The genetic diversity of two contrasting seagrass species using microsatellite analysis

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Abstract:

The goal of this thesis was to examine the genetic diversity of two diverse seagrass species, *Amphibolis antarctica* and *Halodule wrightii*, using microsatellite markers. Microsatellite primers previously did not exist for *Amphibolis antarctica* and were developed as described in the first chapter. From 48 primer candidates, 14 polymorphic loci were arranged into a 3-panel multiplex. The microsatellite primers successfully amplified and distinguished multi-locus genotypes of samples from two test populations. Genotypic richness varied between populations at 0.26 and 0.85, and an $F_{ST} = 0.318$ indicated population differentiation has occurred. Contrary to previous study, genetic diversity was observed in *A. antarctica* meadows. Further studies will be able to use these primers for more extensive analysis of the dispersal and recruitment mechanisms, evolutionary history, and connectivity of *A. antarctica*. The second chapter utilized microsatellite primers in a genetic population study for edge-of-range populations of the tropical/subtropical seagrass *Halodule wrightii*. Sampling occurred at 15 sites representing the Florida gulf coast, Florida Bay, Indian River Lagoon, North Carolina, and Bermuda. Eleven microsatellites were amplified and allelic diversity, genotypic richness, population differentiation, gene flow, principal components analysis, and k-means population clustering analyses were performed. Diploid, triploid, and tetraploid genotypes were observed. Aneuploidy from somatic mutation may be a way for edge-of-range populations to achieve genetic diversity without sexual reproduction, as sites were highly clonal ($R = 0.00 – 0.20$). Population clustering and principal components analysis grouped sites into 2 main populations. Sites were highly structured ($Rho_{ST} = 0.297$) and genetic differentiation occurred between populations following an isolation-by-distance model. The microsatellite analyses of this thesis allowed for the characterization of genetic diversity and population differentiation of the studied species. Such information can allow restoration and conservation managers to infer genetic processes that are important for mitigation success.

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Introduction:

Seagrass beds are among the most productive ecosystems in the world and are found along the coastline of every continent except Antarctica (Duarte and Chiscano, 1999). Seagrasses are an integral component to coastal processes and participate in nutrient exchanges with mangrove forests and coral reefs (Harborne et al., 2006). Further, seagrasses alter the biogeochemistry of the sediment, sequester carbon, and increase water clarity (Short and Short 1984; Bodilier 2003; Fourqurean et al., 2012). The dense root structures of seagrasses stabilize the sediment surface, and the leaves create current drag that reduce erosion (Koch et al., 2006). In addition, seagrass beds provide habitat to hundreds of species, many of which are economically valuable to fisheries or are threatened species with ecological value (Zieman, 1982).

Despite the extensive ecosystem services provided by seagrasses, anthropogenic and environmental threats are resulting in a global decline of seagrass populations (Orth et al., 2006; Waycott et al., 2009). In general, the distribution of submerged aquatic vegetation is limited by light, salinity, temperature, and nutrients (Dennison et al., 1993; Orth et al., 2006; Mazzotti et al., 2007). As a result, conditions such as eutrophication, increased turbidity, climate change, and disease contribute to seagrass loss (Short et al., 2007). Although the number of seagrass species is relatively small compared to other plant taxa (around 60 seagrasses compared to approximately 250,000 terrestrial angiosperms) (Orth et al., 2006), the species are biologically diverse and population responses to environmental conditions can be species-specific (Marba et al., 1996; Carruthers et al., 2007).

The genetic diversity of a seagrass species plays an important role in moderating bed responses to disturbance by enhancing both ecosystem services and resilience (Hughes and Stachowicz, 2009; Reynolds et al., 2012). As seagrass communities are often dominated by only 1-3 plant species, genetic diversity is important for replacing the functional role of species diversity (Duffy, 2006). Genetic diversity contributes to the long-term evolutionary persistence of a species by providing a diverse gene pool from which adaptation to environmental stressors can be selected and passed on to future generations. In this way, seagrass ecosystems with high genotypic diversity show increased resilience to intense disturbance (Hughes and Stachowicz, 2011). Enhanced ecosystem resilience is critical given the current state of global seagrass

decline; therefore, achieving natural levels of genetic diversity can serve as a quantifiable goal for seagrass restoration projects.

Despite these benefits, restoration, augmentation, and conservation projects often do not consider genetic diversity in their project designs. For example, reduced gene pools of transplantation source material have resulted in lower genetic diversity in mitigated areas (Williams, 2001). In the past, donor beds have been sourced from the most cost-effective locations, and performing genetic analyses on such beds would be an additional cost and time component to the project. A short-term benefit of enhanced genetic diversity in restored beds includes increased shoot density, a parameter often used as a measure of success in mitigation projects. In the well-studied species *Zostera marina*, high genetic diversity doubled leaf density compared to low diversity plots over a period of 22 months (Williams, 2001). Though benefits exist for incorporating genetic diversity into mitigation plans, it may fall to the research community to collect baseline genetic information on species of concern (Williams, 2001).

Studies on genetic diversity, particularly those that include multiple spatial scales, provide valuable information for restoration and conservation projects (Escudero et al., 2003). At a local level, spatial genetic knowledge informs management decisions about sampling design for seed stocks, which is important for determining the number of genotypes necessary to avoid inbreeding or outbreeding depression. Understanding the breeding system and clonality of a species can help to determine the optimal size of a conservation area. At a larger scale, information on dispersal and gene flow between populations can be applied to decisions regarding habitat fragmentation and potential corridor populations (Escudero et al., 2003). Studies that extend into peripheral regions of a species extent are important because edge populations often experience reduced genetic diversity (Kendrick et al., 2012). Additionally, populations at the edge of their environmental suitability are most likely to be affected by range shifts due to climate change (Sexton et al., 2009). Understanding the genetic processes of leading or retracting populations will be important for determining the future ecosystem assemblages and services in that area.

This thesis investigates the genetic diversity of two contrasting species, *Amphibolis antarctica* and *Halodule wrightii*, in the family *Cymodoceaceae* using microsatellite primers. *A. antarctica* is a temperate species found in sheltered and exposed environments along the

southern and western coasts of Australia (Carruthers et al., 2007). *H. wrightii* has an extensive tropical and subtropical range spanning the bioregions of the temperate North America, tropical Atlantic, Mediterranean, temperate North Pacific, and tropical Indo-Pacific (Short et al., 2007). *H. wrightii* grows quickly during the spring and summer and becomes dormant during the fall and winter, but the root-rhizome complex is maintained regardless of season and comprises 50-85% of the plant's total biomass (Dunton, 1990; Hall et al., 2006). In contrast, *A. antarctica* has wiry rhizomes from which short shoots extend with branched apical meristem and a number of leaf clusters—a structure allowing for reduced belowground investment (Cambridge, 1999). *A. antarctica* is a late-successional species whereas *H. wrightii* is an early colonizer and is succeeded in some areas by the intermediate species *Syringodium filiforme*, or climax species *Thalassia testudinum* and *Zostera marina* (Orth et al., 2006; Short et al., 2007; Micheli et al., 2008). In addition, *A. antarctica* uses true viviparous reproduction with seedlings that do not have temporal or spatial dormancy (Elmqvist and Cox, 1996), while *H. wrightii* has seed banks with both embryo dormancy and seed dormancy, allowing seeds to survive multiple years before germinating (McMillan 1981).

Figure 0.1. Pictures of *Amphibolis antarctica* (left) and *Halodule wrightii* (right).

All seagrasses can reproduce both sexually through hydrophilous fertilization and asexually via vegetative fragmentation or horizontal elongation of short shoots along a rhizome runner. However, the differences in *A. antarctica* and *H. wrightii* morphology, reproduction, and life history may lend to different allocations of sexual and asexual reproduction for bed

recruitment and maintenance (refer to Appendices A1 and A3 for an overview of genetic terms and processes). High-resolution genetic markers, such as microsatellites, are needed to distinguish the genetic identity of individual short shoots (ramets) to identify ramets for a single clone (genet) (Vallejo-Marín et al., 2010). Microsatellites are single sequence repeats in noncoding regions of the genome that are considered to be neutral to selection (Freeland et al., 2011). Unlike dominant markers such as restriction fragment length polymorphisms (RAPDs) and amplified fragment length polymorphisms (AFLPs), microsatellites are codominant and can directly distinguish homozygotes from heterozygotes. Therefore, microsatellites provide both quantitative (i.e., allelic frequency) and qualitative (i.e., alleles) data, whereas dominant markers provide only presence/absence information and include a higher degree of uncertainty when accounting for heterozygosity and determining allelic frequency (Escudero et al., 2003). Previous studies have used dominant markers for genetic diversity investigations of *A. antarctica* and *H. wrightii*, with the exception of recent work that employed microsatellites along the coastline of Texas for *H. wrightii* (Larkin et al., 2017).

The first chapter of this thesis describes the development of microsatellite markers for *A. antarctica* to allow for better determination of the genetic diversity within the species. The second chapter employs existing microsatellite markers for a genetic population structure survey of *H. wrightii* edge-of-range populations along the western Atlantic coast. The information gathered on the allelic diversity, genotypic diversity, and population differentiation of these diverse focal species will contribute to the growing body of knowledge regarding seagrass genetic processes. This knowledge can be used to further the efficacy of seagrass restoration and conservation.

Literature Cited:

Bodelier, P.L.E., 2003. Interactions between oxygen-releasing roots and microbial processes in flooded soils and sediments. In *Root Ecology*. Springer, Berlin Heidelberg, 331-362

Cambridge, M.L., 1999. Growth strategies of Rottnest Island seagrasses. *The seagrass flora and fauna of Rottnest Island, Western Australia.* Western Australian Museum, Perth, 1-24.

Carruthers, T.J.B., Dennison, W.C., Kendrick, G.A., Waycott, M., Walker, D.I., Cambridge, M.L., 2007. Seagrasses of south–west Australia: A conceptual synthesis of the world's most diverse and extensive seagrass meadows. J. Exp. Mar. Biol. Ecol. 350, 21-45.

Dennison, W. C., Orth, R. J., Moore, K. A., Stevenson, J. C., Carter, V., Kollar, S., Bergstrom, P.W., Batiuk, R. A., 1993. Assessing water quality with submersed aquatic vegetation. BioScience 43, 86-94.

Duffy, J.E., 2006. Biodiversity and the functioning of seagrass ecosystems. Mar. Ecol. Prog. Ser. 311, 233–250.

Duarte, C.M., Chiscano, C.L., 1999. Seagrass biomass and production: a reassessment. Aquat. Bot. 65, 159-174.

Dunton, K.H., 1990. Production ecology of Ruppia maritima L. sl and Halodule wrightii Aschers, in two subtropical estuaries. J. Exp. Mar. Biol. Ecol. 143, 147-164.

Elmqvist, T., Cox, P. A., 1996. The evolution of vivipary in flowering plants. Oikos 3-9.

Escudero, A., 2003. Spatial analysis of genetic diversity as a tool for plant conservation. Biol. Conser. 113, 351–365. doi:10.1016/S0006-3207(03)00122-8

Freeland, J.R., Kirk, H., Petersen, S.D., 2011. *Molecular Ecology 2 Ed*. Wiley-Blackwell, West Sussex, UK.

Fourqurean, J.W., Duarte, C.M., Kennedy, H., Marbà, N., Holmer, M., Mateo, M.A., Apostolaki, E.T., Kendrick, G.A., Krause-Jensen, D., McGlathery, K.J., Serrano, O., 2012. Seagrass ecosystems as a globally significant carbon stock. Nature Geosci. 5, 505–509. doi:10.1038/ngeo1477

Hall, L.M., Hanisak, Md., Virnstein, R.W., 2006. Fragments of the seagrasses Halodule wrightii and Halophila johnsonii as potential recruits in Indian River Lagoon, Florida. Mar. Ecol. Prog. Ser. 310, 109–117.

Harborne, A.R., Mumby, P.J., Micheli, F., Perry, C.T., Dahlgren, C.P., Holmes, K.E., Brumbaugh, D.R., 2006. The functional value of Caribbean coral reef, seagrass and mangrove babitats to ecosystem processes. Adv. Mar. Biol. 57–189.

Hughes, A.R., Stachowicz, J.J., 2009. Ecological impacts of genotypic diversity in the clonal seagrass Zostera marina. Ecology 90, 1412–1419.

Hughes, A.R., Stachowicz, J.J., 2011. Seagrass genotypic diversity increases disturbance response via complementarity and dominance. J. Ecol. 99, 445-453.

Kendrick, G.A., Waycott, M., Carruthers, T.J.B., Cambridge, M.L., Hovey, R., Krauss, S.L., Lavery, P.S., Les, D.H., Lowe, R.J., Vidal, O.M. i, Ooi, J.L.S., Orth, R.J., Rivers, D.O., Ruiz-Montoya, L., Sinclair, E.A., Statton, J., van Dijk, J.K., Verduin, J.J., 2012. The central role of dispersal in the maintenance and persistence of seagrass populations. BioScience 62, 56–65. doi:10.1525/bio.2012.62.1.10

Koch, E. W., Ackerman, J. D., Verduin, J., & van Keulen, M., 2007. Fluid dynamics in seagrass ecology—from molecules to ecosystems. In *Seagrasses: Biology, Ecology and Conservation*. Springer, Netherlands, 193-225

Larkin, P.D., Maloney, T.J., Rubiano-Rincon, S., Barrett, M.M., 2017. A map-based approach to assessing genetic diversity, structure, and connectivity in the seagrass Halodule wrightii. Mar. Ecol. Prog. Ser. 567, 95-107. doi: 10.3354/meps12037

Marba, N., Cebrian, J., Enriquez, S., Duarte, C.M., 1996. Growth patterns of Western Mediterranean seagrasses: species-specific responses to seasonal forcing. Mar. Ecol. Prog. Ser. 133, 203-215.

Mazzotti, F.J., Pearlstine, L.G., Chamberlain, R., Barnes, T., Chartier, K., DeAngelis, D., 2007. Stressor response models for the seagrasses, Halodule wrightii and Thalassia testudnium. In: *Final report to the South Florida Water Management District and the US Geological Survey*. University of Florida, Florida Lauderdale Research and Education Center, FL, USA, 19.

McMillan, C. 1981. Seed reserves and seed germination for two seagrasses, Halodule wrightii and Syringodium filiforme, from the Western Atlantic. Aquat. Bot. 11, 279-296.

Micheli, F., Bishop, M.J., Peterson, C.H., Rivera, J., 2008. Alteration of seagrass species composition and function over two decades. Ecol. Monogr. 78, 225–244.

Orth, R.J., Carruthers, T.J., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Olyarnik, S., others, 2006. A global crisis for seagrass ecosystems. BioScience 56, 987–996.

Reynolds, L.K., McGlathery, K.J., Waycott, M., 2012. Genetic diversity enhances restoration success by augmenting ecosystem services. PLoS ONE 7, e38397. doi:10.1371/journal.pone.0038397

Sexton, J.P., McIntyre, P.J., Angert, A.L., Rice, K.J., 2009. Evolution and Ecology of Species Range Limits. Annu. Rev. Ecol. Evol. Syst.40, 415–436. doi:10.1146/annurev.ecolsys.110308.120317

Short, F.T., Short, C.A., 1984. Seagrass Filter: Purification of Estuarine and Coastal Waters. In *The Estuary as a Filter.* Academic Press, Orlando, FL, USA, 395-413

Short, F., Carruthers, T., Dennison, W., Waycott, M., 2007. Global seagrass distribution and diversity: A bioregional model. J. Exp. Mar. Biol. Ecol. 350, 3–20. doi:10.1016/j.jembe.2007.06.012

Vallejo-Marín, M., Dorken, M. E., Barrett, S.C., 2010. The ecological and evolutionary consequences of clonality for plant mating. Annu. Rev. Ecol. Evol. Syst. 41, 193.

Waycott, M., Duarte, C.M., Carruthers, T.J., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck, K.L., Hughes, A.R., others, 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proc. Nat. Acad. Sci. 106, 12377–12381.

Williams, S.L., 2001. Reduced genetic diversity in eelgrass transplantations affects both population growth and individual fitness. Ecol. Appl. 11, 1472–1488.

Zieman, J.C., 1982. *Ecology of the seagrasses of south Florida: a community profile*. No. FWS/OBS-82/25. Charlottesville, VA, USA.

Chapter 1. New genetic markers for the seagrass *Amphibolis antarctica* reveal genetic diversity[1]

Overview:

The temperate seagrass *Amphibolis antarctica* previously displayed little genetic diversity in allozyme, RFLP, and other DNA fingerprinting analyses. The goal of the work described here was to develop microsatellite primers. The development of primers will facilitate more in-depth studies of the genetic structure, clonality, and relatedness of *A. antarctica* populations than have been done previously. Forty-eight primer candidates were screened using TypeIT Microsatellite PCR reageants (Qiagen). Fourteen primers displayed polymorphism and were arranged into multiplex panels for further refinement of PCR conditions across population DNA samples, as well as characterization of allelic frequency and genotypic diversity. The number of alleles per locus ranged from 2-7, and the presence of polymorphism was the first time genetic diversity was detected in *A. antartica*. These microsatellite primers can be used for future studies regarding the evolutionary ecology and population structure of *A. antarctica*.

Highlights:

- Fourteen microsatellite primers were developed for the temperate seagrass *A. antarctica*
- Contrary to prior studies, polymorphism was identified in *A. antarctica* populations
- Microsatellite primers and multiplexes were developed for future use in population studies

Keywords: microsatellite; polymorphism; seagrass

[1] Work was performed with Kor-jent van Dijk and Michelle Waycott from The University of Adelaide, Australia

Introduction:

Amphibolis antarctica is a temperate seagrass endemic to southern and western Australia that forms dense, monospecific beds as well as mixed stands (Carruthers et al., 2017). Genetic diversity enhances seagrass ecosystem services and is an important component to increasing bed resilience to disturbance (Hughes and Stachowicz, 2009; Reynolds et al., 2012). Therefore, genetic diversity should be a consideration for conservation and restoration decisions (Williams, 2001). Accurate diversity information for restoration projects is especially important given the multiple stressors facing seagrasses and subsequent population declines (Waycott et al., 2009), such as the catastrophic (>90%) dieback of *A. antarctica* beds in Shark Bay, Australia following a marine heat wave in 2011 (Thomson et al., 2014). *A. antarctica* failed to show rapid recovery following this event, attributed to the lack of nearby seedling recruitment and persistent rhizome bed, whereas *Halodule uninervis* increased cover (Nowicki et al., 2017).

Genetic studies can give insight into bed recruitment mechanisms and, thus, allow for better predictions of ecosystem recovery following seagrass loss. In addition, genetic information can provide direction for restoration efforts that may hasten bed recovery. The only study to date assessing the genetic structure of *A. antarctica* showed genetic invariance between individuals across a range of over 1,100 km (Waycott et al., 1996). This result was unexpected given observations of flowering and copious seed production (Waycott et al., 1996). It is possible that the type of genetic marker system used (allozymes, restriction fragment length polymorphisms or RFLPs, and other DNA fingerprinting techniques) lacked the resolution necessary to detect existing polymorphisms.

Microsatellites are highly polymorphic genetic markers commonly used for the study of seagrass clonality and genetic diversity (Freeland et al., 2011). The aim of this study was to develop a set of species-specific microsatellite markers that would enable genet detection and reassessment of genetic diversity in *A. antarcica* in the future. Considering the presence of asexual and sexual reproduction in other seagrass species, I hypothesized that the previously used markers did not have the necessary resolution to observe patterns of genetic structure or to distinguish genetic individuals (genets). I expected the microsatellites developed to be variable and suitable for population structure studies and clonality assessment. To test if this was so, the potential of the developed markers was tested on a small number of populations.

Methods:

1.1 Sampling:

For marker development and testing, 12 plant samples with herbarium vouchers were collected from a range of populations along the South Australian coast (Semaphore, Seaford, Rapid Bay, Ardrossan, and Stenhouse Bay) (Figure 1.1). The variety of locations was chosen to best identify potential polymorphisms during marker development and promote amplification in future studies. To test for microsatellite neutrality and intra- and inter-population variability, three populations were sampled (Robe and Marino Rocks in South Australia and Penguin Island in Western Australia). At each site 50 samples were taken haphazardly. The Robe samples were collected as wrack and, as a result, do not represent a meadow but what aggregated in that location. Both Marino Rocks and Penguin Island were collected by free diving and samples covered an area of a homogeneous meadow of approximately 50 x 50 m.

1.2 Primer development:

Genomic DNA of the 12 test samples was isolated from the meristematic leaf sheath tissue using the Qiagen DNeasy Plant Kits (Qiagen, Valencia, California, USA) according to manufacturer instructions. An equimolar mix of the DNA samples was sent to GenoScreen (Lille, France) for library preparation and followed the methods described by Malausa et al. (2011). Briefly; the samples were fragmented and enriched for GA, CTT, AC, CA, TC, TTC, AGAT, TCT, AGG, CT, AG, GGT, ATGT, GTG, GAG, AAG, and GAA repeats. The enriched library was then amplified and prepared for sequencing using 454 GS-FLX Titanium chemistry (454 Life Sciences, a Roche Company, Bradford, Connecticut, USA). A total of 2344 contigs were isolated using the QDD software (Meglécz et al., 2010) and a shortlist of 207 validated loci with primers were provided. Forty-eight loci with various repeat motifs were selected and ordered for testing. For cost saving purposes the forward primers were ordered with M13 labeled tags (5′-TGTAAAACGACGGCCAGT-3′) preceding the 5’-end of the forward primes. Additionally, universal M13 primers with fluorescent tags (6-FAM, VIC, NED, and PET) were used to allow for nested PCRs according to Schuelke (2000). Reverse primers were ordered with an additional PIG-tail (5′-GTTTCT-3′) added to the 5’ end to reduce stutters (Brownstein et al., 1996).

1.3 Population diversity assessment with developed primers:

Polymerase Chain Reactions (PCR) were performed in 15 µL volumes using the TypeIT® (Qiagen) chemistry and by adding 7.5 µL of DNA template (diluted 1:5), 0.24 µL M13-fluorecent labeled primer, 0.24 µL reverse primer, 0.06 µL forward primer, and 5.96 µL RNase-free water (Qiagen). PCR conditions followed the manufactures instructions. Amplification was verified using gel electrophoresis on a 1.5% agarose gel. The amplicons were sequenced using a capillary-based 3730xl DNA Analyzer (Applied Biosystems, Foster City, California, USA) at the University of Georgia Genomics Facility (Athens, Georgia, USA). The electropherograms were analyzed with Geneious R9.0.5 software using microsatellite plugin v1.4. Following initial diversity assessment, 35 loci with potential multiple peaks suggesting polymorphism were tested using eight samples from varying populations. A selection of 24 variable loci was tested further to assess genetic diversity and linkage disequilibrium (Table 1.1; Table 1.2). The loci were selected based on characters that allowed for future multiplexing, and primer combinations were tested with Multiplex Manager v1.2 (Holleley and Geerts, 2009). The DNA from 48 samples from Henley Bay, South Australia was extracted and amplified with the selected loci, of which 14 were then arranged into 3 multiplexes to reduce laboratory expenses and labor (Table 1.3). The suggested multiplexes were tested on 48 samples from Penguin Island and Marino Rocks.

Multilocus lineages and multilocus genotypes were determined using GenClone2.0 and the ρ_{gen} and ρ_{sex} were calculated (Arnaud-Haond and Belkhir, 2007). Duplicated genotypes were removed from allelic frequency and genetic diversity analyses performed in GenoDive 2.0 b14 (Meirmans and Van Tienderen, 2004). Loci were tested for heterozygote excess, heterozygote deficit, and linkage disequilibrium in GenePop 4.2 against a Bonferroni corrected p-value (Raymond and Rousset, 1995; Rousset, 2008).

Results:

An annealing temperature of 60° C effectively amplified the 14 microsatellite primers. Linkage equilibrium p-values were greater than the Bonferroni corrected significance of 0.0036 for all primer pairs. Allelic diversity ranged from 2-7 alleles with 0.038 to 3.258 effective number of alleles (Table 1.1). Observed heterozygosity ranged from 0.038 to 0.730 across loci. Contrary to the expectation for neutral markers, AmA_09 and AmA_36 showed significant

heterozygote deficiencies from Hardy-Weinberg Equilibrium and may be used with greater caution. Penguin Island showed fewer unique multi-locus genotypes (13) than Marino Rocks (41), resulting in genotypic richness values of 0.26 and 0.85, respectively. Observed heterozygosity was similar between sites at 0.381 for Marino Rocks and 0.390 for Penguin Island. Both populations showed slight inbreeding as indicated by positive G_{IS} values, with a lower level of inbreeding displayed by Marino Rocks (G_{IS} = 0.064) compared to Penguin Island (G_{IS} = 0.124). The F_{ST} of 0.318 indicated great differentiation occurred between sites.

Discussion:

The 14 microsatellite primers developed showed comparable allelic diversity to the 2 to 8 alleles per locus seen in *Syringodium filiforme* (*Cymodoceaceae*) (Bijak et al., 2014) but fewer than the 3 to 17 observed in *Thalssia testudinum* (*Cymodoceaceae)* (van Dijk et al. 2007). All loci showed linkage disequilibrium, and the significant heterozygote deficiencies of 2 loci may be due to characteristics of the tested populations instead of characteristics of the primer pairs. Multi-locus genotypes were distinguished in *A. antarctica* samples due to the polymorphic nature of the developed microsatellites. The lower genotypic richness of Penguin Island suggests a greater allocation of asexual reproduction at that site.

Waycott et al. (1996) suggested inbreeding as a potential reason for the lack of diversity previously detected. Inbreeding increases the proportion of homozygotes beyond what is expected from a randomly mating population and, if common, can cause alleles to become fixed within a population (Freeland et al., 2011). *A. antarctica* populations were slightly inbred, which could be the result of local bed characteristics that prevent establishment of seedlings. Sexual reproduction in *A. antarctica* utilizes true vivipary where offspring germination occurs while attached to the parent plant (Kuo and Kirkman, 1990). Successful establishment of viviparous seedlings relies upon local bed characteristics because hydrodynamic forcing, lack of substrata roughness, and contact with adult plants can dislodge seedlings and impede recruitment (Rivers et al., 2011). Unlike seeds that display dormancy, viviparous plants do not experience fitness benefits from dispersal through time or space (Elmqvist and Cox, 1996), such that gene flow between populations is likely to be limited and population differentiation enhanced. Further study would need to be conducted to determine the extent to which inbreeding and population

differentiation are occurring across the species range and to identifying driving factors of genetic patterns.

Contrary to previous studies, microsatellite marker screening of three populations provided evidence of the presence of genetic polymorphism in *A. antarctica*. This result has implications for restoration and conservation decisions regarding *A. antarctica* beds. For example, areas with high genotypic richness may be identified as potential donor beds to reduce the risk of inbreeding depression. Future studies will be able to utilize the 14 microsatellite markers developed to improve our understanding of the population processes and evolutionary ecology of *A. antarctica*.

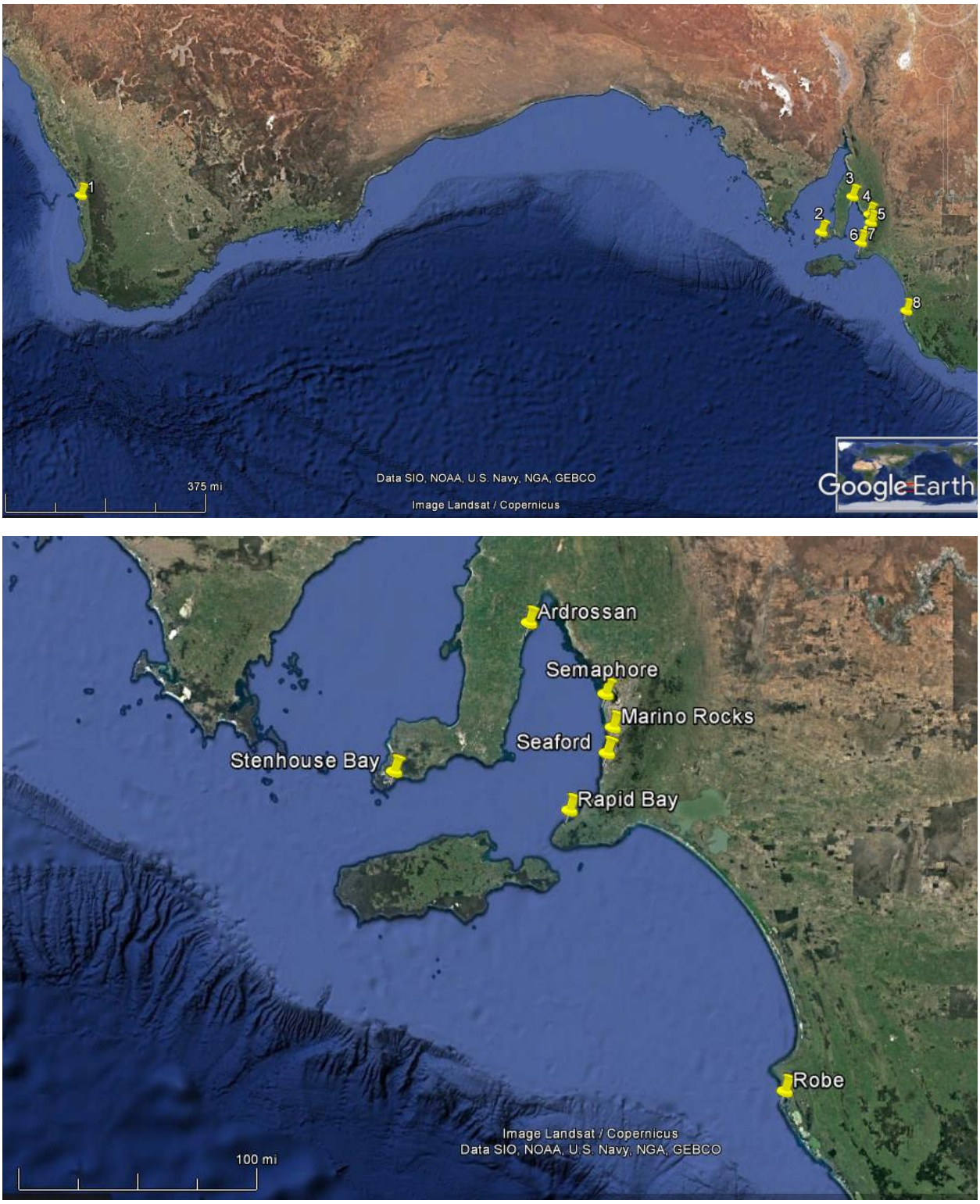


Figure 1.1. Sampling locations for *A. antarctica*. Top panel shows all sampling locations where 1= Penguin Island, 2 = Stenhouse Bay, 3 = Ardrossan, 4 = Semaphore, 5 = Marino Rocks, 6 = Seaford, 7 = Rapid Bay, and 8 = Robe. The bottom panel highlights the South Australia populations.

Table 1.1. Developed microsatellite loci information. N = number of samples, N_a = number of alleles, N_{eff} = effective number of alleles, H_o = observed heterozygosity, H_s = heterozygosity of the subpoulation, H_t = total heterozygosity, H'_t = total corrected heterozygosity, and G_{IS} = inbreeding coeffient.

Locus	Primer sequences (5'-3')	Repeat motif	Ascension number	Range	Multiplex	Tag	N	N_a	N_{eff}	H_o	H_s	H_t	H'_t	G_{IS}
AmA_05	F: AGAAAACACCTTTTCTTGCTGC R: ATCCATGCGATCCAAGAGTC	(CTT)16	KX707436	267-288	1	PET	96	4	1.308	0.203	0.243	0.256	0.268	0.166
AmA_07	F: TCTCCATACATCTTTGCACAACT R: GGATTTTGTCGTTGAGCAGG	(TC)13	KX707437	87-105	3	NED	96	5	2.082	0.462	0.535	0.768	1.000	0.136
AmA_09	F: GGTTGCGATTGAAAGAAACAA R: CCTGAATGTCTCCTATCTCCG	(GA)13	KX707438	86-100	2	6-FAM	96	5	2.358	0.538	0.592	0.622	0.652	0.092
AmA_10	F: TTTTCACTAGAACTTGGAACTTGA R: ACTCATTGACCGATGGATGC	(TTC)12	KX707439	78-103	3	PET	96	4	1.919	0.473	0.492	0.530	0.568	0.038
AmA_17	F: GTTCGGGCGTGTTTATTCC R: ATTATGGTACCCTCGGAGCC	(TTC)11	KX707440	152-176	3	6-FAM	96	5	1.809	0.536	0.457	0.649	0.841	-0.173
AmA_20	F: GCAATGCCATAAGCATCAGA R: ATCTCCCAATGACCACGAAG	(TCT)11	KX707441	95-140	3	6-FAM	96	7	2.922	0.522	0.679	0.714	0.750	0.232
AmA_21	F: GTCATGTAGATAGATGTCGTCATTG R: CCAAATTGCAAATGCAAACA	(TCT)11	KX707442	71-89	1	6-FAM	96	5	3.258	0.730	0.711	0.735	0.758	-0.027
AmA_25	F: CCCTCTCTTTTCTCCGCCT R: GTGCAGCTTGTGATGCAGTT	(CTT)10	KX707443	113-137	2	NED	96	7	2.156	0.511	0.551	0.700	0.848	0.072
AmA_26	F: GTCAACACCATGGGACCTTC R: CATGACTTCTGTATGTGACCCA	(TC)10	KX707444	140=156	1	PET	96	4	1.417	0.190	0.305	0.464	0.622	0.376
AmA_27	F: CGTGCAGATTCATTCCCTTC R: CACCACAAGTACCTTTGCCA	(CTT)10	KX707445	138-153	1	VIC	96	2	1.302	0.171	0.240	0.437	0.634	0.288
AmA_36	F: TCCTTCTCTTTCTTGCTCGC R: CACCCATTTGATTTCCATCC	(AGG)9	KX707446	104-134	2	PET	96	7	2.177	0.389	0.559	0.698	0.837	0.304
AmA_38	F: AAAGAGAAGAGTTCCATCTCCG R: GCTGGGAGGACCTGACAGTA	(GAG)9	KX707447	136-142	1	6-FAM	96	3	1.337	0.264	0.259	0.258	0.257	-0.019
AmA_39	F: TCTCTTCTTCTTGCCGATCC R: GTTTCTGACAAGGAGAACCTCCCTCC	(TGG)8	KX707448	107-116	3	VIC	96	2	1.493	0.225	0.342	0.500	0.658	0.341
AmA_40	F: TGCACATATAGATCTGTTTACAACCC R: TCGTTACTAATCTAAATATCGTCGGA	(AAG)8	KX707449	109-111	2	VIC	96	2	1.038	0.038	0.038	0.038	0.038	-0.014

Table 1.2 Additional *A. antarctica* primers developed.

Locus	Primer Sequence (5'-3')	Repeat motif	Ascension number
AmA_04	F:AATACATACTTCAACACTCTCCCTCG	(AC)16	KX822162
	R: TTCTCCTATTCTCTTATTCTCAGAGC		
AmA_06	F: CTCACGTCCTTCCTCTACTTTGA	(CA)14	KX822163
	R: GGTGCGGTTTTCTAACAGAAT		
AmA_13	F: CACTGCCTTCACCGATATGT	(AGG)12	KX822164
	R: CAAGAAACCAACGAGCAGAA		
AmA_18	F: CTACAGCGGGGTTGCTGG	(TC)11	KX822165
	R: AAACCCAAAACCATGACCTG		
AmA_19	F: CCCAAACAACAGGCACTTTC	(AG)11	KX822166
	R: CATCACCACAACCACAGGTC		
AmA_22	F: AACGGTCGCAAGTAAGCTG	(GGT)10	KX822167
	R: AACCTTGAATGGTTGATGTAAAA		
AmA_23	F: CTTTCTCTAGTTTTGGCGGC	(TC)10	KX822168
	R: CGATGTTCCATTGAGATTGTTG		
AmA_28	F: TTGAAGATGACCAAACTATTTATCG	(ATGT)10	KX822169
	R: GCAGTGATACCTGTTGTTGCAT		
AmA_47	F: TCTTATTACCAAACATACCCAGCA	(AAG)8	KX822170
	R: CGTACTTTATGCATATGTAATGCC		
AmA_48	F: CCACAACAACAGCAGGAACA	(GAG)8	KX822171
	R: TGGACAAGTTCCACCTCCTT		

Table 1.3. *Amphibolis antactica* multiplex panel assignments.

	Primer	Tag
	AmA_21	6-FAM
	AmA_38	6-FAM
	AmA_27	VIC
	AmA_26	PET
	AmA_05	PET
Multiplex 2		
	AmA_09	6-FAM
	AmA_40	VIC
	AmA_25	NED
	AmA_36	PET
Multiplex 3		
	AmA_20	6-FAM
	AmA_17	6-FAM
	AmA_39	VIC
	AmA_07	NED
	AmA_10	PET

Literature Cited:

Arnaud-Haond, S., Belkhir, K., 2006. genclone: a computer program to analyse genotypic data, test for clonality and describe spatial clonal organization: program note. Mol. Ecol. Notes 7, 15–17. doi:10.1111/j.1471-8286.2006.01522.x

Bijak, A.L., van Dijk, K., Waycott, M., 2014. Development of microsatellite markers or a tropical seagrass, Syringodium filiforme (Cymodoceaceae). Appl. Plant Sci. 10.

Brownstein, M.J., Carpten, J.D., Smith, J.R., 1996. Modulation of non-templated nucleotide addition by Taq DNA polymerase: primer modifications that facilitate genotyping. Biotechniques 20, 1004–1006.

Carruthers, T.J.B., Dennison, W.C., Kendrick, G.A., Waycott, M., Walker, D.I., Cambridge, M.L., 2007. Seagrasses of south–west Australia: A conceptual synthesis of the world's most diverse and extensive seagrass meadows. J. Exp. Mar. Biol. Ecol. 350, 21-45.

Elmqvist, T., Cox, P.A., 1996. The evolution of vivipary in flowering plants. Oikos 3-9.

Freeland, J.R., Kirk, H., Petersen, S.D., 2011. *Molecular Ecology 2 Ed.* Wiley-Blackwell, West Sussex, UK.

Holleley, C., Geerts, P., 2009. Multiplex Manager 1.0: a cross-platform computer program that plans and optimizes multiplex PCR. Biotechniques 46, 511–517. doi:10.2144/000113156

Hughes, A.R., Stachowicz, J.J., 2009. Ecological impacts of genotypic diversity in the clonal seagrass Zostera marina. Ecology 90, 1412–1419.

Kuo, J., Kirkman, H., 1990. Anatomy of viviparous seagrasses seedlings of Amphibolis and Thalassodendron and their nutrient supply. Botanica marina 33, 117-126.

Malausa, T., Gilles, A., Meglécz, E., Blanquart, H., Duthoy, S., Costedoat, C., Dubut, V., Pech, N., Castagnone-Sereno, P., Délye, C., Feau, N., Frey, P., Gauthier, P., Guillemaud, T., Hazard, L., Le Corre, V., Lung-Escarmant, B., Malé, P.-J.G., Ferreira, S. & Martin, J.-F. (2011) High-throughput microsatellite isolation through 454 GS-FLX Titanium pyrosequencing of enriched DNA libraries. Mol. Ecol. Resour. 11, 638-644.

Meirmans, P.G., and Van Tienderen, P.H., 2004. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. Mol. Ecol. Notes 4, 792-794.

Meglecz, E., Costedoat, C., Dubut, V., Gilles, A., Malausa, T., Pech, N., Martin, J.F., 2010. QDD: a user-friendly program to select microsatellite markers and design primers from large sequencing projects. Bioinformatics 26, 403–404. doi:10.1093/bioinformatics/btp670

Nowicki, R.J., Thomson, J.A., Burkholder, D.A., Fourqurean, J.W., Heithaus, M.R., 2017. Predicting seagrass recovery times and their implications following an extreme climate event. Mar. Ecol. Prog. Ser. 567, 79-93. doi: 10.3354/meps12029

Raymond, M., Rousset F., 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Hered. 86, 248-249.

Reynolds, L.K., McGlathery, K.J., Waycott, M., 2012. Genetic diversity enhances restoration success by augmenting ecosystem services. PLoS ONE 7, e38397. doi:10.1371/journal.pone.0038397

Rivers, D. O., Kendrick, G. A., Walker, D. I., 2011. Microsites play an important role for seedling survival in the seagrass Amphibolis antarctica. J. Exp. Mar. Biol. Ecol. 40, 29-35.

Rousset, F., 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Mol. Ecol. Resour. 8,103-106.

Schuelke, M., 2000. An economic method for the fluorescent labeling of PCR fragments. Nature Biotechniques 18, 233–234.

Thomson, J.A., Burkholder, D.A., Heithaus, M.R., Fourqurean, J.W., Fraser, M.W., Statton, J., Kendrick, G.A., 2015. Extreme temperatures, foundation species, and abrupt ecosystem change: an example from an iconic seagrass ecosystem. Glob. Change Biol. 21, 1463–1474. doi:10.1111/gcb.12694

Waycott, M., Walker, D.I., James, S.H., 1996. Genetic uniformity in Amphibolis antarctica, a dioecious seagrass. Heredity 76.

Waycott, M., Duarte, C.M., Carruthers, T.J., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Short F.T., Williams, S. L., 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proc. Natl. Acad. Sci. 106, 12377–12381.

Williams, S.L., 2001. Reduced genetic diversity in eelgrass transplantations affects both population growth and individual fitness. Ecol. Appl. 11, 1472–1488.

Van Dijk, J. K., Waycott, M., Van Tussenbroek, B. I., Ouborg, J., 2007. Polymorphic microsatellite markers for the Caribbean seagrass Thalassia testudinum Banks ex König. Mol. Ecol. Notes 1, 89-91.

Chapter 2. The genetic population structure of *Halodule wrightii* in Atlantic edge-of-range populations

Overview:

Seagrass genetic diversity decreases along edge-of-range populations, making them more susceptible to biotic and abiotic stressors. However, the tropical/subtropical species *Halodule wrightii* has the potential to expand in range along the northeast coast of the United States under climate change scenarios. Due to the important role this species plays in seagrass bed colonization, the goal of this study was to investigate the genetic population structure of *H. wrightii* at edge-of-range locations in Florida, North Carolina, and Bermuda. Eleven microsatellites were amplified on samples representing 15 sites. Allelic diversity, genotypic richness, population differentiation, gene flow, principal components analysis, and k-means population clustering were performed. *H. wrightii* sites were highly clonal, with a mean genotypic richness of 0.09. Many clones included samples that displayed aneuploid genotypes, which could have positive implications for clonal persistence. Genetic differentiation followed a statistically significant isolation-by-distance relationship. Two main populations were identified as Bermuda and Florida Bay, and the Indian River Lagoon, Gulf of Mexico, and North Carolina. Genetic results from this study can inform our understanding of the origins of *H. wrightii* edge populations, and the mechanisms of bed recruitment and maintenance used in the case of range expansion.

Highlights:

- Edge-of-range *H. wrightii* sites were highly clonal (R = 0.00 – 0.20)
- Aneuploidy was observed in 27% of samples
- Bermuda populations were related most closely to Florida Bay while the North Carolina and Indian River Lagoon populations were related most closely to one another

Keywords: seagrass; genetic diversity; microsatellites

Introduction:

Haldoule wrightii is a tropical/subtropical species of seagrass that is an effective colonizer of bare sediment due to its fast growth and wide tolerance for a variety of temperatures, depths, and nutrient loads (Phillips, 1960; Gallegos et al, 1994; Short et al., 2007). These characteristics allow *H. wrightii* to occupy an extensive geographic range, reaching from Brazil to North Carolina in the western Atlantic Ocean (Figure 2.1). Under climate change scenarios, the range of *H. wrightii* has the potential to expand northward along the coast of North Carolina due to increased temperatures, nutrient loads, and subsequent dieback of *Zostera marina,* though encroachment of *H. wrightii* into previous *Z. marina* habitat has yet to be observed (Micheli, 2008). Regardless, *H. wrightii* is one of five seagrass species with an increasing range during a time of global seagrass loss (Short et al., 2011; IUCN redlist. http://www.iucnredlist.org/details/173372/0. Accessed Jan 11, 2017).

Bermuda and North Carolina populations of *H. wrightii* are at the extreme northern edge-of-range and are isolated from other populations, the nearest of which is in the Indian River Lagoon along the eastern coast of Florida (Figure 2.1). The reasons behind the absence of *H. wrightii* along the coasts of Georgia and South Carolina are unclear but high wave energy may be a contributing factor (Robert Virnstein, personal communication). Measures of population divergence and gene flow can give insight into the origin of isolated populations. Genetic processes also contribute to the evolutionary potential of edge-of-range populations (Sexton et al., 2009) For example, marginal populations with sufficient genetic diversity may adapt to novel edge environments, but adaptation can be disrupted by gene flow (Sexton et al., 2009). The long-term persistence of peripheral *H. wrightii* populations also may be affected by genotypic diversity, as seen by the increased resilience of *Z. marina* meadows with high genotypic diversity (Reusch et al., 2005; Hughes and Stachowicz, 2009).

The genetic structure of *H. wrightii* has been investigated along the Texas Gulf Coast using a variety of molecular markers. Results with restriction length fragment profile (RAPD) markers showed each ramet (sampled at 3 meter intervals) as being genetically distinct (Angel, 2002). However, other studies from amplified fragment length profiles (AFLPs), RAPDs, and microsatellites detected low to moderate levels of genetic diversity (Travis and Sheridan, 2006; Larkin et al., 2008; Larkin et al., 2017) *Halodule wrightii* population differentiation was first

detected in the Texas region as following the genetic isolation-by-distance model (Travis and Sheridan, 2006; Larkin et al., 2008). This result is unsurprising given that *H. wrightii* seeds are negatively buoyant and are released at the sediment surface, encouraging local dispersal, and that dioecious plants such as seagrasses are more likely to experience isolation-by-distance genetic structuring than monoecious plants (Nazareno et al., 2013). However, isolation-by-distance in seagrasses is highly regionally specific (Muñez-Salazar et al., 2005), and later study showed population structure in the region was more closely related to tidal range than to barriers or distance (Larkin et al., 2017). Further investigation of *H. wrightii* population structure is needed to assess the generalizability of these results outside of the Texas region (Larkin et al., 2008).

Challenges remain for obtaining an accurate estimate of *H. wrightii* genetic diversity. Though seagrasses are typically considered to be diploid organisms, the number of chromosomes in *H. wrightii* varied from 2n = 24 to 2n = 39 with the most common arrangement of 2n = 38 in 45% of cells sampled using intraspecific analysis (da Silva et al., 2017). Other studies, such as the original description of chromosome number in *H. wrightii*, describe the basic chromosome number to be as high as 2n = 44 (den Hartog et al. 1979). Due to the lack of relationship between the number of chromosomes detected and full genome duplication, it appears unlikely that true polyploidy is occurring. Ito and Tanaka (2011) also refuted the presence of polyploids in the *Halodule* genus based on the number of copies of the phyB gene in *Halodule pinifolia*.

Rather, the major process driving karyotype variation appears to be cytomixis, where crossover of DNA is occurring between cells in the meristematic tissue of *H. wrightii* during prophase (da Silva et al., 2017). As a result, cells can have an abnormal number of chromosomes (aneuploidy) and individuals contain varied genotypes (genetic mosaicism). Determining the correct allelic dosage, or number of copies of each allele at a particular locus, is a major challenge for analyzing aneuploid data (Dufresne et al., 2014). Genetic mosaicism has been observed in *Zostera marina* and *Posidonia australis* with microsatellites (Reusch and Boström, 2011; Sinclair et al., 2015). In angiosperms, genetic mosaicism is facilitated by the stratified arrangement of the basal meristem (Klekowski, 2003), which is a characteristic of *H. wrightii*. However, the presence of genetic mosaicism and aneuploidy was not noted in previous *H. wrightii* genetic population studies, likely due to the use of methods such as AFLPs and RAPDs, which do not examine specific alleles.

The goal of this study was to assess the genetic diversity, recruitment processes, and regional differentiation of *H. wrightii* from 15 sites along the species edge-of-range in Florida, North Carolina, and Bermuda. My hypotheses were as follows: first, due to the location of study sites along the northern edge of the distribution range, I expect populations to rely predominantly on asexual reproduction rather than sexual reproduction, following the edge-of-range effect observed in other seagrasses. This effect will be reflected in low local genetic diversity with a high proportion of clonality within populations. Second, because *H. wrightii* is a dioecious plant with negatively buoyant seeds, the expectation is that genetic connectivity should be limited resulting in a strong isolation-by-distance relationship. If so, one would expect strong genetic structure among the regions where *H. wrightii* samples were collected: Florida, North Carolina, and Bermuda.

Methods:

2.1 Sample collection:

Samples were collected from 15 sites along the U.S. Atlantic coast in the Gulf of Mexico (1 site), Florida Bay (5), Indian River Lagoon (3), North Carolina (4), and Bermuda (2) (Figure 2.2, see Appendix A2 for site descriptions and individual collection-site maps). Sites in the Indian River Lagoon, Florida Bay, and North Carolina were established along distance gradients to capture spatial structure and connectivity of populations (Table 2.1). At each site, seagrass was sampled at two quadrat locations spaced between 35 – 200 m apart. Next, GPS coordinates were collected for each quadrat. Then, each quadrat was sectioned with dive flags into a 5-m by 5-m grid. At each quadrat a shoot was collected every meter of the grid for a total of 72 shoots per site (Figure 2.3). Notable exceptions include Research Dock (Florida Bay) where three quadrats were established for greater spatial coverage, Wabasso Causeway (Indian River Lagoon) where quadrats were sampled randomly due to seagrass patchiness, and Bermuda locations that were collected by previous researches. Bailey's Bay (Bermuda samples were haphazardly gathered across an area of 70 m^2 (Bijak, 2016), and Well Bay (Bermuda) samples came from two 180-m transects spaced 55 m apart, with 15 samples collected every 15 m along each transect (Dr. Kimberley Holzer, under Bermuda Department of Environment and Natural Resources permit number 130607). Shoots were cleaned of epiphytes and placed in silica gel beads for desiccation and storage until DNA extraction (Chase and Hills, 1991).

2.2 DNA extraction, amplification, and sequencing:

Following collection, the youngest available dried tissue was placed in a 96 deep-well plate (USA Scientific, Ocala, Florida, USA) and sealed with strip caps (Qiagen,Valencia, California, USA) for DNA extraction at the University of Wisconsin-Madison Biotechnology Center DNA Sequencing Facility (Madison, Wisconsin, USA). The Cetyltrimethyl Ammonium Bromide (CTAB) method, as described in Saghai-Maroof et al., (1984), was performed with minimal modification to extract DNA from 40-50 mg of tissue. Following elution, a 1.5:1 (v:v) mixture of Axygen Clean-Seq beads (Corning Life Sciences, Corning, New York, USA) to extracted DNA sample was used to clean the DNA of any inhibitory compounds. Quant-IT PicoGreen fluorescent dye (Thermo Fisher, Waltham, Massachusetts, USA) was used to quantify the DNA (Thermo Fisher). The DNA was standardized to 5 ng μL^{-1}, except in a final rerun of samples where 9:1 and 3:1 dilutions of RNase-free water:DNA were made. Results of representative samples were verified with comparisons to in-house extractions following the manufacture's protocol of Qiagen DNeasy extraction kits.

DNA regions of interest were targeted and amplified in a three-panel multiplex setup using 13 microsatellite primers developed by Larkin et al. (2012; personal communication) (Table 2.1). Primer combinations were organized into multiplex panels using Multiplex Manager v1.2 (Holleley and Geerts, 2009). PIG-tails (5′-GTTTCT-3′) were attached to reverse primers to reduce stutters, and fluorescent and M13 labeled tags (5′-TGTAAAACGACGGCCAGT-3′) were added to forward primers to allow for a nested Polymerase Chain Reaction (PCR) protocol (Brownstein et al., 1996; Schuelke, 2000). Each reaction well contained 1 µL of DNA, and 9 µL of master mix consisting of 0.1 µL forward and reverse primer, 5 µL TypeIt (Qiagen), and 3.2 µL RNase-free water. PCR protocol was optimized using a touch-down approach from 60˚C to 54˚C to target better primer annealing temperatures. Amplification was confirmed with gel electrophoresis using a 1.5% agarose gel.

PCR products were sequenced at the Georgia Genomics Facility (Athens, Georgia, USA) using a capillary-based 3730xl DNA analyzer (Applied Biosystems, Foster City, California, USA) with an internal GFFLIZ500 size standard. Electropherograms were processed and scored in Geneious R9.0.5. Some electropherograms displayed aneuploidy and were rerun to reduce the risk of scoring PCR artifacts. Additionally, to determine if different genotypes existed across cell

lineages of a single ramet (indicating genetic mosaicism), the leaves of 10 samples displaying aneuploidy were sectioned vertically and each vertical section was analyzed separately, as described in Reusch and Boström (2011) and Sinclair et al. (2015). However, due to the thin width (approximately 1 mm) of *H. wrightii* samples, the leaves were only split into 2 longitudinal sections rather than 8-10 sections of 0.4 mm as recommended by Reusch and Boström (2011).

2.3 Statistical analyses:

Data formatting

The presence of aneuploidy was challenging for statistical analyses due to the uncertainty in allelic dosing, as mentioned above. For example, an electropherogram displaying alleles ABC could be AABC, ABBC, ABCC, or ABCX (where X is a null allele) (Dufresne et al., 2014). Allelic dosage assignments affect allelic frequency statistics, which is the basis for other genetic diversity measures. Thus, discrepancies in allelic dosage will propagate throughout statistical analyzes. Several techniques have been suggested for overcoming this deficiency (Dufresne et al., 2014). First, microsatellites may be scored as dominant data, resulting in the loss of information about heterozygosity, but avoiding the problem of dosage uncertainty. Second, dosage uncertainty can be incorporated into the inferences of population genetic parameters through programs such as GenoDive 2.0 (Meirmans and Van Tienderen, 2004). To preserve the maximum amount of information about the allelic composition of the populations, I chose to use the second approach for the analysis of my data.

GenoDive 2.0 vb15 allows for correction of unsure allele dosage for certain allele frequency tests (described below), population fixation indexes, AMOVA testing, and Principal Components Analysis (Meirmans and Van Tienderen, 2004). A maximum likelihood method for every locus and population, heavily modified from De Silva et al. (2005) by Meirmans and Van Tienderen (2004), was used to resample aneuploid alleles into diploid identities for STRUCTURE analysis that required data to be of a single ploidy level (Meirmans and Van Tienderen, 2004). Resampling overestimates the number of heterozygotes and therefore was not performed for other analyses. Missing data may bias the results of Principal Components Analysis (PCA) and STRUCTURE tests by artificially grouping samples with missing data at the same loci (Meirmans and Van Tienderen, 2004). For this reason, the missing values (<8%) were

filled in using randomly drawn alleles from relative allelic frequencies for each population in those analyses, as recommended by Meirmans and Van Tienderen (2004).

Clonal identity

Preliminary clonal identity of all diploid samples was determined in GenClone and the probabilities of randomly drawing the genotype, ρ_{gen}, and of a clone resulting from a sexual event, ρ_{sex}, were calculated (Arnaud-Haond and Belkhir, 2006). Aneuploid genotypes and samples with missing values were manually assigned to clones during initial screening. Manual assignment was performed in Microsoft Excel 2013 using sorting functions to identify clonal individuals. Samples with null alleles that could not be confidently assigned to a clone were reanalyzed. If adequate amplification did not occur the sample was removed from the dataset. The resulting list of potentially unique genotypes was reanalyzed to increase the dataset robustness. Final clonal identity for each population was determined in RClone (Bailleul et al., 2016). Replicate genotypes were removed from further analyses to avoid biasing allelic frequencies.

Within population diversity measures

Allelic frequencies and common genetic diversity parameters including the number of alleles (N), effective number of alleles (N_{eff}), expected subpopulation heterozygosity (H_s), and expected total heterozygosity (H_t) were calculated with correction for unsure allele dosage in GenoDive. Observed heterozygosity, inbreeding coefficients, and population divergence from Hardy-Weinberg equilibrium could not be accurately calculated with the presence of aneuploidy.

Between population diversity measures

Partitioning of genetic diversity between and among populations was performed in an Analysis of Molecular Variance (AMOVA) using a ploidy-independent Infinite Allele Model without permutations. Matrices of population differentiation were calculated based on Nei's Corrected Fixation Index, G'_{ST} (Nei, 1987), the Corrected Standardized Fixation Index, G''_{ST} (Hendrick, 2005; Meirmans and Hedrick, 2011), and Jost's D (Jost, 2008). Jost's D population differentiation matrix was converted in a manner similar to Sundqvist et al. (2016) to estimate relative gene flow and depict population connectivity over evolutionary time periods. Bermuda sites were excluded from the analysis due to small sample size. Gene flow was plotted using the

qgraph function in the qgraph package in R (Epskamp et al., 2016). A standard Mantel test was performed using Jost's D differentiation matrix and pairwise geographic distances, excluding Bermuda sites, to test for isolation-by-distance. Geographic distances were determined from Google Earth using the shortest in-water path between sites. PCA covariance matrices were developed in GenoDive and plotted in DataGraph.

An admixture model of assumed population size K was run in STRUCTURE for population sizes K=1 to K=8 (Pritchard et al., 2000). Individuals were probabilistically assigned to populations based on allelic frequencies using a Markov Chain Monte Carlo (MCMC) method with burnin length of 50,000 steps, run length of 250,000 steps, and 15 iterations per K. Variations of dataset and parameter set inputs were tested to compare between potentially biased runs due to resampling and missing data fills. Two datasets were run with no missing data, resampled genotypes, and with and without location set as an a priori assumption. An additional dataset was run under the same settings but contained missing data. Resulting files were further analyzed with CLUMPAK software to visualize population structure and estimate Best K based on the ΔK method from Evanno et al., (2005) (Kopelman et al., 2015).

Results:

Aneuploidy was found in 27% (30/112) of genets. Anueploidy was seen for all sites except Wabasso Causeway (Indian River Lagoon), St. Joseph's Bay (Gulf of Mexico), and both Bermuda sites. Terrapin Bay (Florida Bay) and the Middle Marsh sites (North Carolina) had a large proportion of samples with an abnormal number of alleles. Samples that displayed aneuploidy were generally triploid, with a small number (< 7%) of tetraploids. The consistency of aneuploid data across runs, samples, and as many as 7 loci make it unlikely that the unusual number of alleles were due to scoring error (Figure 2.4). Nine of the 10 aneuploid samples that were vertically divided did not amplify. The remaining sample displayed different genotypes across the two sections of the ramet.

Initial screening of samples showed the presence of clones across quadrats so samples were pooled for each site during clone assignment. Across all sites, 112 unique genotypes were identified. While the pooled data increased sample size, Terrapin Bay only had 2 unique genotypes and both Bermuda locations were excluded from some analyzes because only a single clone was found at each site. Genotypic richness was constrained between 0 for Bermuda sites to

0.20 for Pelican Key (Table 2.1). While a higher proportion of sites in Bermuda and North Carolina showed extensive clonality, other regions showed variable genotypic richness.

Eleven of the 13 microsatellite loci were used because 1 locus was the same simple sequence repeat but with different flanking regions and another locus had poor amplification (Table 2.2). The number of alleles per locus ranged from 2 to 25 (Table 2.3), as compared with 2 to 8 for *Syringodium filiforme* (*Cymodoceaceae*) (Bijak et al., 2014) and 3 to 17 for *Thalassia testudinum* (*Cymodoceaceae*) (van Dijk et al., 2007). The locus with 25 alleles showed frequent presence of polyploidy. Per population, the average number of alleles per locus ranged from 1.2 for the Bermuda sites to 4.82 for Pelican Key (Florida Bay). In general, the effective number of alleles followed a similar pattern where N_{eff} was highest in Florida Bay sites and lowest in Bermuda. No observed heterozygosity was calculated due to the presence of polyploidy, and therefore could not be compared to expected within-population or total heterozygosity values. Average total hetereozygosity over all populations was 0.50.

G'_{ST}, G''_{ST}, and Jost's D numbers varied in absolute value but showed similar trends (Tables 2.4, 2.5, 2.6). Nei's G'_{ST} consistently had the lowest values and G''_{ST} the highest (Figure 2.5), as expected given that G''_{ST} is standardized to the maximum theoretical G_{ST} to account for the underestimation of G'_{ST} in populations with more than 2 alleles. All values were positive except between Pelican Key and Research Dock, and between Middle Marsh 1, 2, and 3. Both G_{ST} variants excluded Bermuda due to low sample size and showed St. Joseph's Bay as the most differentiated site. The highest differentiation for Jost's D occurred between St. Joseph's Bay and Bermuda, with Bermuda being the most differentiated overall. In the connectivity analysis, St. Joseph's Bay and Florida Bay displayed the weakest gene flow (Figure 2.6). The highest relative gene flow occurred within the Indian River Lagoon and between the Indian River Lagoon and North Carolina. A statistically significant ($p = 0.005$) isolation by distance relationship occurred with a slope of 0.178 for the linear regression of genetic distance by geographic distance. Geographic distance accounted for 22% of genetic distance variance ($R^2 = 0.220$).

The Rho_{ST} of 0.297 from the AMOVA indicated population structuring is occurring. The majority of AMOVA variance (70.3%) was attributed to the within population level. Principal component axes 1 and 2 represented 27.7% and 9.0% of the observed variance (Figure 2.7).

Graphically, there was general clustering within sites. Closest between-site grouping occurred with Bermuda and Florida Bay, with visual separation from other sites. In accordance with the PCA results, STRUCTURE output for missing, non-missing, location prior and no a prior settings indicated a best population structure at K= 2, followed by K = 3, where K is the number of populations (Figure 2.8). There was a consistent order of magnitude difference between the ΔK values of K = 2 and K= 3 across runs. At K= 2, Florida Bay and Bermuda sites were grouped as a population and other sites were grouped as the second population. North Carolina separated from Florida Bay at K= 3, and the Indian River Lagoon sites separated from St. Joseph's Bay at K= 4.

Discussion:

Populations with higher numbers of unique genotypes generally had greater allelic diversity, as indicated by higher average number of alleles per locus and effective number of alleles. Total heterozygosity of 0.50 was similar to mean heterozygosity of 0.56 reported in the Texas Gulf of Mexico (Larkin et al., 2017). While the presence of aneuploidy has not been noted in previous population diversity studies of *H. wrightii*, genetic mosaicism was positively correlated with clonal reproduction in edge-of-range populations of *Z. marina* (Reusch and Boström, 2011). These populations experienced a sharp decrease in the proportion of non-mosaic genotypes below a clonal richness of 0.20. Triploid genotypes can coexist with diploid and tetraploid genotypes in species with mixed reproductive modes such as seagrasses (Neiman et al., 2011), but results from this study support the observation of somatic mutation as the source of aneuploidy (da Silva et al., 2017). First, ploidy level was not consistent across all loci within aneuploid individuals. Second, the presence of two genotypes along the stratified meristem tissue of an aneuploid ramet could be caused by nuclei transfer across cell lines, as suggested in da Silva et al. (2017), but would not be seen in a sample with full polyploidy.

Differences between the sampling schemes of locations may have impacted the results. Random sampling occurred in Bermuda by previous researchers and in Wabasso Causeway (Indian River Lagoon) due to bed patchiness. Bailey's Bay and Well Bay on the Bermuda platform only had one unique genotype represented, and though fewer samples were collected they were obtained from a larger quadrat area. However, those samples were not combined with a second quadrat as in other sites and may not be representative of other sections of the seagrass

beds within those bays, or of processes beyond the island platform. Additionally, Wabasso Causeway genotypic richness was lower than other Indian River Lagoon sites that were sampled as blocks, which could be due to the closer spacing of the randomly collected samples or to other environmental factors.

Consistent with the hypothesis that edge-of-range populations rely predominantly on asexual reproduction, sites displayed low genotypic richness (R = 0.00 – 0.20). Along the Texas Gulf coast, in latitudes comparable to the Indian River Lagoon sites in this study, more variable clonal richness of *H. wrightii* was observed (R = 0.02 – 0.81) (Larkin et al., 2017). These sites may not capture the dynamics of extreme edge-of-range populations such as North Carolina and Bermuda, and samples analyzed in Larkin et al. (2017) from a Bermuda site had a relatively low genotypic richness of 0.03 consistent with this study. The lower genotypic richness observed in other regions of the present study compared to Larkin et al. (2017) may be due to the spatial scale at which sampling occurred. *Syrigodium filiforme* samples gathered over a similar scale to this study, across six 70-m^2 areas in northeast Florida Bay, also showed low genotypic richness (R = 0.03) in comparison to higher richness (R= 0.37 – 0.62) over 2500-m^2 sampling areas (Bijak, 2016). The low genotypic richness observed at the 25-m^2 quadrat and 30 – 200-m site scale for all areas in this study suggests rhizome extension plays a larger role than seeds in local bed recruitment of *H. wrightii* edge habitat.

Clones were present in varying proportions within and across quadrats, indicating a mixture of closely clustered, "phalanx", and interspaced, "guerilla", clonal architecture (Doust, 1981). Rhizome expansion can allow for persistence in suboptimal conditions (Kendrick et al., 2012). In particular for self-incompatible plants with low pollination success, vegetative expansion allows plants to "wait" for more optimal conditions for seed recruitment (Kudoh et al., 1999; Honnay and Jacquemyn 2008). The common multi-locus genotype between Research Dock, Pelican Key, and Porjoe Key (Florida Bay) indicates clonal spread across hundreds to thousands of meters. This extensive clonal expansion has been shown for *T. testudinum* in Mexico where a clone extended nearly 250 m (van Dijk and van Tussenbroek, 2009). Over further distances that exceed the maximal rhizome growth rate, clonal dispersal can be facilitated by vegetative fragmentation. *H. wrightii* vegetative fragments can remain viable for up to 4 weeks, with declines in viability after 2 weeks, and have shown the ability to settle and re-root (Hall et al., 2006). Vegetative

fragments may be especially important for areas lacking sexual reproduction or seed banks. *Halodule wrightii* seed banks have been observed in Texas, Panama, and parts of North Carolina, but seed reserves have not been reported for other areas of the range, such as the Indian River Lagoon (McMillan, 1981, 1985; Ferguson et al., 1993; Hall et al., 2006; Darnell et al., 2015).

Sexual reproduction is not the only source of genetic variation. Somatic mutations such as cytomixis can cause diversity within individual ramets and clonal lineages. Cellular mechanisms are subject to environmental cues and may use stress as a cue to create new genetic variation through polyploidy, aneuploidy, somatic doubling, etc. (De Storme and Mason, 2014). In small populations, somatic mutations can increase heterozygosity by a factor of two (Gill et al., 1995). Genetic mosaicism is often assumed to have negative fitness consequences including germ cell parasitism, cancerous growth, and intra-individual competition (Pineda-Krch and Lehtiliä, 2004). However, the increased diversity, synergism between variants, and selection through heterosis may have fitness advantages for genetically heterogeneous individuals, processes that are lesser studied (Pineda-Krch and Lehtiliä, 2004). Edge populations often do not display reduced fitness (Sexton et al., 2009), and highly clonal populations of seagrasses may be using somatic mutations as a means for increasing genetic diversity to maintain fitness. However, further study of the role somatic mutations play in clonal survival of *H. wrightii* edge populations is needed.

Given the high clonality of *H. wrightii* within my study sites, investigation of genetic differentiation and presence of gene flow across sites is important to understanding long-term diversity trends. Under some rhizome branching patterns, *H. wrightii* aneuploidy and genetic mosaicism has the potential to revert during vegetative expansion and increase differentiation (Reusch and Boström, 2011). Genetic homogeneity can be restored within a new shoot if it is the result of a single dividing cell line. However, this process would increase differentiation because genetic homogeneity would not extend to the original clone (Reusch and Boström, 2011). The rhizome of *H. wrightii* is highly branched during vegetative expansion (Bell et al., 2014) and might facilitate such differentiation. Across sites, moderate genetic structure ($Rho_{ST} = 0.297$) was detected in AMOVA results despite higher variability within populations than between populations. *H. wrightii* seeds are released below the sediment and are negatively buoyant (McMillan, 1985), which limits dispersal capabilities and can lend to population structuring.

Population fixation indices showed similar patterns of regional differentiation across F-statistics. The differentiation of St. Joseph's Bay (Gulf of Mexico) and Bermuda sites from other locations is to be expected given the statistically significant isolation-by-distance relationship between genetic distance and geographic distance. Low fixation values typically occurred within regions, which is consistent with low differentiation at local scales (< 100 km) observed across other seagrasses (Kendrick et al., 2012). Departing from this trend, Mosquito Lagoon and Parrish Park (both northern Indian River Lagoon) showed lower differentiation with North Carolina than expected given the distance from the nearest inlet and modeled flushing period of 230 days (Smith, 1993). This discrepancy could indicate a more recent evolutionary divergence between the Indian River Lagoon and North Carolina sites, which is supported by the highest relative gene flow occurring between the Indian River Lagoon and North Carolina, as well as the clustering of those regions in PCA and STRUCTURE analyses. However, the distinction of North Carolina sites at STRUCTURE population assignment K = 3 indicates some degree of differentiation has occurred.

Although geographically distant, Florida Bay and Bermuda clustered together through K = 8. A potential explanation for this would be if vegetative fragments from the Florida Bay region were transported to Bermuda and successfully established in a founder effect, resulting in a genetic bottleneck that contributed to the extreme clonality observed. It is also possible that additional genotypes existed in the region but were lost to the population over time because they were unable to persist in the conditions of the edge habitat. However, the latter hypothesis is less likely due to the recent establishment of *H. wrightii* in Bermuda. *H. wrightii* is excluded in papers on the ecology of Bermuda from 1918, 1935, and 1936 (Bernatowicz, 1952). The first report of *H. wrightii* in Bermuda was by Ostenfelt in 1927, though it was not substantiated with specimens (Bernatowicz, 1952). More confidence in the presence of *H. wrightii* in Bermuda is seen during the late 1940s, when *H. wrightii* was noted in several Bermuda bays, mentioned in a paper by Moore (n.d. ca 1947), and collected for specimen comparison (Bernatowicz, 1952). The lack of aneuploidy in the highly clonal Bermuda sites also suggests that the establishment of *H. wrightii* was too recent for major accumulation of somatic mutations within the populations.

If persistent over evolutionary timescales, genetic differentiation can lead to speciation events. Species debates already exist for the genus *Halodule*. For example, the characterization of *H. bermudensis* in Bermuda is based solely on small morphological differences in leaf tips

(den Hartog, 1964). Consequently, the species is currently referred to as *Halodule sp.*, though recent genetic barcoding studies have suggested the species identity to be *H. wrightii* (Bilewitch et al. http://www.researchgate.net/publication/267722329. Accessed Jan 11, 2017). Microsatellite markers amplified for *Halodule sp*. samples in this study and corroborate the likely identity of those samples as *H. wrightii*. Additional species debates remain regarding the *Halodule* genus. Next-generation sequencing techniques will be valuable for detangling these lineages, as well as for identifying drivers of genetic diversity and gene flow.

In conclusion, *H. wrightii* edge-of-range populations displayed a low number of clones but diversity within clonal lineages. In this way, somatic mutations replaced sexual reproduction as the prevailing force behind diversity. Given the cryptic nature of *H. wrightii* flowers and seed banks, it is difficult to determine if sexual reproduction is selected against or is lost in these populations. The future implications of genetic mosaicism on long-term clonal fitness are also unclear (Pineda-Krch and Lehtiliä, 2004). The geographically isolated populations of Bermuda and North Carolina showed few unique genotypes and may have resulted from founder effects of different source populations. While *H. wrightii* is the likely species identity of all samples in this study, population differentiation at geographically distant locations could have the potential to facilitate evolutionary-scale speciation events. Genetic variability is an important factor for the adaptability of edge-of-range populations, though further incorporation of genetics with models, empirical studies, and other evolutionary drivers is needed (Sexton et al., 2009). Next-generation sequencing can further the knowledge generated by this study on the important physiological and reproductive processes of *H. wrightii*.

Literature Cited:

Angel, R., 2002. Genetic diversity of Halodule wrightii using random amplified polymorphic DNA. Aquat. Bot. 74, 165–174.

Arnaud-Haond, S., Belkhir, K., 2006. GenClone: a computer program to analyse genotypic data, test for clonality and describe spatial clonal organization: program note. Mol. Ecol. Notes 7, 15–17. doi:10.1111/j.1471-8286.2006.01522.x

Bailleul, D., Stoeckel, S., Arnaud-Haond, S., 2016. RClone: a package to identify MultiLocus Clonal Lineages and handle clonal data sets in R . Methods Ecol. Evol. 7, 966–970. doi:10.1111/2041-210X.12550

Bell, S.S., Middlebrooks, M.L., Hall, M.O., 2014. The value of long-term assessment of restoration: support from a seagrass investigation. Restoration Ecol. 22, 304-310.

Bernatowicz, A.J., 1952. Marine monocotyledonous plants of Bermuda. Bull. Mar. Sci. 2, 338-345.

Bijak, A.L., 2016. Genetic diversity and population connectivity of Syringodium filiforme in the Florida Keys, USA and Northeastern Subtropical Atlantic Region (Unpublished Master's Thesis). University of Virginia, Virginia, USA.

Bijak, A.L., Dijk, K. van, Waycott, M., 2014. Development of Microsatellite Markers for a Tropical Seagrass, Syringodium filiforme (Cymodoceaceae). Appl. Plant Sci. 2, 1400082. doi:10.3732/apps.1400082

Brownstein, M., Carpten, J., Smith, J., 1996. Modulation of non-templated nucleotide addition by *Taq* DNA polymerase: primer modifications that facilitate genotyping. Biotechniques 11, 1004-1110.

Chase, M. W., Hills, H. H., 1991. Silica gel: an ideal material for field preservation of leaf samples for DNA studies. Taxon 215-220.

Darnell, K.M., Booth, D.M., Koch, E.W., Dunton, K.H., 2015. The interactive effects of water flow and reproductive strategies on seed and seedling dispersal along the substrate in two sub-tropical seagrass species. J. Exp. Mar. Biol. Ecol. 471, 30–40. http://doi.org/10.1016/j.jembe.2015.05.006

Da Silva, S.L., Magalhães, K.M., and de Carvalho, R., 2017. Karyotype variations in seagrass (Halodule wrightii Ascherson—Cymodoceaceae). Aquat. Bot. 136, 52-55.

De Silva, H.N., Hall, A.J., Rikkerink, E., McNeilage, M.A., Fraser, L.G., 2005. Estimation of allele frequencies in polyploids under certain patterns of inheritance. Heredity 95, 327-334.

De Storme, N., Mason, A., 2014. Plant speciation through chromosome instability and ploidy change: Cellular mechanisms, molecular factors and evolutionary relevance. Curr. Plant Biol. 1, 10–33. doi:10.1016/j.cpb.2014.09.002

Den Hartog, C., 1964. An approach to the taxonomy of the sea-grass genus Halodule Endl. (Potamogetonaceae). Blumea 12, 290.

Den Hartog, C., Van Loenhoud, P.J., Roelofs, J.G.M., Van de Sande, J.C.P.M., 1979. Chromosome numbers of three seagrasses from the Netherlands Antilles. Aquat. Bot. 7, 267-271.

Doust, L. L., 1981. Population dynamics and local specialization in a clonal perennial (Ranunculus repens): I. The dynamics of ramets in contrasting habitats. J. Ecol. 743-755.

Dufresne, F., Stift, M., Vergilino, R., Mable, B.K., 2014. Recent progress and challenges in population genetics of polyploid organisms: an overview of current state-of-the-art molecular and statistical tools. Mol. Ecol. 23, 40–69. doi:10.1111/mec.12581

Epskamp, S., Cramer, A.O., Waldorp, L.J., Schmittmann, V.D., Borsboom, D., 2012. qgraph: Network visualizations of relationships in psychometric data. J. Stat. Softw. 48, 1-18.

Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. Mol. Ecol.14, 2611–2620. doi:10.1111/j.1365-294X.2005.02553.x

Ferguson, R.L., Pawlak, B.T., Wood, L.L., 1993. Flowering of the seagrass Halodule wrightii in North Carolina, USA. Aquat. Bot. 46, 91-98.

Gallegos, M.M., Merino, M., Rodriguez, A., Marbá, N., Duarte, C. 1994. Growth patterns and demography of pioneer Caribbean seagrasses Halodule wrightii and Syringodiun filiforme K. Mar. Ecol. Prog. Ser. 109, 99-104.

Gill, D. E., Chao, L., Perkins, S. L., Wolf, J. B., 1995. Genetic mosaicism in plants and clonal animals. Annu. Rev. Ecol. Syst. 423-444.

Hall, L.M., Hanisak, M.D., Virnstein, R.W., 2006. Fragments of the seagrasses Halodule wrightii and Halophila johnsonii as potential recruits in Indian River Lagoon, Florida. Mar. Ecol. Prog. Ser. 310, 109–117.

Hedrick, P. W., 2005. A standardized genetic differentiation measure. Evol. 59, 1633-1638.

Holleley, C., Geerts, P., 2009. Multiplex Manager 1.0: a cross-platform computer program that plans and optimizes multiplex PCR. Biotechniques 46, 511–517. doi:10.2144/000113156

Honnay, O., Jacquemyn, H., 2008. A meta-analysis of the relation between mating system, growth form and genotypic diversity in clonal plant species. Evol. Ecol. 22, 299-312.

Hughes, A.R., Stachowicz, J.J., 2009. Ecological impacts of genotypic diversity in the clonal seagrass Zostera marina. Ecology 90, 1412–1419.

Ito, Y., Tanaka, N., 2011. Hybridisation in a tropical seagrass genus, Halodule (Cymodoceaceae), inferred from plastid and nuclear DNA phylogenies. Telopea 13, 219–231.

Jost, L., 2008. GST and its relatives do not measure differentiation. Mol. Ecol. 17, 4015-4026.

Kendrick, G.A., Waycott, M., Carruthers, T.J.B., Cambridge, M.L., Hovey, R., Krauss, S.L., Lavery, P.S., Les, D.H., Lowe, R.J., Vidal, O.M. i, Ooi, J.L.S., Orth, R.J., Rivers, D.O., Ruiz-Montoya, L., Sinclair, E.A., Statton, J., van Dijk, J.K., Verduin, J.J., 2012. The central role of dispersal in the maintenance and persistence of seagrass populations. BioSciences 62, 56–65. doi:10.1525/bio.2012.62.1.10

Klekowski, E.J., 2003. Plant clonality, mutation, diplontic selection and mutational meltdown. Biol. J. Linnean Soc. 79, 61-67.

Kopelman, N.M., Mayzel, J., Jakobsson, M., Rosenberg, N.A., Mayrose, I., 2015. CLUMPAK : a program for identifying clustering modes and packaging population structure inferences across *K*. Mol. Ecol. Resour. 15, 1179–1191. doi:10.1111/1755-0998.12387

Kudoh, H., Shibaike, H., Takasu, H., Whigham, D. F., Kawano, S., 1999. Genet structure and determinants of clonal structure in a temperate deciduous woodland herb, Uvularia perfoliata. J. Ecol. 87, 244-257.

Larkin, P., Heideman, K.L., Parker, J.E., Hardegree, B., 2008. Genetic structure of Halodule wrightii populations from the Laguna Madre region in the Western Gulf of Mexico. Gulf Mexico Sci. 124-129

Larkin, P.D., Maloney, T.J., Rubiano-Rincon, S., Barrett, M.M., 2017. A map-based approach to assessing genetic diversity, structure, and connectivity in the seagrass Halodule wrightii. Mar. Ecol. Prog. Ser. 567, 95-107. doi:10.3354/meps12037

Larkin, P., Schonacher, T., Barrett, M., Paturzzio, M., 2012. Development and characterization of microsatellite markers for the seagrass Halodule wrightii. Conserv. Genet. Resour. 4, 511–513. doi:10.1007/s12686-011-9587-0

McMillan, C. 1981. Seed reserves and seed germination for two seagrasses, Halodule wrightii and Syringodium filiforme, from the Western Atlantic. Aquat. Bot. 11, 279-296.

McMillan, C., 1985. The seed reserve for Halodule wrightii, Syringodium filiforme and Ruppia maritima in Laguna Madre, Texas. Contrib. Marine Sci. 28, 141-149.

Meirmans, P.G., Hedrick, P.W., 2011. Assessing population structure: FST and related measures: Invited technical review. Mol. Ecol. Resour. 11, 5–18. doi:10.1111/j.1755-0998.2010.02927.x

Meirmans, P.G., Van Tienderen, P.H., 2004. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. Mol. Ecol. Notes 4, 792–794. doi:10.1111/j.1471-8286.2004.00770.x

Micheli, F., Bishop, M.J., Peterson, C.H., Rivera, J., 2008. Alteration of seagrass species composition and function over two decades. Ecol. Monogr. 78, 225–244.

Moore, H.B., n.d. Ecological guide to Bermuda inshore water. Bermuda Biol. Sta. Res.

MuñIz-Salazar, R., Talbot, S.L., Sage, G.K., Ward, D.H., Cabello-Pasini, A., 2005. Population genetic structure of annual and perennial populations of Zostera marina L. along the Pacific coast of Baja California and the Gulf of California: Population genetics of eelgrass in Mexico. Mol. Ecol. 14, 711–722. doi:10.1111/j.1365-294X.2005.02454.x

Nazareno, A.G., Alzate-Marin, A.L., Pereira, R.A.S., 2013. Dioecy, more than monoecy, affects plant spatial genetic structure: the case study of Ficus. Ecol. Evol. 3, 3495-3508.

Nei, M., 1987. Molecular Evolutionary Genetics. Columbia University Press, New York

Neiman, M., Paczesniak, D., Soper, D. M., Baldwin, A. T., Hehman, G., 2011. Wide variation in ploidy level and genome size in a New Zealand freshwater snail with coexisting sexual and asexual lineages. Evolution 65, 3202-3216.

Phillips, R.C., 1960. Observations on the ecology and distribution of the Florida sea-grasses. Florida State Board of Conservation, Prof. Pap, 2, 1-72.

Pineda-Krch, M., Lehtilä, K., 2004. Costs and benefits of genetic heterogeneity within organisms. J. Evol. Biol. 17, 1167-1177.

Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.

Reusch, T.B., Ehlers, A., Hämmerli, A., Worm, B., 2005. Ecosystem recovery after climatic extremes enhanced by genotypic diversity. Proc. Natl. Acad. Sci. U.S.A.102, 2826–2831.

Reusch, T.B.H., Boström, C., 2011. Widespread genetic mosaicism in the marine angiosperm Zostera marina is correlated with clonal reproduction. Evol. Ecol. 25, 899–913. doi:10.1007/s10682-010-9436-8

Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A., Allard, R.W., 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc. Nat. Acad. Sci. 81, 8014-8018.

Schuelke, M., 2000. An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnol. 18, 233–234.

Sexton, J.P., McIntyre, P.J., Angert, A.L., Rice, K.J., 2009. Evolution and ecology of species range limits. Annu. Rev. Ecol. Evol. Syst. 40, 415–436. doi:10.1146/annurev.ecolsys.110308.120317

Short, F., Carruthers, T., Dennison, W., Waycott, M., 2007. Global seagrass distribution and diversity: A bioregional model. J. Exp. Mar. Biol. Ecol. 350, 3–20. http://doi.org/10.1016/j.jembe.2007.06.012

Short, F.T., Polidoro, B., Livingstone, S.R., Carpenter, K.E., Bandeira, S., Bujang, J.S., Calumpong, H.P., Carruthers, T.J., Coles, R.G., Dennison, W.C., others, 2011. Extinction risk assessment of the world's seagrass species. Biol. Conser. 144, 1961–1971.

Sinclair, E.A., Statton, J., Hovey, R., Anthony, J., Dixon, K.W., Kendrick, G.A., 2015. Reproduction at the extremes: pseudovivipary and genetic mosaicism in Posidonia australis (Posidoniaceae). Annals Bot.

Smith, N.P., 1993. Tidal and nontidal flushing of Florida's Indian River Lagoon. Estuaries 16, 739-746.

Sundqvist, L., Keenan, K., Zackrisson, M., Prodöhl, P., Kleinhans, D., 2016. Directional genetic differentiation and relative migration. Ecol. Evol. 6, 3461-3475.

Travis, S.E., Sheridan, P., 2006. Genetic structure of natural and restored shoalgrass Halodule wrightii populations in the NW Gulf of Mexico. Mar. Ecol. Prog. Ser. 322, 117–127.

Van Dijk, J.K., van Tussenbroek, B.I., 2010. Clonal diversity and structure related to habitat of the marine angiosperm Thalassia testudinum along the Atlantic coast of Mexico. Aquat. Bot. 92, 63–69. doi:10.1016/j.aquabot.2009.10.005

Van Dijk, J. K., Waycott, M., Van Tussenbroek, B. I., Ouborg, J., 2007. Polymorphic microsatellite markers for the Caribbean seagrass Thalassia testudinum Banks ex König. Mol. Ecol. Notes 1, 89-91.

Table 2.1 *H. wrightii* sampling locations coordinates, collection dates, and distances. Refer to Figures A2.1-A2.3 for detailed site maps.

Location	Site	Abbreviation	Latitude (N)	Longitude (W)	Collection	Appx. distance (m) 1 to 2:	Appx. Distance (m) from:
Florida Bay							Research Dock 1
	Research Dock 1	RD1	25°5'15.58"	80°27'11.12"	2015	0	0
	Research Dock 2	RD2	25°5'17.48"	80°27'6.94"	2015	130	130
	Research Dock 3	RD3	25°5'14.14"	80°27'12.63"	2015	80	80
	Pelican Key 1	PK1	25°5'31.31"	80°27'20.57"	2015	0	550
	Pelican Key 2	PK2	25°5'31.83"	80°27'18.72"	2015	55	550
	Porjoe Key 1	PJ1	25°8'9.80"	80°28'24.43"	2015	0	5,750
	Porjoe Key 2	PJ2	25°8'11.27"	80°28'25.20"	2015	50	5,750
	Duck Key 1	DK1	25°10'47.11"	80°29'24.49"	2015	0	10,825
	Duck Key 2	DK2	25°10'46.23"	80°29'23.51"	2015	35	10,825
	Terrapin Bay 1	TRP1	25°9'52.50"	80°44'3.1"	2015	0	30,000
	Terrapin Bay 2	TRP2	25°9'56.0"	80°44'3.2"	2015	100	30,000
Gulf of Mexico	St. Joseph's Bay 1	SJ1	29°45'59.75"	85°24'14.47"	2016	0	N/A
	St. Joseph's Bay 2	SJ2	29°46'1.94"	85°24'15.03"	2016	70	N/A
Indian River Lagoon							Wabasso Causeway 1
	Wabasso Causeway 1	WC1	27°45'15'56"	80°25'40.99"	2016	0	0
	Wabasso Causeway 2	WC2	27°45'16.69"	80°25'38.47"	2016	75	45
	Parrish Park 1	PP1	28°37'30.98"	80°47'33.24"	2016	0	100,000
	Parrish Park 2	PP2	28°37'34.93"	80°47'27.26"	2016	200	100,000
	Mosquito Lagoon 1	ML1	28°52'9.17"	80°50'12.91"	2016	0	135,000
	Mosquito Lagoon 2	ML2	28°52'7.92"	80°50'12.53"	2016	40	135,000
North Carolina							Middle Marsh 1 Q1
	Middle Marsh 1 Q1	MM1_1	34°41'24.74"	76°37'10.27"	2015	0	0
	Middle Marsh 1 Q2	MM1_2	34°41'25.55"	76°37'12.24"	2015	55	55
	Middle Marsh 2 Q1	MM2_1	34°41'25.68"	76°37'9.97"	2015	0	30
	Middle Marsh 2 Q2	MM2_2	34°41'26.94"	76°37'8.62"	2015	50	80
	Middle Marsh 3 Q1	MM3_1	34°41'51.45"	76°35'48.16"	2015	0	2,250
	Middle Marsh 3 Q2	MM3_2	34°41'51.82"	76°35'46.26"	2015	50	2,300
	North Carolina 1	NC1	34°40'32.30"	76°34'33.81"	2016	0	4,300
	North Carolina 2	NC2	34°40'31.49"	76°34'32.71"	2016	40	4,340
Bermuda	Well Bay	WB	32°21'9.00"	64°39'44.51"	2013	N/A	N/A
	Bailey's Bay	BRM	32°20'16.27"	64°43'26.05"	2015	N/A	N/A

Table 2.2 Microsatellite primers for *H. wrightii* population study. Primer development is described in Larkin et al., 2012.

Locus	Genbank	Primer sequence (5'-3')	Repeat	Range	Multiplex	Dye
HW166	JN609256	F: AGCACTCGCTTACTCCAACAC	CA	151-229	2	VIC
		R: TCCCATTCTTTAGGTTCAACG				
HW180	JN614999	F: GTGGAGGCCGAACTGTATCT	ATG	239-257	1	PET
		R: CGACCTTCATCCTAATCATCG				
HW188	JN615000	F: ACCTTCATAAATGGCAACTTG	CA	113-161	3	FAM
		R:CAACTTGGTTCTGGTAGTCATC				
HW190	JN615001	F: ATGACGAATCCCGAGGTAT	GA	N/A	1	FAM
		R: CTCACCCACGTTAAAGCACAAT				
HW190b	KT002048	F: ATGACGAATCCCGAGGTAT	GA	262-278	1	FAM
		R: CACCCACGTTAAAGCACAAT				
HW196	JN615002	F: ACAACCTAGATCATCCTCACAC	CAT	183-192	1	NED
		R: AGCAGGAAGTCAAGAGATAGG				
HW200	JN615003	F: ACAACCTAGATCATCCTCACAC	TAGA	232-268	2	NED
		R: AGCAGGAAGTCAAGAGATAGG				
HW208	KT278676	F: TGCCTTTCCCAACTTTTC	CT	191-205	3	NED
		R: CTAGGGGTGCTTATGTAGGGT				
HW212	JN615005	F: ATGGATGTTCATTGAGTTTGAC	CAT	284-305	3	VIC
		R: CAAGGCTAAGGTAGTGGACC				
HW214	JN615006	F: TCCTCTATCAATGGGATTTAGA	CA	212-244	2	PET
		R: GGGTGGCTATGTATCGA				
HW222	KT002049	F: CCAGCAACAAGACAAATGTAT	TAGA	N/A	1	VIC
		R: CTATAAGGATTAGGACAAGCACAC				
HW228	KT002050	F: AAGACGGCATTGGAAAATAAG	CAT	262-304	2	FAM
		R: CTGGTATCATCGGAAGCACTGT				
HW232	KT002051	F: AGCACCCTTCATTCCAAC	CT	265-267	3	PET
		R: CTCTGCCAATCTTCTTCTTCTACA				

Table 2.3 Allelic diversity of *H. wrightii*. Statistics based using 11 loci where N = number of samples, G = number of genets, G_p = number of aneuploidy genets, R = genotypic richness, N_a = average number of alleles per locus N_{eff} = effective number of alleles, H_s = heterozygosity within populations, and H_t = total heterozygosity. Location abbreviations represent Florida Bay (FB), Indian River Lagoon (IRL), Gulf of Mexico (GOM), North Carolina (NC) and Bermuda (BRM).

Population	Location	N	G	G_p	R	N_a	N_{eff}	H_s	H_t
Research Dock	FB	102	13	1	0.12	4.46	2.81	0.63	0.63
Pelican Key	FB	70	15	4	0.20	4.82	2.93	0.59	0.59
Porjoe Key	FB	70	10	2	0.13	4.36	2.64	0.62	0.62
Duck Key	FB	69	10	1	0.13	3.55	2.12	0.48	0.48
Terrapin Bay	FB	68	2	2	0.01	2.55	1.86	0.54	0.54
Wabasso Causeway	IRL	71	5	0	0.06	3.09	2.38	0.60	0.60
Parrish Park	IRL	66	10	3	0.14	3.64	2.50	0.52	0.52
Mosquito Lagoon	IRL	66	13	4	0.18	4.55	3.42	0.64	0.64
St. Joseph's Bay	GOM	70	5	0	0.06	2.55	1.91	0.38	0.38
Middle Marsh 1	NC	59	6	3	0.09	2.55	2.17	0.57	0.57
Middle Marsh 2	NC	67	3	3	0.03	2.36	2.00	0.56	0.56
Middle Marsh 3	NC	58	6	5	0.09	2.91	2.56	0.57	0.57
North Carolina	NC	70	12	2	0.16	3.09	1.86	0.45	0.45
Bailey's Bay	BRM	19	1	0	0.00	1.20	1.27	0.20	0.20
Well Bay	BRM	30	1	0	0.00	1.20	1.27	0.20	0.20
Overall:		955	112	30	Average: 0.09	3.12	2.25	0.50	0.50

Table 2.4 Pairwise population differentiation using G'$_{ST}$. Abbreviations along the left column represent regions where FB = Florida Bay, IRL = Indian River Lagoon, GOM = Gulf of Mexico, and NC = North Carolina.

	Research Dock	0.00												
	Pelican Key	-0.01	0.00											
FB	Porjoe Key	0.03	0.01	0.00										
	Duck Key	0.07	0.10	0.14	0.00									
	Terrapin Bay	0.15	0.15	0.09	0.29	0.00								
	Wabasso Causeway	0.16	0.18	0.16	0.30	0.14	0.00							
IRL	Parrish Park	0.23	0.25	0.20	0.39	0.21	0.04	0.00						
	Mosquito Lagoon	0.11	0.14	0.10	0.22	0.14	0.06	0.08	0.00					
GOM	St. Joseph's Bay	0.38	0.41	0.37	0.53	0.32	0.30	0.23	0.24	0.00				
	Middle Marsh 1	0.15	0.18	0.15	0.30	0.19	0.11	0.11	0.08	0.30	0.00			
NC	Middle Marsh 2	0.16	0.18	0.15	0.29	0.20	0.05	0.11	0.08	0.30	-0.09	0.00		
	Middle Marsh 3	0.18	0.21	0.19	0.32	0.22	0.09	0.11	0.10	0.28	-0.07	-0.03	0.00	
	North Carolina	0.33	0.35	0.32	0.45	0.33	0.17	0.24	0.22	0.40	0.15	0.10	0.10	0.00
		RD	PK	PJ	DK	TRP	WC	PP	ML	SJ	MM1	MM2	MM3	NC

$x \leq 0$	Very little differentiation
$0 < x < 0.05$	Little differentiation
0.05 - 0.09	Moderate differentiation
0.1 - 0.25	Great differentiation
$0.25 < x$	Very great differentiation

Table 2.5 Pairwise genetic differentiation using Jost's D. Abbreviations along the left column represent regions where FB = Florida Bay, IRL = Indian River Lagoon, GOM = Gulf of Mexico, and NC = North Carolina.

$x \leq 0$	Very little differentiation
$0 < x < 0.05$	Little differentiation
0.05 - 0.09	Moderate differentiation
0.1 - 0.25	Great differentiation
0.25 < x	Very great differentiation

		RD	PK	PJ	DK	TRP	WC	PP	ML	SJ	MM1	MM2	MM3	NC	BRM	WB
FB	Research Dock	0.00														
	Pelican Key	-0.01	0.00													
	Porjoe Key	0.05	0.01	0.00												
	Duck Key	0.10	0.13	0.20	0.00											
	Terrapin Bay	0.26	0.24	0.15	0.45	0.00										
IRL	Wabasso Causeway	0.30	0.33	0.30	0.50	0.22	0.00									
	Parrish Park	0.40	0.43	0.34	0.63	0.33	0.05	0.00								
	Mosquito Lagoon	0.21	0.26	0.18	0.39	0.24	0.10	0.12	0.00							
GOM	St. Joseph's Bay	0.64	0.66	0.58	0.85	0.42	0.40	0.25	0.34	0.00						
NC	Middle Marsh 1	0.27	0.31	0.27	0.48	0.32	0.17	0.14	0.13	0.38	0.00					
	Middle Marsh 2	0.29	0.30	0.26	0.44	0.33	0.07	0.14	0.14	0.39	-0.11	0.00				
	Middle Marsh 3	0.34	0.39	0.35	0.54	0.40	0.15	0.15	0.17	0.35	-0.09	-0.05	0.00			
	North Carolina	0.56	0.59	0.51	0.70	0.50	0.21	0.28	0.32	0.46	0.18	0.11	0.12	0.00		
BRM	Bailey's Bay	0.36	0.25	0.26	0.15	0.49	0.74	0.82	0.66	1.07	0.69	0.66	0.76	0.85	0.00	
	Well Bay	0.36	0.25	0.26	0.15	0.49	0.74	0.82	0.66	1.07	0.69	0.66	0.76	0.85	0.00	0.00

Table 2.6 Pairwise genetic differentiation using G''$_{ST}$. Abbreviations along the left column represent regions where FB = Florida Bay, IRL = Indian River Lagoon, GOM = Gulf of Mexico, and NC = North Carolina.

	Research Dock	0.00											
	Pelican Key	-0.02	0.00										
FB	Porjoe Key	0.07	0.02	0.00									
	Duck Key	0.16	0.22	0.31	0.00								
	Terrapin Bay	0.37	0.35	0.22	0.61	0.00							
	Wabasso Causeway	0.41	0.45	0.42	0.65	0.33	0.00						
IRL	Parrish Park	0.54	0.58	0.47	0.77	0.48	0.10	0.00					
	Mosquito Lagoon	0.30	0.36	0.25	0.52	0.34	0.15	0.19	0.00				
GOM	St. Joseph's Bay	0.77	0.80	0.73	0.93	0.61	0.58	0.43	0.50	0.00			
	Middle Marsh 1	0.38	0.44	0.38	0.63	0.45	0.26	0.23	0.20	0.57	0.00		
NC	Middle Marsh 2	0.41	0.43	0.37	0.60	0.46	0.12	0.24	0.20	0.57	-0.21	0.00	
	Middle Marsh 3	0.46	0.51	0.47	0.69	0.54	0.22	0.24	0.25	0.53	-0.17	-0.08	0.00
	North Carolina	0.70	0.73	0.67	0.83	0.67	0.34	0.45	0.47	0.68	0.30	0.20	0.20
		RD	PK	PJ	DK	TRP	WC	PP	ML	SJ	MM1	MM2	MM3

$x \leq 0$	Very little differentiation
$0 < x < 0.05$	Little differentiation
0.05 - 0.09	Moderate differentiation
0.1 - 0.25	Great differentiation
$0.25 < x$	Very great differentiation

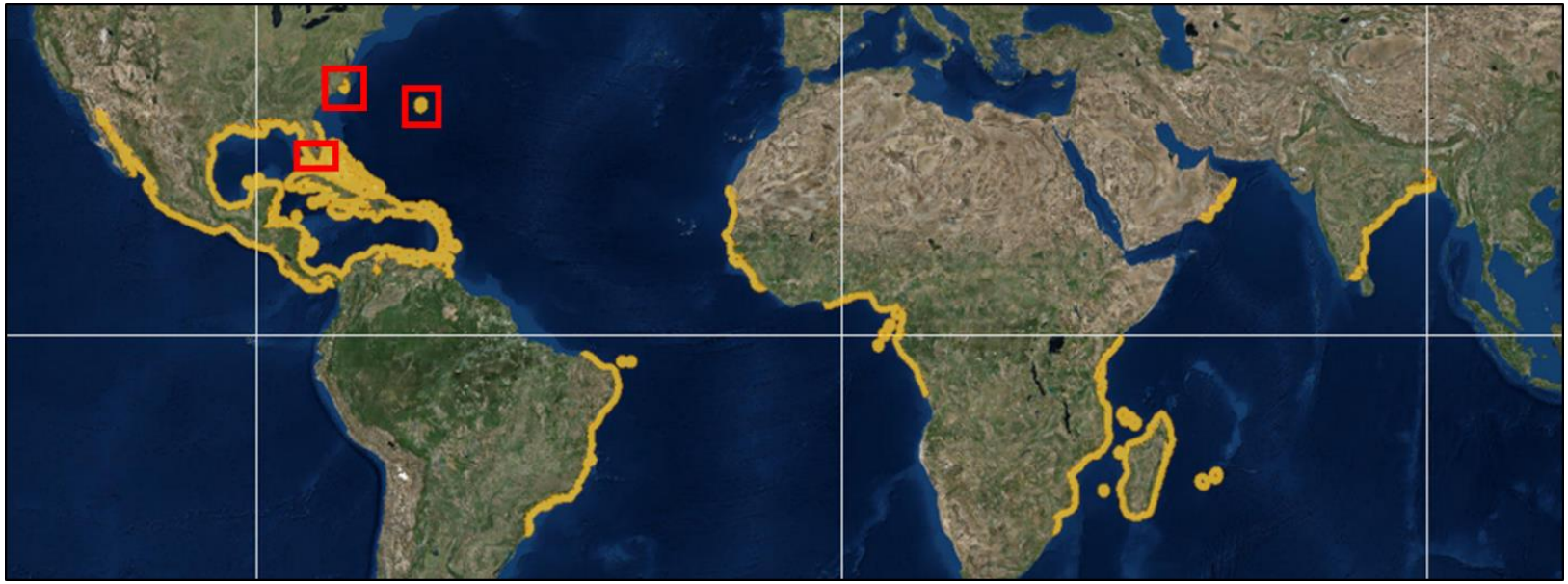


Figure 2.1 Range of *Halodule wrightii*. Yellow lines indicate the range of *H. wrightii* as depicted by the IUCN redlist, and red boxes show general sampling regions with Bermuda and North Carolina populations representing the extreme northern edge-of-range (The IUCN Red List of Threatened Species. www.iucnredlist.org. Accessed March 3, 2017).

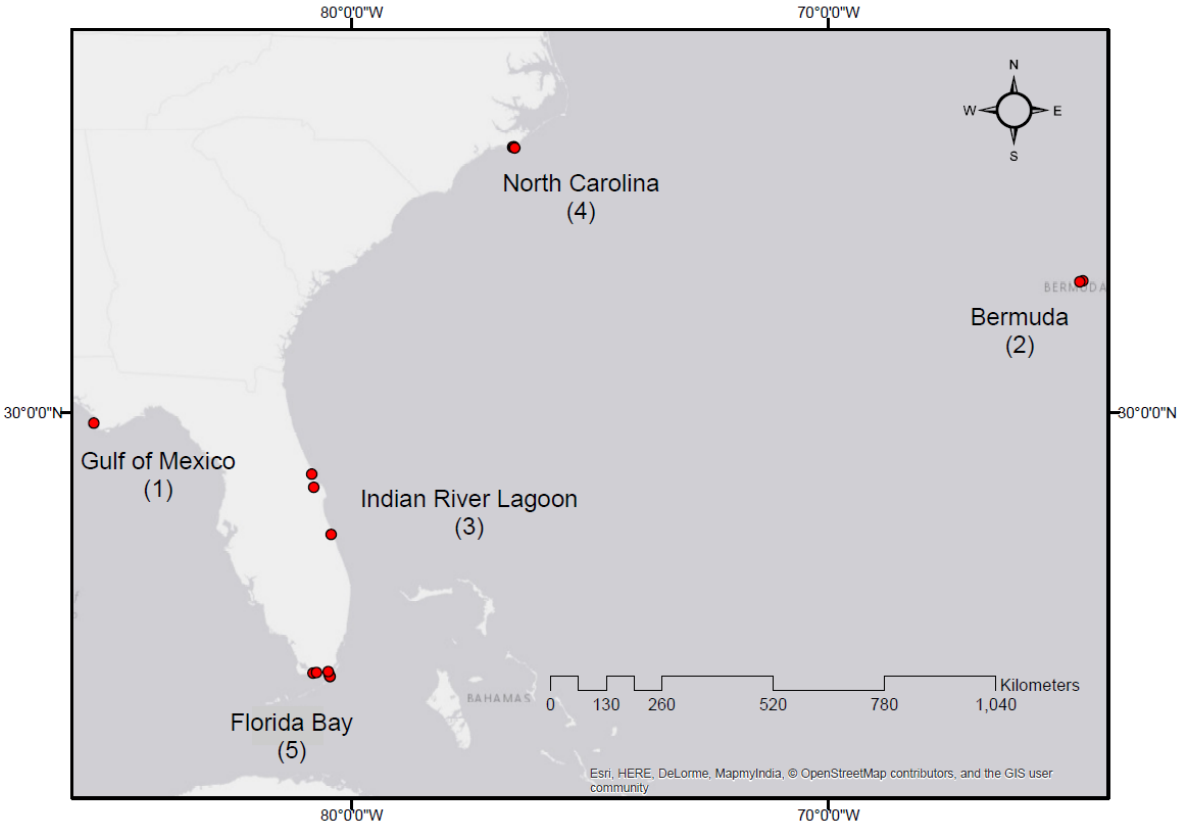


Figure 2.2 Overview of *H. wrightii* sample collection locations. The number of sites sampled per location is listed in the parentheses.

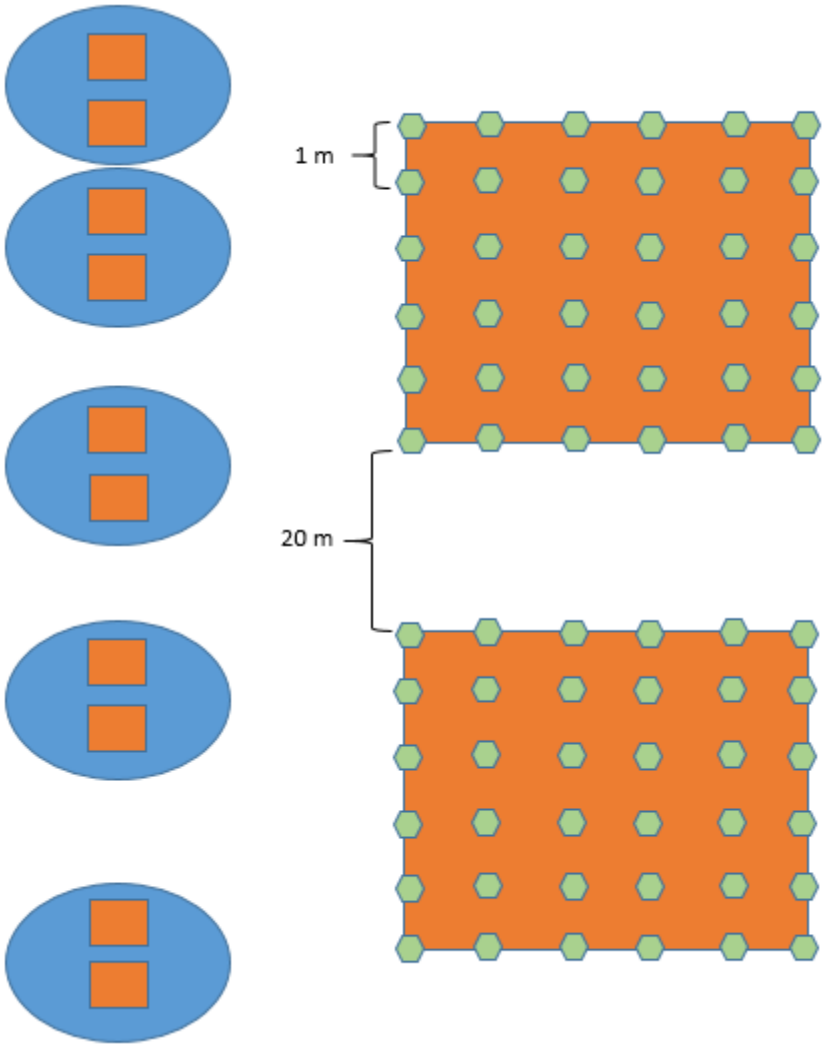
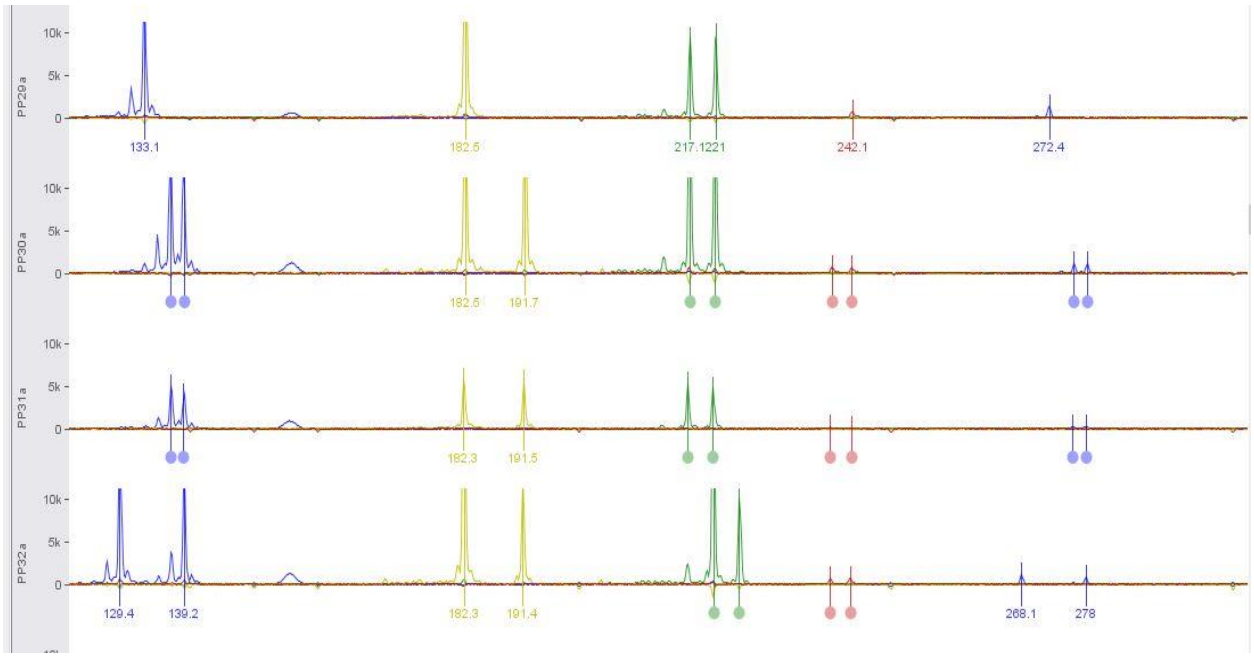


Figure 2.3 Schematic of sampling protocol. Sites, represented by blue circles, were located along a distance gradient for Florida Bay, the Indian River Lagoon, and North Carolina. Two 5-m by 5-m quadrats, represented by orange squares, were sampled at known distances for each site. A shoot was collected at each meter of the quadrats, shown by the green hexagons.

A



B

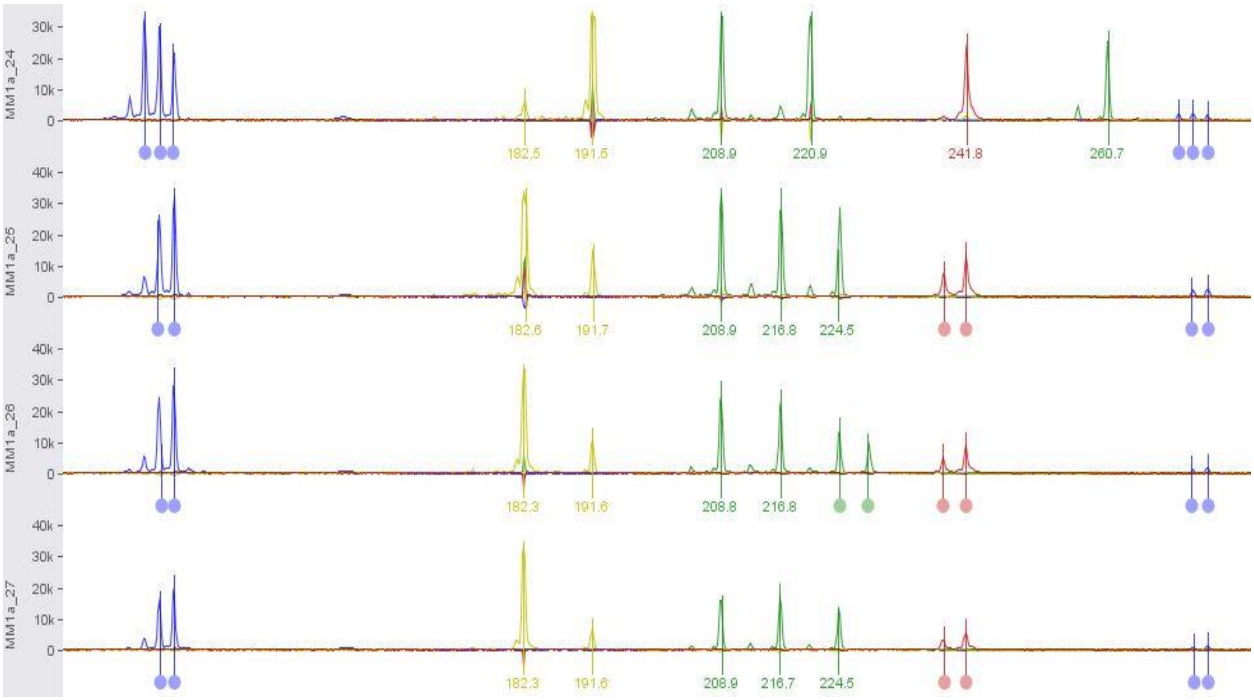


Figure 2.4 Scoring examples of *H. wrightii* diploid and aneuploid samples. Both panels show amplification of 5 loci (colored above in blue, yellow, green, and red) form Multiplex 1. Panel A shows homozygous (1 peak per locus) and heterozygous (2 peaks per locus) diploid samples from Parrish Park. Panel B shows samples from Middle Marsh with diploid, triploid, and tetraploid genotypes.

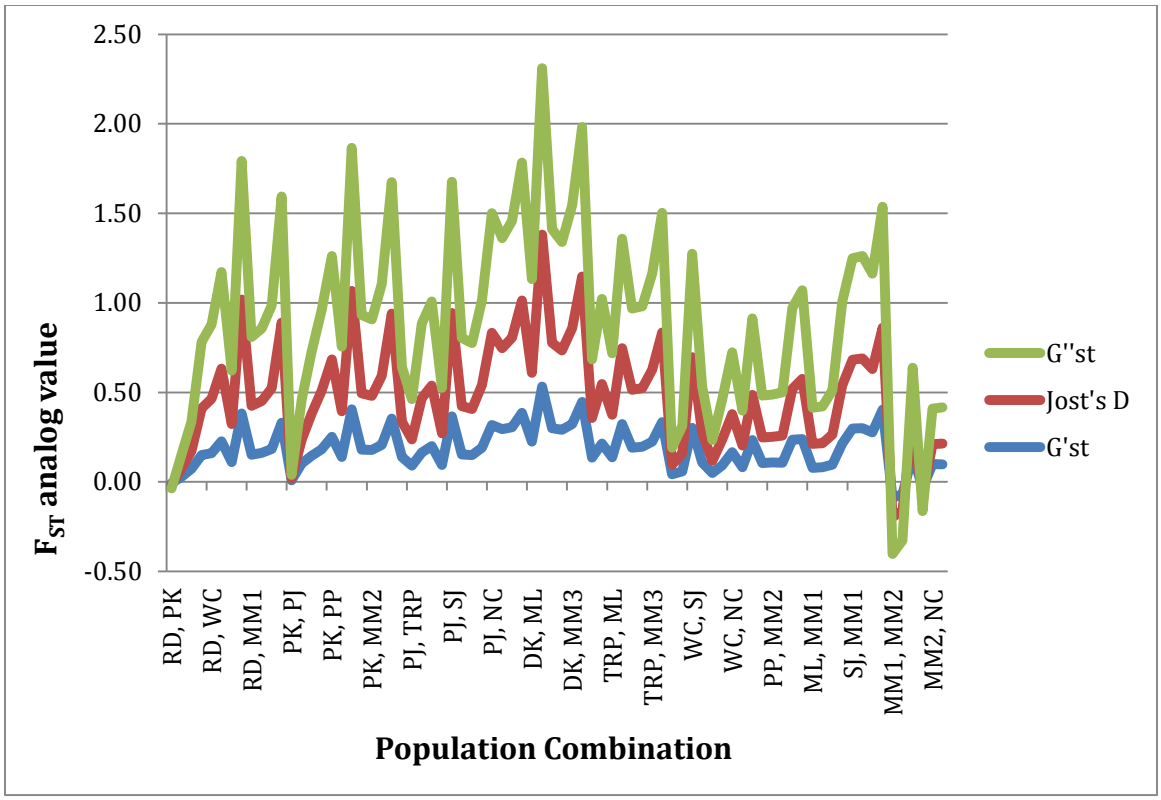


Figure 2.5. Comparison of F_{ST} analogs across *H. wrightii* population pairs

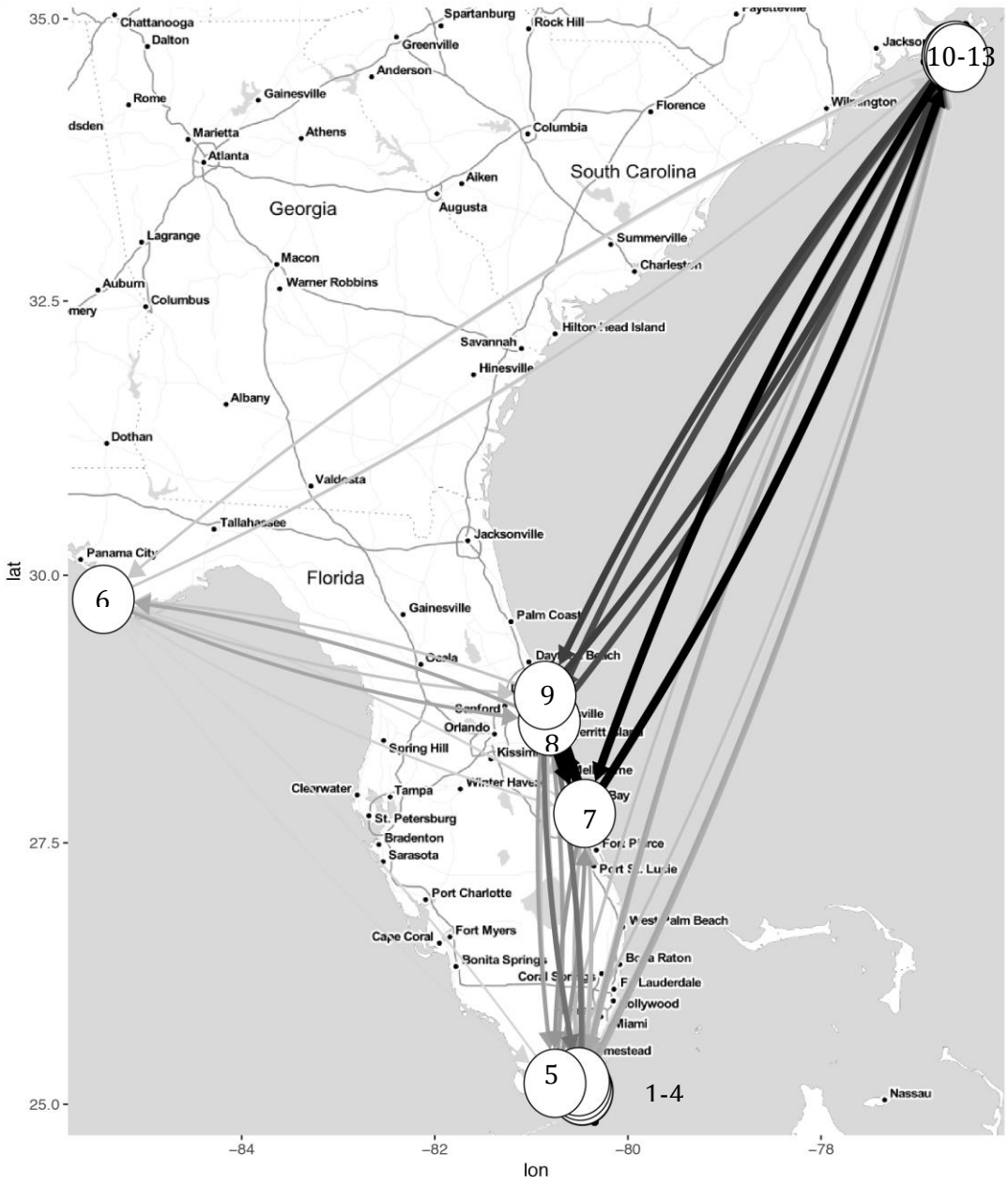


Figure 2.6 Relative gene flow between *H. wrightii* populations. Connectivity results are based off Jost's D. Thicker arrows depict greater gene flow. Population codes are as follows: (1) Research Dock, (2) Pelican Key, (3) Porjoe Key, (4) Duck Key, (5) Terrapin Bay, (6) St. Joseph's Bay, (7) Wabasso Causeway, (8) Parrish Park, (9) Mosquito Lagoon, (10) Middle Marsh 1, (11) Middle Marsh 2, (12) Middle Marsh 3, and (13) North Carolina.

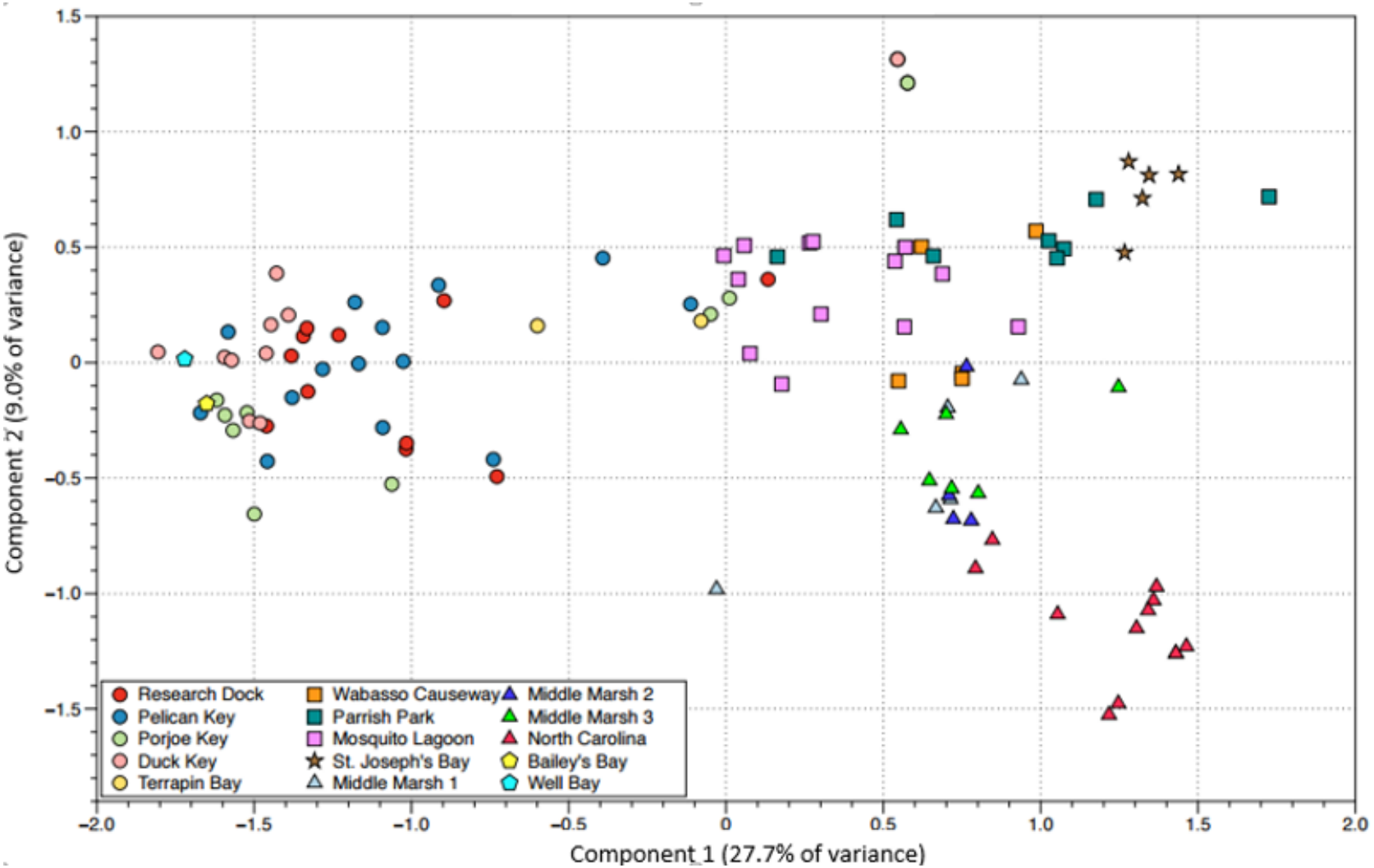


Figure 2.7 Principal components analysis of *H. wrightii* populations. Different colors represent sites while different shapes represent locations: Florida Bay (circle), Indian River Lagoon (square), Gulf of Mexico (star), North Carolina (triangle) and Bermuda (pentagon).

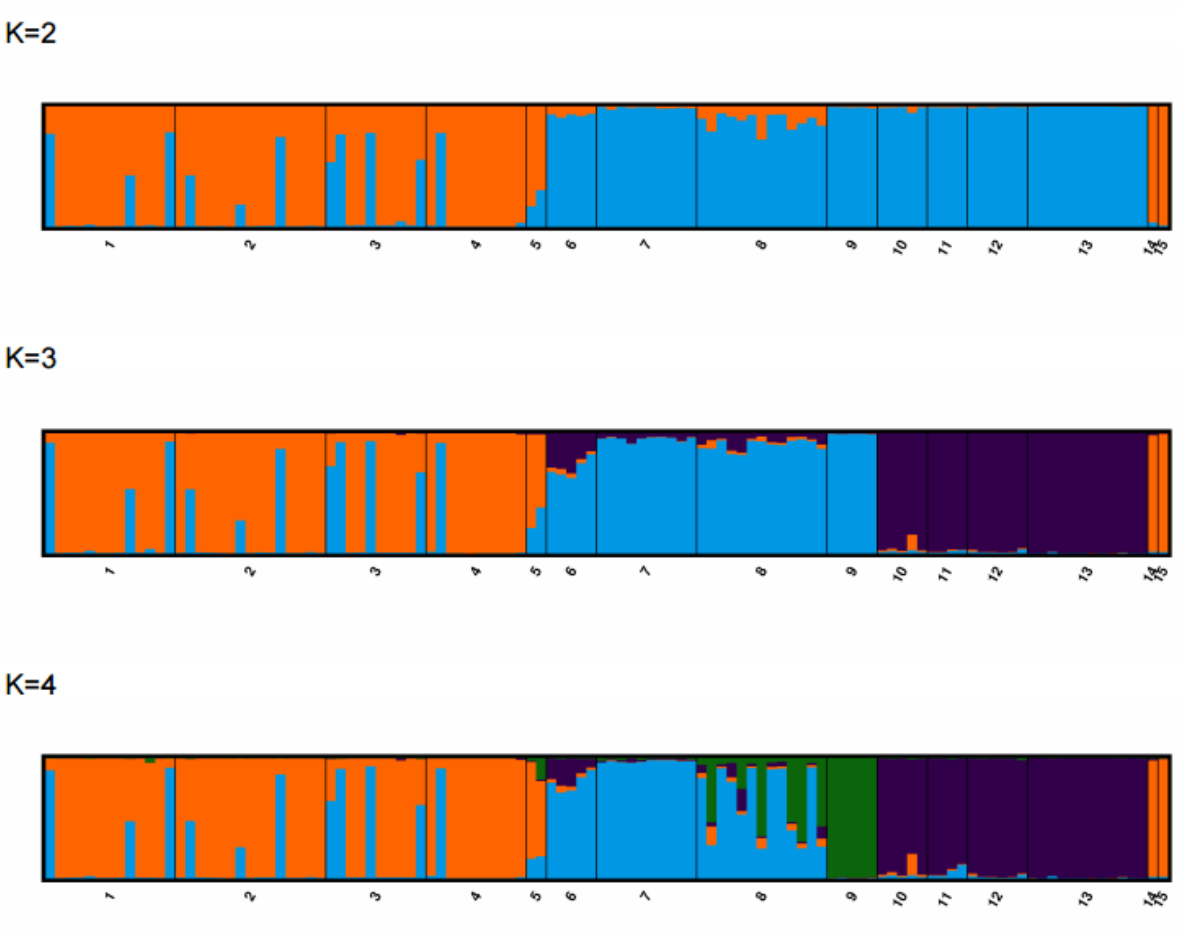


Figure 2.8 STRUCTURE assignment of populations for K =1 to K =4. Population numbers align with site order in Table 2.1.

Summary and potential for future work:

In this thesis microsatellites were used to enhance our knowledge of the genetic processes in two diverse seagrass species. In the first chapter, microsatellite development allowed for the detection of polymorphism in *A. antarctica*. A broader examination into the genetic processes of *A. antarctica* can be performed using the developed markers. The discrepancies between microsatellite and previous marker results indicate re-assessments of the genetic diversity of other species may need to be performed. In the second chapter, the genetic population structure of *H. wrightii* was investigated at edge-of-range locations. Edge populations experience dynamic conditions, particularly as climate change progresses. Sites in this study were along the subtropical and tropical western Atlantic Ocean, and it would be valuable to compare these results with genetic diversity at the center and southern regions of the *H. wrightii* range. It is expected that the high clonality and presence of aneuploidy found in this study would be mirrored in southern regions of *H. wrightii*, while populations at the core of the range would display greater sexual reproduction. However, comparisons are needed because southern edge populations may experience distinctive biotic and abiotic stressors and have a different set of reproductive responses. Genetic mosaicism was present in *H. wrightii* clones, and while the processes underlying aneuploidy have been investigated, the fitness consequences remain unclear. Next-generation sequencing would be valuable for examining drivers of aneuploidy, genetic diversity, and gene flow in *H. wrightii*. Speciation is a particularly important question for *Halodule* due to debates over *H. bermudensis* in Bermuda, *H. beaudettei* in Florida (now called *H. wrightii s.l.*), and *H. emarginata* in Brazil. Morphology is unreliable for distinguishing *Halodule* species; therefore, next-generation sequencing of species lineages should be employed for more accurate categorization.

Microsatellite markers are valuable tools for increasing our understanding of the population genetic structure of ecologically and economically important seagrass beds. In this thesis, microsatellite analyses contributed information on allelic diversity, genotypic richness, and population differentiation. These factors are important for understanding basic physiological processes employed by seagrasses during recruitment, bed maintenance. The knowledge gained by these studies can be applied in mitigation projects for the growth and persistence of seagrass populations.

Appendix:

A1. Glossary of genetic terms*

Allelic diversity – The average number of alleles per locus for a population

Amplified fragment length polymorphisms (AFLP) – A dominant molecular marker that genotypes individuals at multiple loci through the digestion of amplified DNA by restriction enzymes.

Effective population size (N_e) – The size of an ideal population (under Hardy-Weinberg conditions) that will lose genetic variation through drift at the same rate as an actual population.

F_{ST} – An overall inbreeding coefficient that compares the heterozygosity of an individual to that of the total population under consideration. Calculated as:

$$F_{ST} = \frac{H_T - H_S}{H_T}$$

Where H_T is the expected heterozygosity of the total population and H_S is the heterozygosity that would be expected if the subpopulation is in Hardy Weinberg Equilibrium. Analogs of F_{ST} include G'_{ST}, G''_{ST}, Jost's D, and Rho_{ST}.

Genetic bottleneck – A severe, temporary reduction in the size of a population that also reduces the number of alleles in a population

Gene flow – The transfer of genetic material from one population involving successful dispersal and subsequent reproduction

Hardy-Weinberg equilibrium- A predictable ratio of genotype frequencies in a sexually reproducing population of infinite size with random mating and no selection

Homozygous- An individual that has only one type of allele at a particular locus (ex: aa)

Heterozygous- An individual that has more than one type of allele at a particular locus (ex: Aa)

Isolation-by-distance – A pattern of population differentiation where genetic dissimilarity of populations is correlated with the geographic distance that separates them

Locus – The location of a particular gene or region of DNA on a chromosome

Microsatellite – A stretch of DNA consisting of short tandem repeats of up to 5 base pairs

Nuclear DNA- The complement of DNA that is arranged in chromosomes and located in the nucleus of a cell; used in microsatellite analysis

Polymerase chain reaction (PCR) – A procedure that denatures DNA to amplify specific segments of DNA

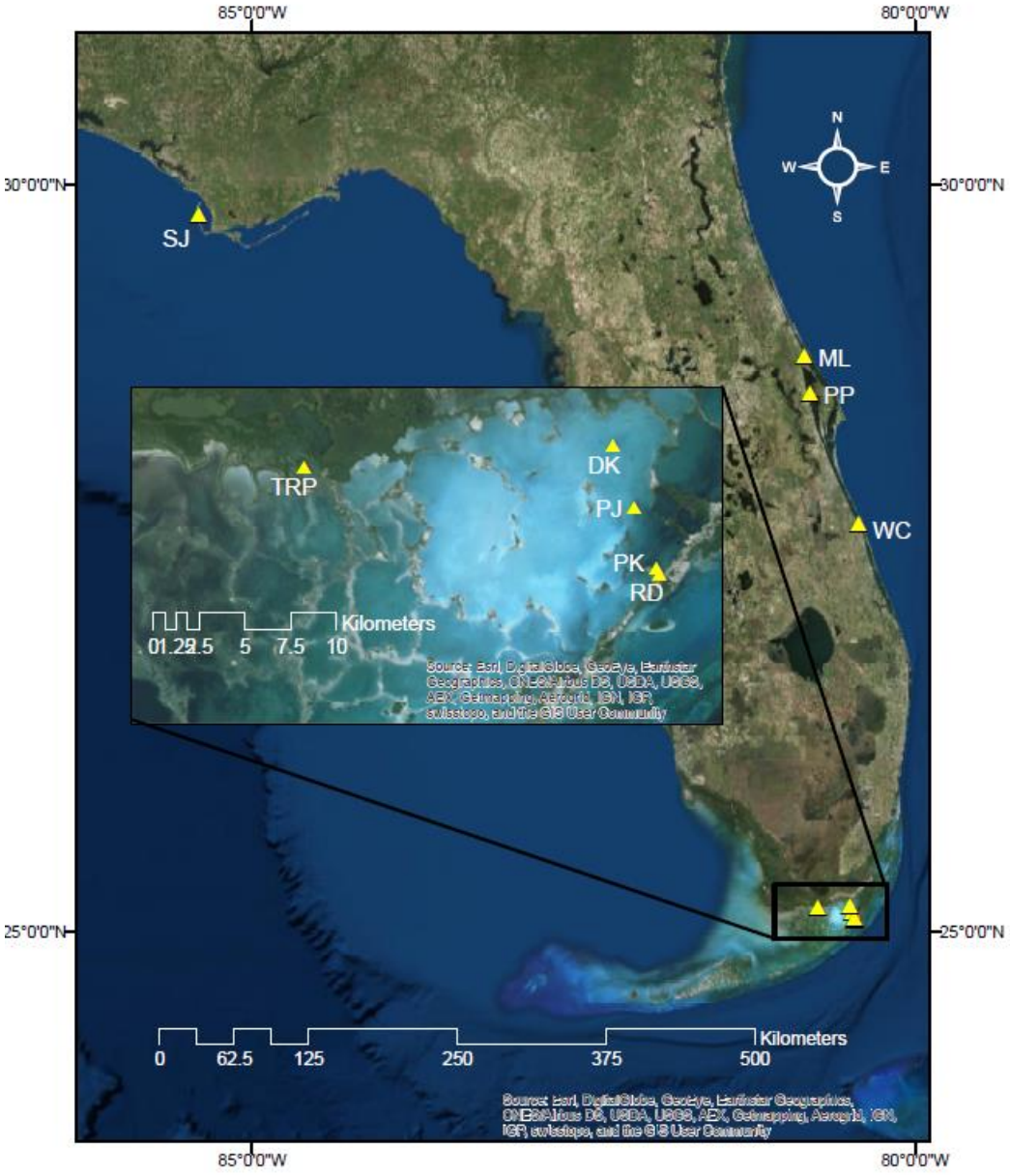
Restriction fragment length polymorphisms (RFLP) – Dominant molecular markers that generate multiple fragments of DNA through digestion with restriction enzymes.

* All definitions provided in the Glossary are from Freeland et al., 2011

A2. Site descriptions

A2.1 Site descriptions for the Gulf of Mexico, Florida Bay, and the Indian River Lagoon:

Figure A2.1 *Halodule wrightii* Florida site map. Population codes are described in Table 2.1.



As in other regions, *H. wrightii* habitat in Florida Bay is determined by a variety of biotic and abiotic factors. *H. wrightii* is more tolerant to variations in salinity and temperature than *S. filiforme* and *T. testudinum* (Zieman, 1982). Lack of fresh water supply is an issue for Florida Bay due to the rerouting of water supplies. This resulted in

a large die-off of seagrass around the northwest portion of the Bay during the low-rainfall summer of 2015, shortly after sample collection for this study. Hypersaline conditions are not uncommon in Florida Bay due to cyclic droughts and freshwater diversion, which resulted in large areas averaging over 50 psu during 1990 (Fourqurean and Robblee, 1999). Die-offs of seagrass in Florida Bay have also occurred historically, such as the 1987 decline that resulted from hypoxia and sulfide toxicity (Carlson et al. 1994).

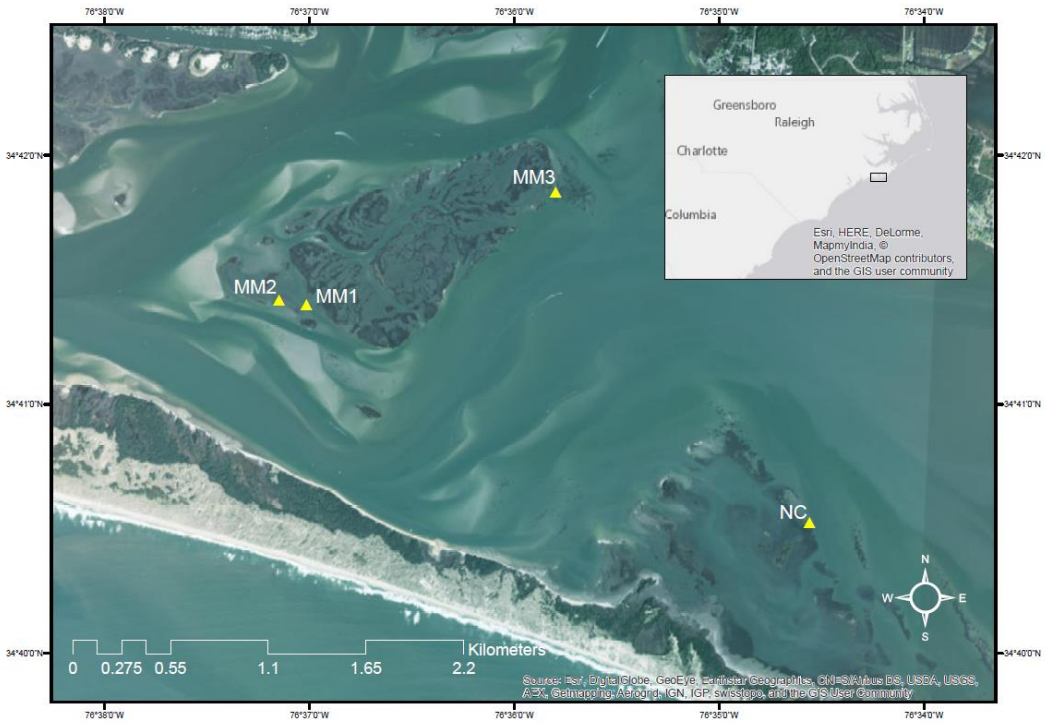
Hydrodynamics play an important role in the dispersal of seeds and fragments, and thus the genetic connectivity of populations. There is a net Gulf of Mexico flow down the southwest Florida shelf and through the Florida Keys to the Atlantic allowing for hydrological connections of the greater Florida region (Lee et al., 2016). Water inflow occurs through the Flamingo Channel in the northwest section of the bay with wind-driven, clockwise circulation patterns in the western interior portion (Lee et al., 2016). The mean current speed along the gulf coast of Florida to Florida Bay is generally less than 20 cm sec^{-1} throughout the year, as compared to the approximately 100 cm sec^{-1} current of the Gulf Stream around the outside of the Florida Keys and west coast of Florida (Naval Research Laboratory. http://www7320.nrlssc.navy.mil/GLBhycom1-12/skill.html. Accessed July 17, 2015). Despite mild current velocity within Florida Bay, the 2-4 week viability of *H. wrightii* fragments and hydraulic connection of the area may allow for long-distance dispersal and genetic connectivity of Florida populations (Obeysekera et al., 2000; Hall et al., 2006).

The Indian River Lagoon (IRL) a shallow (1-3 m deep) lagoon stretching 250 km along the eastern coast of Florida (Dawes et al., 1995; Hall et al., 2006). The IRL was formed in the past 6,000 years, with seagrass communities establishing after geological and oceanic changes during the past 3,000 years (Dawes et al., 1995). The three openings into the Atlantic Ocean are the Sebastian, Fort Pierce, and St. Lucie Inlets with limited tidal flushing beyond the inlet (Dawes et al., 1995). The northern inlet is flood-dominated seasonally from late fall to early spring whereas the central and southern inlets are largely ebb-dominated throughout the year (Smith, 2001). Wind forcing is the driving circulation factor and causes water storage in the northern lagoon during the summer months and removal during the winter from a southwestern wind (Dawes et al, 1995; Smith, 2001).

The IRL hosts approximately 30,000 ha of seagrass and has the highest seagrass species richness of any estuary in the western hemisphere (Morris and Virnstein, 2004; Virnstein, 1999). *H. wrightii* is the most abundant of the 7 seagrass species in the lagoon (Dawes et al., 1995). Seagrass coverage completely crashed in 1997 at the Turnbull site, along the Mosquito Lagoon in the northernmost region of the IRL, perhaps due to natural cyclical processes (Morris and Virnstein, 2004). More recently, seagrass areal extent decreased across the lagoon in 2011 due to an algal superbloom driven by extreme climactic conditions (Phlips et al., 2015). Additionally, a brown tide event occurred shortly before sample collection in the upper and middle IRL during January and February 2016, with seagrass surveys showing a 60% decline in percent cover from December values (Indian River Lagoon Conditions Update). Seed reserves allow for rapid recolonization of disturbed areas, but have not been reported for *H. wrightii* in the Indian River Lagoon, so vegetative fragmentation may play a larger role in dispersal and recruitment (Hall et al., 2006).

A2.2 Site descriptions for North Carolina:

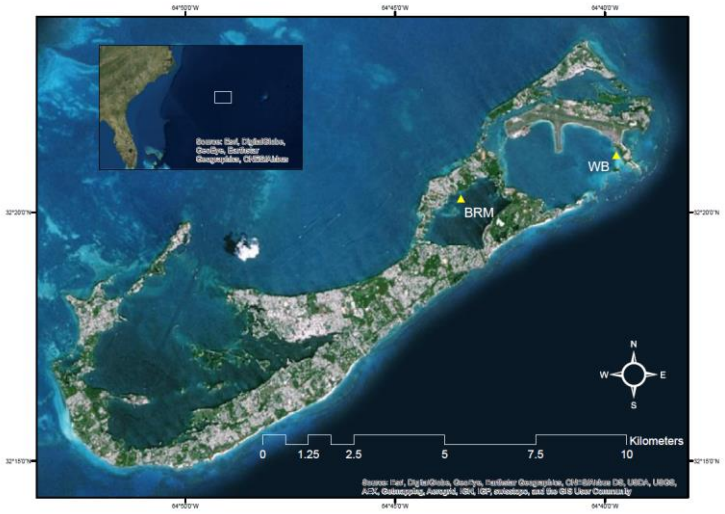
Figure A2.2 *Halodule wrightii* North Carolina site map. Population codes are described in Table 2.1.



Dare County, North Carolina hosts the northernmost population of *H. wrightii* (Ferguson et al., 1993). Further south, the Bogue Sound-Back Sound region of North Carolina marks an overlap of the southernmost *Zostera marina* habitat and northern *H. wrightii* habitat on the U.S. eastern coast (Micheli et al., 2008). Where these species co-occur, *H. wrightii* occupies the upper intertidal zone and *Z. marina* is found at greater depths (Thayer et al., 1984). Over the past 2 decades in the Bogue Sound-Back Sound area, *Zostera* declines have been associated with increased spring water temperature and nutrient loads. By contrast, *H. wrightii* has remained stable (Micheli et al., 2008). Changes in species composition at the ecotone will directly affect faunal assemblages and ecosystem functions (Micheli et al., 2008); therefore, it will be important to understand the genetic processes of *H. wrightii* occurring at this leading edge population. *H. wrightii* sample acquisition from the Back Sound region occurred in the Rachel Carson Reserve, a North Carolina National Estuarine Research Reserve. Three sites were collected at Middle Marsh, an island about 3 km long and less than 1.6 km wide separated from the land by the North River Channel (Rachel Carson. http://www.nccoastalreserve.net/web/crp/rachel-carson. Accessed Nov 3, 2016). While *H. wrightii* seeds have been found in Bogue Sound in 1991, they have not been observed for the northern Oregon Inlet or southern Cape Lookout populations (Ferguson et al., 1993).

A2.3 Site descriptions for Bermuda:

Figure A2.3. ***Haldoule wrightii* Bermuda site map.** Population codes are described in Table 2.1.



The Bermuda platform in the Sargasso Sea is a shallow, 650-km^2 limestome island 1,000 km from the nearest land of Cape Hatteras, North Carolina (Vacher and Rowe, 1997). A 1 km wide forereef terrace of 50 m depth extends along the rim surrounding the Bermuda platform, followed by a precipitous drop to 1000 m and gradual decline to the seafloor surface (Murdoch et al., 2007). *H. wrightii*, *S. filiforme*, and *T. testudinum* are found in waters shallower than 12 m, while *Halophila decipiens* extends to 18 m depths (Murdoch et al., 2007). The early reports of *H. wrightii* in Bermuda referred to the species as *Diplantheria wrightii* (Acherson) (Bernatowicz, 1952). *S. fililforme* is the most prevalent seagrass, followed by *H. decipiens*, *T. testudinum*, and *Halodule sp.* (Manuel et al., 2013). There has been a loss in seagrass spatial extent by approximately 23% since the 1990s, most of which has occurred in offshore areas due to overgrazing by green sea turtles (Murdoch et al. 2007; Fourqurean et al., 2010; Fourqurean et al., 2015). The dense human populations of Bermuda do not have a wastewater collection system, resulting in land-based nitrogen and phosphorus inputs to the nearshore ocean (Fourqurean et al., 2015). Enriched $\delta\ ^{15}N$ levels nearby wastewater outflows declined rapidly at a rate of 1.8 ‰ km^{-1}, suggesting use of these nutrients in the benthos and water column and resulting in a dramatic nutrient isoscape (Fourqurean et al., 2015).

A3. Factors influencing genetic diversity

Genetic diversity is influenced by genetic drift, natural selection, reproductive mode, and gene flow. Genetic drift occurs since not all individuals within a population reproduce, and therefore allelic diversity decreases because some alleles are not passed on to the next generation. The degree to which allelic diversity is reduced is determined by the effective population size, with more alleles contained within larger populations of reproducing individuals. Natural selection also affects allelic frequencies, but can either act to increase or decrease diversity over time (Freeland et al., 2011). Some molecular markers, including microsatellites, should not be affected by natural selection since they are in the noncoding region of DNA. However, their variation can be used to infer gene flow, which is the driver for natural selection events (Hardy and Vekemans, 1999; Escudero et al., 2003). Molecular markers can also provide insights into reproductive mode, which is an important consideration for seagrasses due to their ability to reproduce both sexually and asexually.

The allocation of asexual and sexual reproduction is a driving force behind genetic diversity and can be influenced by a variety of environmental conditions. Due to the combination of asexual and sexual reproduction within seagrasses, a given shoot may be a part of a large clone or may be genetically distinct. The number of genetically different individuals in a population is called the genotypic richness and is a common measure of genetic diversity. High resolution genetic markers are needed to distinguish the genetic identity of an individual short shoots (ramet) in order to identify ramets of a single clone (genet) (Vallejo-Marín et al., 2010). Trade-offs associated with the allocation of asexual versus sexual reproduction occur at the ramet level and primarily involve the commitment of resources required for sexual reproduction against the reduced opportunities for mating (Vallejo-Marín et al., 2010). However, more research needs to be performed to understand the environmental triggers responsible for reproductive allocation.

Asexual reproduction occurs via vegetative fragmentation or horizontal elongation of short shoots along a rhizome runner. Vegetative fragments are potential propagules that can contribute to long-distance clone dispersal. Aquatic leaves are more

easily fragmented than terrestrial counterparts since plants require less structural support in the water (Eckert et al., 2016). Most bed maintenance and new shoot production occurs through rhizome elongation (Phillips, 1960). While production of new short shoots via rhizome runners is energetically less expensive than sexual reproduction, asexual reproduction does not contribute new alleles to the gene pool, which can decrease resilience to environmental changes.

Inbreeding can also lead to a reduction in population fitness. Inbreeding decreases genetic diversity by increasing the proportion of homozygotes beyond what is expected from a randomly mating population (Freeland et al., 2011). If inbreeding is common, the higher prevalence of homozygotes can cause alleles to become fixed within a population. Inbreeding in seagrass communities is reduced by the separation of male and female plants. However, sexual reproduction relies on the production of seeds or seedlings following successful hydrophilous fertilization of one plant by another. Seagrasses have been known to have patchy flowering distributions, and the processes underlying flowering events are poorly understood. Accurate estimates of sexual reproduction are difficult to achieve due to the variable timing and cryptic nature of some flowers, such as for *H. wrightii*

The seed banks of seagrasses are also hidden but are important reservoirs of genetic information and allow for dispersal through both time and space (Bakker et al., 1996; Darnell et al., 2015). Following fertilization and flowering, fruits containing seeds are buried underneath the sediment. As the fruit ripens and decomposes, the seed is left behind at the level of the rhizome. Seed germination is stimulated under favorable genetic/environmental conditions or when the bed is disturbed (McMillan, 1981). Seeds can survive for multiple years before germinating, which allows them to survive in disturbed sites and colonize denuded beds (McMillan, 1981).

Gene flow counteracts genetic drift and is the primary mechanism through which genetic material is exchanged between populations. Populations that do not engage in gene flow become increasingly differentiated, and over long time scales may become reproductively isolated and diverge evolutionarily. Very little gene flow, as low as one migrant per generation, is needed to reduce the rate of genetic drift and maintain

connectedness of population units (Wright, 1931; Freeland et al., 2011). Gene flow relies on the successful dispersal and establishment of propagules away from their parent populations. Unidirectional transport of seeds or vegetative propagules in the direction of current flow is often expected for aquatic plants but backflow has also been shown to occur, and human-mediated transport can cause dispersal far beyond the natural expected range (Eckert et al., 2016).

Dispersal is limited spatially by the distance the seed or fragment travels from the parent to the substrate (primary dispersal) and the distance it travels across the substrate before establishing (secondary dispersal) (Darnell et al., 2015). The primary dispersal distance of a seed is influenced by factors such as the height of the parent plant, the buoyancy of the seed, and hydrodynamic flow (Darnell et al., 2015). The seeds of *H.e wrightii* are negatively buoyant and are released at the sediment surface, which encourages local dispersal near the mother plant (Kendrick et al., 2012). *H. wrightii* plants that reproduce asexually via vegetative fragmentation increase their primary dispersal distance since fragments are viable for 2 weeks in the fall and 4 weeks in the spring (Hall et al., 2006). Often vegetative fragmentation is used as a means of colonizing space where seed banks are absent (Campbell, 2003).

Literature Cited:

Bakker, J.P., Poschlod, P., Strykstra, R.J., Bekker, R.M., Thompson, K., 1996. Seed banks and seed dispersal: important topics in restoration ecology. Acta Botanica Neerlandica 45, 461-490.

Bernatowicz, A.J., 1952. Marine monocotyledonous plants of Bermuda. Bull. Mar. Sci. 2, 338-345.

Campbell, M.L., 2003. Recruitment and colonisation of vegetative fragments of Posidonia australis and Posidonia coriacea. Aquat. Bot. 76, 175-184.

Carlson Jr, P.R., Yarbro, L.A., Barber, T.R., 1994. Relationship of sediment sulfide to mortality of Thalassia testudinum in Florida Bay. Bull. Mar. Sci. 54, 733-746.

Darnell, K.M., Booth, D.M., Koch, E.W., Dunton, K.H., 2015. The interactive effects of water flow and reproductive strategies on seed and seedling dispersal along the substrate in two sub-tropical seagrass species. J. Exp. Mar. Biol. Ecol. 471, 30–40. doi:10.1016/j.jembe.2015.05.006

Dawes, C.J., Hanisak, D., Kenworthy, J.W., 1995. Seagrass biodiversity in the Indian river lagoon. Bull. Mar. Sci. 57, 59–66.

Eckert, C.G., Dorken, M.E., Barrett, S.C., 2016. Ecological and evolutionary consequences of sexual and clonal reproduction in aquatic plants. Aquat. Bot.

Escudero, A., Iriondo, J.M., Torres, M.E., 2003. Spatial analysis of genetic diversity as a tool for plant conservation. Biol. Conserv. 113, 351–365. doi:10.1016/S0006-3207(03)00122-8

Ferguson, R. L., Pawlak, B.T., Wood, L.L., 1993. Flowering of the seagrass Halodule wrightii in North Carolina, USA. Aquat. Bot. 46, 91-98.

Fourqurean, J.W., Manuel, S.A., Coates, K.A., Kenworthy, W.J., Boyer, J.N., 2015. Water quality, isoscapes and stoichioscapes of seagrasses indicate general P limitation and unique N cycling in shallow water benthos of Bermuda. Biogeosciences 12, 6235–6249. doi:10.5194/bg-12-6235-2015

Fourqurean, J.W., Manuel, S., Coates, K.A., Kenworthy, W.J., Smith, S.R., 2010. Effects of excluding sea turtle herbivores from a seagrass bed: overgrazing may have led to loss of seagrass meadows in Bermuda. Mar. Ecol. Prog. Ser. 419, 223-232.

Fourqurean, J.W., Robblee, M.B., 1999. Florida Bay: a history of recent ecological changes. Estuaries 22, 345–357.

Freeland, J.R., Kirk, H., Petersen, S.D., 2011. Molecular Ecology 2 Ed. Wiley-Blackwell, West Sussex, UK.

Hall, L.M., Hanisak, M.D., Virnstein, R.W., 2006. Fragments of the seagrasses Halodule wrightii and Halophila johnsonii as potential recruits in Indian River Lagoon, Florida. Mar. Ecol. Prog. Ser. 310, 109–117.

Hardy, O.J., Vekemans, X., 1999. Isolation by distance in a continuous population: reconciliation between spatial autocorrelation analysis and population genetics models. Heredity 83, 145-154.

Indian River Lagoon Conditions Update, 2016. Internal communications report, SJRWML WRI Bureau, Palm Bay, Fl.

Kendrick, G.A., Waycott, M., Carruthers, T.J.B., Cambridge, M.L., Hovey, R., Krauss, S.L., Lavery, P.S., Les, D.H., Lowe, R.J., Vidal, O.M. i, Ooi, J.L.S., Orth, R.J., Rivers, D.O., Ruiz-Montoya, L., Sinclair, E.A., Statton, J., van Dijk, J.K., Verduin, J.J., 2012. The central role of dispersal in the maintenance and persistence of seagrass populations. Bioscience 62, 56–65. doi:10.1525/bio.2012.62.1.10

Lee, T.N., Melo, N., Smith, N., Johns, E.M., Kelble, C.R., Smith, R.H., Ortner, P.B., 2016. Circulation and water renewal of Florida Bay, USA. Bull. Mar. Sci. 92, 153–180. doi:10.5343/bms.2015.1019

Manuel, S.A., Coates, K.A., Kenworthy, W.J., Fourqurean, J.W., 2013. Tropical species at the northern limit of their range: Composition and distribution in Bermuda's benthic habitats in relation to depth and light availability. Mar. Environ. Res. 89, 63-75.

McMillan, C. 1981. Seed reserves and seed germination for two seagrasses, Halodule wrightii and Syringodium filiforme, from the Western Atlantic. Aquat. Bot. 11, 279-296.

Micheli, F., Bishop, M.J., Peterson, C.H., Rivera, J., 2008. Alteration of seagrass species composition and function over two decades. Ecol. Monogr. 78, 225–244.

Morris, L.J., Virnstein, R.W., 2004. The demise and recovery of seagrass in the northern Indian River Lagoon, Florida. Estuaries 27, 915–922.

Murdoch, T.J.T., Glasspool, A.F., Outerbridge, M., Ward, J., Manuel, S., Gray, J., Nash, A., Coates, K.A., Pitt, J., Fourqurean, J.W., others, 2007. Large-scale decline in offshore seagrass meadows in Bermuda. Mar. Ecol. Prog. Ser. 339, 123–130.

Obeysekera, J., Browder, J., Hornung, L., Harwell, M.A., 1999. The natural South Florida system I: Climate, geology, and hydrology. Urban Ecosyst. 3, 223–244.

Phillips, R.C., 1967. On species of the seagrass, Halodule, in Florida. Bull. Mar. Sci. 17, 672–676.

Phlips, E.J., Badylak, S., Lasi, M.A., Chamberlain, R., Green, W.C., Hall, L.M., Hart, J.A., Lockwood, J.C., Miller, J.D., Morris, L.J., Steward, J.S., 2015. From red tides to green and brown tides: bloom dynamics in a restricted subtropical lagoon under shifting climatic conditions. Estuar. Coasts 38, 886–904. doi:10.1007/s12237-014-9874-6

Smith, M.D., 2011. An ecological perspective on extreme climatic events: a synthetic definition and framework to guide future research: Defining extreme climate events. J. Ecol. 99, 656–663. doi:10.1111/j.1365-2745.2011.01798.x

Thayer, G.W., Bjorndal, K.A., Ogden, J.C., Williams, S.L., Zieman, J.C., 1984. Role of Larger Herbivores in Seagrass Communities. Estuaries 7, 351. doi:10.2307/1351619

Vallejo-Marín, M., Dorken, M. E., Barrett, S.C., 2010. The ecological and evolutionary consequences of clonality for plant mating. Annu. Rev. Ecol. Evol. Syst. 41, 193.

Vacher, H.L., Rowe, M.P., 1997. Geology and hydrogeology of Bermuda. In: Vacher HL, Quinn T (eds) Geology and hydrogeology of carbonate islands, 54 Developments in sedimentology. Elsevier, Amsterdam, 35–90

Virnstein, R.W., 1999) Seagrass meadows: fish and wildlife factories. Fla Nat. 72, 18–19.

Wright, S., 1931. Evolution in Mendelian populations. Genetics 16, 97-159.

Zieman, J.C., 1982. *Ecology of the seagrasses of south Florida: a community profile*. No. FWS/OBS-82/25. Charlottesville, VA, USA.