might obtain decent estimates of architecture, in the form of intermingling, or evenness at smaller scales richness could be greatly underestimated. This may not be especially surprising, given the well-established relationship between area and species diversity (Rosenzweig, 1995),but it is reassuring in the context of asexually reproducing plants which can vary widely in terms of clone size and growth pattern (Eckert et al., 2016).

Genotypic diversity patterns were also consistent across locations. Sites with the highest values at the largest scale of sampling also tended to have them at smaller scales and vice versa. One exception occurred with genotypic richness, where *R* was highest in the ULM at the largest scale of sampling. However, at the medium and small scales richness was highest in the LLM population. This could be a sampling effect or a reflection of greater sexual reproduction and/or recruitment at this site. In spite of this, the largest scale of sampling seemed most efficient for capturing diversity attributes. For *H. wrightii* this appears to be in the vicinity of 2 m, the approximate length their rhizomes are able to grow per year (Marba and Duarte, 1998). A grid pattern based on such a scale would provide a good chance of accurately characterizing genotypic diversity even in fairly young populations. For other objectives, such as the gathering of vegetative material for restoration projects, a better scale might be the mean clone size or clonal subrange. This would improve the chances that unique genotypes were collected (Arnaud-Haond et al., 2007).

Smaller sampling scales were better at revealing patterns of sexual and vegetative recruitment. The visual maps and quantitative attributes of richness, evenness, and architecture showed unique genotypes and intermingling at even the smallest scale of sampling in the Laguna Madre populations. This is consistent with a RSR form of

recruitment (Eriksson, 1993). While more often dominated by fewer, or single genotypes, the intermingling found at the medium and small scales indicates a high degree of guerilla-type vegetative recruitment (Lovett-Doust, 1981). Both of these features are predicted to contribute to high levels of sexual reproduction and genetic diversity (Watkinson and Powell, 1993; Honnay and Jacquemyn, 2008). The level of intermingling at the different scales may also indicate something about mortality rates or competition among genotypes. Most of the intermingling occurred among genotypes found at all three levels of sampling. Relatively little involved unique genotypes found at the smaller scales. For example, only 6 of the 12 unique genotypes found exclusively at the medium or small scale occurred more than once. While this may only reflect clone age, it could also indicate an increased mortality among smaller clones or an inability to compete with larger ones for resources.

Overall, the results are similar to those of another dioecious, perennial species (*Cymodocea nodosa*) sampled at multiple scales from a single bed in the Mediterranean Sea (Ruggiero et al., 2005). They also found high clonal richness, a skewed distribution of clone sizes (many small, few large), and significant intermingling even at small sampling scales. They attributed their findings to three interacting processes: (i) a balance of persistence between older, founder genets and sexual recruitment (ii) poor seed dispersal in association with RSR, and (iii) a guerilla growth strategy. Given the similarities, including poor seed dispersal (Darnell et al., 2015), these processes may be at work in *H.wrightii* as well.

Genotypic diversity and seedbank density also differed across locations. The northern-most location examined (CB) exhibited very low diversity at all three scales, and the lowest seedbank density. This is concerning because the CB site is near a major commercial port (Corpus Christi) and approaches the edge of *H. wrightii's* range on the Texas coast. Populations towards the edge of their species' range tend to be more clonal, and the results found here are consistent with the general trend for this region (Larkin et al., 2017). Lower reproductive success is expected when clones are large and *R* is low, due to a shortage of compatible pollen (Honnay and Jacquemyn, 2008). This can be especially acute in dioecious species if one sex is under-represented (McMillan, 1981). Clonal diversity has also been shown to decline with increasing population age, perhaps as a consequence of competition among genotypes or lack of disturbance that can create opportunities for recruitment (Eckert et al., 2016). The Coastal Bend is a popular destination for recreational fisherman and

propeller scars within seagrass beds are common (Dunton and Schonberg, 2002). Scars create gaps in the canopy. This may have contributed to the pattern observed at the largest scale, where unique genotypes were found interspersed within an otherwise monoclonal bed. It suggests that RSR and guerilla recruitment, while rare, may still be operating at this location. This is significant because it implies that both types of recruitment are possible even in the presence of a dominant clone and relatively low levels of sexual reproduction.

Genotypic diversity and seedbank density in the ULM largely fell between that of the CB and LLM. The ULM showed an intermingled, guerilla type architecture but not to the same extent as the LLM. The presence of unique and intermingled genotypes in two of the ULM cores suggests that new recruitment still occurs, and is capable of following an RSR and guerilla strategy. Historically high salinity levels in the ULM means that widespread seagrass growth in this basin is probably a relatively recent phenomenon (Tunnell and Judd, 2002; Onuf, 2007). Recent population expansions are expected to result in greater clustering among genotypes (Arnaud-Haond et al., 2007). While not highly clumped the reduced extent of intermingling at all scales, greater proportion of smaller clones, and the smallest clonal subrange suggests a less mature successional stage. Even though the difference in mean seedbank density with the LLM was not significant, the ULM had less than half as many seed-containing cores. This suggests some limitation to fertilization or seed development. Given time and further RSR and guerilla recruitment, evenness and intermingling at this location may approach that of the LLM, potentially contributing to greater seed production.

Diversity and intermingling were generally highest in the LLM. This is important because it also had the largest and most consistent seedbank. Intermingled genet distributions are thought to improve the opportunities for outcrossing (Eckert et al., 2016) but the factors responsible for fertility (e.g. flowering and pollen production) probably also covary with latitude (Eckert, 2002; Blok et al., 2018). As the southern-most location, the LLM may experience earlier, or longer, warming periods that have been shown to be conducive to tropical seagrass flowering (McMillan, 1982). The LLM, however, is something of a paradox. It possesses good conditions for seagrass growth in terms of temperature, salinity, and protected shorelines but it's water quality has been variable and the basin as a whole has witnessed a serious decline in seagrass cover over the past 50 years (Onuf, 2007). Largely

attributed to the effects of maintenance dredging, many beds have become fragmented. This disturbance, however, may have produced gaps in the canopy that facilitated seedling and vegetative recruitment .

The role of disturbance may be key to the patterns observed at the different scales and sites. Disturbance is frequently invoked as a factor contributing to recruitment and has been shown to be associated with greater reproductive effort in seagrasses (Connell, 1978; Cabaco and Santos, 2012). Although it is often associated with declines in genetic diversity due to reductions in population size, it may also be critical for processes that result in new genotypes (Reusch, 2006; McMahon et al., 2017). Disturbance occurred in different forms (boat traffic, salinity, dredging) across the different sites, but in each case we found evidence of seedling and vegetative recruitment that influenced genotypic diversity patterns. In this age of seagrass decline any meadow loss is cause for concern. However, small levels of disturbance may form gaps in the canopy that facilitate recruitment.

Despite a low predicted capacity for pollen and seed dispersal (McMahon et al., 2014; Darnell et al., 2015), spatial genetic structure at all locations was weak. This is similar to other studies, where significant SGS was only observed at the shortest scale investigated (Vekemans and Hardy, 2004). A lack of SGS is also consistent with expectations for species that exhibit a high degree of guerilla growth (Alberto et al., 2005) where unrelated genets are in closer proximity compared to a more clumped, phalanx-type arrangement. Pairwise relatedness among unique genotypes was also low across populations and sampling scales. Both features indicate that mating among related individuals is not high at any of the locations. A lack of inbreeding is good news. While levels of diversity at certain locations may be low, recruits appear novel and capable of contributing to the evolutionary potential of each population

Because genotypic richness, evenness, and architecture have the potential to influence sexual reproduction we also examined seedbank density at each location. *H. wrightii* is a seed-banking species, producing seeds that are viable for up to four years (McMillan, 1991). These banks could be important for re-establishment following storms, colonization of newly opened habitat, or as donor material for restoration projects (Orth et al., 2006). As such, they represent an important component of seagrass bed condition. Estimates for seed bank

densities in *H. wrightii* are highly variable. An early study by McMillan (1981) found mean values of 260 seeds m^{-2} in the CB while Darnell and Dunton (2016) found reserves as high as 3,950 seeds m^{-2}, although most sites ranged from 0 to 611 seeds m^{-2}. Additional surveys by our laboratory has found no further evidence of seed banks at CB sites (PD Larkin, unpublished). Similarly, McMillan (1981) found mean values of 260 seeds m^{-2} in the LLM. Kowalski and DeYoe (2016) found LLM densities to range from 0 to over 4000 seeds m^{-2}, with a mean value of 890. Our values fall within these ranges. However, we found higher densities and a greater number of seed-containing cores in populations with larger genotypic diversity estimates. We reason that more intermingled, even distributions of unique genotypes, resulting in more successful fertilization events, could explain this result. The low number of sampling sites and a latitudinal gradient, however, confound this interpretation. Additional sites across a range of latitude need to be characterized to clarify this relationship.

Future studies could benefit from concurrent sampling at all spatial scales. Apart from similarities in the amount of cover observed for the two sampling dates, we cannot exclude the possibility of large scale changes in population structure at each site. However, the similarities in genotypic composition, richness, and evenness between the medium grids sampled at different time periods suggests the beds remained fairly consistent, indicative of the stability that often characterizes seagrass populations (Reynolds et al., 2017). Many of the same genotypes were also found across spatial and temporal scales. Only the medium grids from the LLM appeared different, although their locations occurred in a highly variable region of the large grid.

Conclusions

H. wrightii populations from the northwestern Gulf of Mexico differ considerably in seedbank density and genotypic diversity attributes such as clonal richness, evenness, and architecture. While patterns of diversity were consistent across sampling scales, the largest (2 m) scale of sampling captured the greatest amount diversity and is recommended for routine genetic analysis. It could be used in conjunction with coring techniques and other protocols that simultaneously measure seedbank density or vegetative characteristics important for assessing bed condition. Medium and smaller scales of sampling were more useful for

assessing seedling and vegetative recruitment patterns, features that can help explain the amount of genetic diversity or sexual reproduction at a site. Spatial genetic structure, in the form of kinship coefficients and pairwise relatedness estimates, was uniformly low among genotypes across scales and sites. It suggests that *H. wrightii* may have a mechanism for avoiding inbreeding or the germination of seedlings from closely related parents.

If this study had only considered clonal richness it would have missed other aspects of genotypic diversity that may be also be important. Seedbank density was highest and most consistent in a population that showed greater genotypic evenness and intermingling, especially at smaller scales where pollen exchange is likely to be most significant (Van Tussenbroek et al., 2016). Additional populations and locations need to be examined, but preliminary evidence suggests that aspects of genotypic diversity besides richness may be important contributors to seed production.

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Location	Sampling Scale	Year	*N*	*G*	*R*	*ED*	Pareto β	A_c	*M* (m)	*CR* (m)	Seed Density (seeds·m^{-2})	Unique Genotypes[1]	Pairwise Relatedness
CB	Large	2008	48	4	0.06	0.12	1.03	0.26*	12.8 +/- 10.0	22.8	19 +/- 9	3	-0.33 +/- 0.19
ULM	Large	2008	47	23	0.48	0.88	1.78	0.21	4.6 +/- 0.6	6.3	133 +/- 43	16	-0.05 +/- 0.02
LLM	Large	2008	46	20	0.42	0.93	1.90	0.20	6.6 +/- 1.2	14.1	188 +/- 30	14	-0.05 +/- 0.02
CB	Medium 1	2008	20	1	0.00	-	-	-	-	-	-	0	-
	Medium 2	2010	20	1	0.00	-	-	-	-	-	-	0	-
	Combined	-	40	1	0.00	-	-	-	-	-	-	0	-
ULM	Medium 1	2008	20	3	0.11	0.17	1.06	0.28*	-	-	-	0	-0.50 +/- 0.20
	Medium 2	2010	20	4	0.16	0.16	1.07	0.04*	-	-	-	0	-0.33 +/- 0.11
	Combined	-	40	5	0.10	0.20	1.04	-	-	-	-	0	-0.25 +/- 0.09
LLM	Medium 1	2008	20	4	0.16	0.42	-	0.00*	-	-	-	2	-0.33 +/- 0.06
	Medium 2	2010	19	6	0.28	0.65	1.23	0.36	-	-	-	3	-0.20 +/- 0.09
	Combined	-	39	9	0.21	0.67	1.22	-	-	-	-	5	-0.14 +/- 0.07
CB	Core 1	2010	8	1	-	-	-	-	-	-	-	-	-
	Core 2	2010	8	1	-	-	-	-	-	-	-	-	-
	Core 3	2010	8	1	-	-	-	-	-	-	-	-	-
	Core 4	2010	8	1	-	-	-	-	-	-	-	-	-
	Core 5	2010	8	1	-	-	-	-	-	-	-	-	-
	Combined	-	40	1	0.00	-	-	-	-	-	-	0	-
ULM	Core 1	2010	8	1	0.00	-	-	-	-	-	-	-	-
	Core 2	2010	8	1	0.00	-	-	-	-	-	-	-	-
	Core 3	2010	8	4	0.43	-	-	-	-	-	-	2	-0.33 +/- 0.08
	Core 4	2010	8	1	0.00	-	-	-	-	-	-	-	-
	Core 5	2010	8	3	0.29	-	-	-	-	-	-	1	-0.50 +/- 0.20
	Combined	-	40	8	0.18	0.90	1.38	-	-	-	-	3	-
LLM	Core 1	2010	8	3	0.29	-	-	-	-	-	-	-	-0.50 +/- 0.13
	Core 2	2010	8	4	0.43	-	-	-	-	-	-	3	-0.33 +/- 0.06
	Core 3	2010	8	1	0.00	-	-	-	-	-	-	-	-
	Core 4	2010	8	3	0.29	-	-	-	-	-	-	1	-0.5 +/- 0.07
	Core 5	2010	6	1	0.00	-	-	-	-	-	-	-	-
	Combined	-	38	10	0.24	0.90	1.38	-	-	-	-	4	-

[1]genotypes found only at specified scale of sampling *not significant

Table 1. Genotypic diversity results for *Halodule wrightii* at 3 different sampling scales. CB: Coastal Bend; ULM: Upper Laguna Madre; LLM: Lower Laguna Madre; *N*: number of samples; *G*: number of genets; *R*: genotypic (clonal) richness; *ED*: Simpson's evenness index; β: slope of the Pareto distribution of clonal membership; A_c: aggregation index; *M*: mean clone size; *CR*: clonal subrange. Mean clone size, seed density, and pairwise relatedness values reported as mean +/- SE.

570

571 **Figure 1.** Sampling locations along the Texas coast in the northwestern Gulf of Mexico.

572

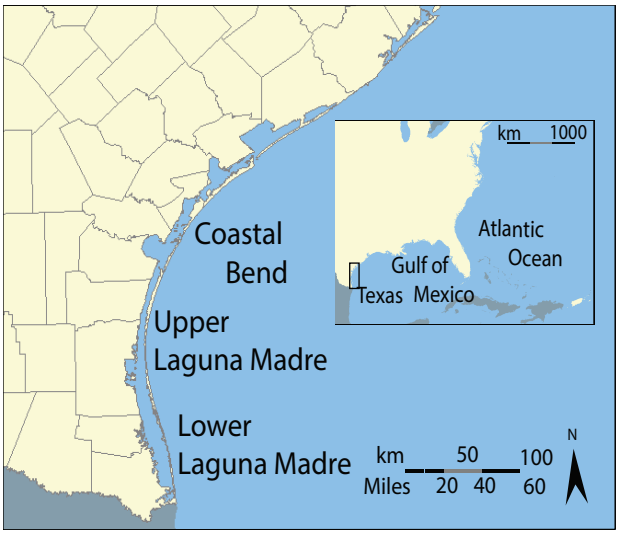
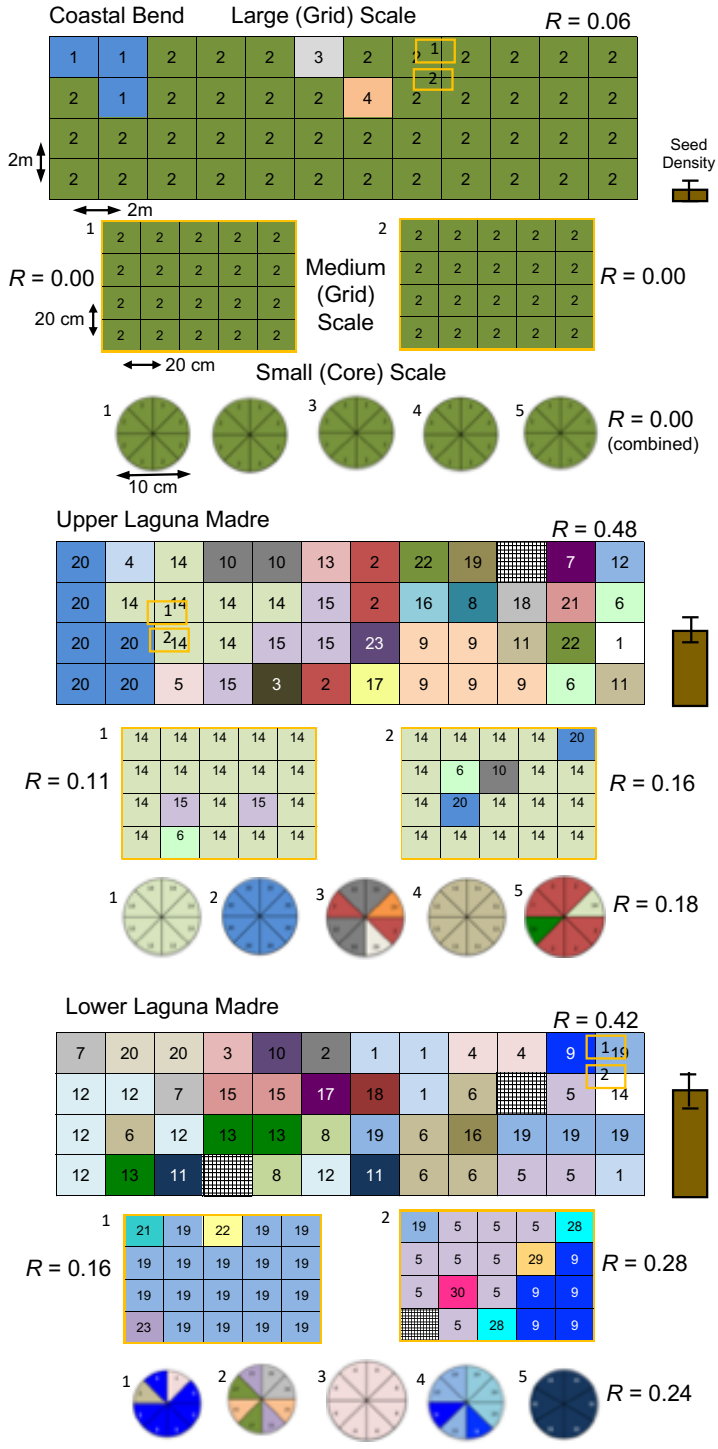


Figure 2. Clonal diversity and distribution at large, medium, and small (core) sampling scales. Colored boxes or sections (cores) represent unique genotypes at each site. Hatched boxes represent missing data. Small yellow rectangles inside of large grids represent approximate positions of medium scale sampling grids. Genotypic (clonal) richness values (*R*) are indicated for each scale (values were combined for core samples). Columns to right of large grid indicate relative seedbank densities (mean +/- SE).



588 **Figure 3.** Frequency distribution of *H. wrightii* clone sizes from Coastal Bend, Upper and Lower
589 Laguna Madre sites in northwestern Gulf of Mexico.

590