Genetic diversity and population connectivity of *Syringodium filiforme* in the Florida Keys, USA and northeastern subtropical Atlantic region

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Abstract

Syringodium filiforme is a seagrass that populates extensive meadows in South Florida and other coastal areas throughout the tropical and subtropical Atlantic. Such meadows are habitat-forming, support an abundance of biota, and confer economic and ecological benefits. The ability of this species to colonize bare areas following disturbance has been attributed to its fast rhizome elongation rates. The central research problem addressed in this thesis is how this rapid clonal growth strategy influences sexual reproduction and overall population survival in *S. filiforme,* which I indirectly evaluated through genetic diversity analyses. Measures of population genetic diversity and population genetic structure provide insight into the number and distribution of genetically distinct or related individuals within and among populations, thereby indirectly estimating the prevalence of sexual reproduction. In this thesis, I assess genetic diversity, population structure and gene flow in the tropical seagrass, *Syringodium filiforme,* from populations across the Florida Keys as well as additional sites in South Florida and the northeastern subtropical Atlantic.

A total of 17 polymorphic microsatellite markers were developed for population genetic analysis of *S. filiforme* at the onset of this study. Sixteen populations from the Florida Keys and single populations from Florida Bay, Tampa Bay, Bermuda and the Bahamas were sampled and analyzed using these markers to determine how genetic diversity partitions within and among sampled populations. Allelic diversity and heterozygosity-based statistics were used to measure genetic diversity, and population structure and gene flow were analyzed using F_{ST}- and model-based methods.

The markers used in this study proved to be powerful, as the probability of genet identity (clonal membership) due to random chance was very low among the samples analyzed because

of the high number of loci (17) amplified and the moderate to high allelic diversity (A = 35 – 106) detected in all populations. In most cases, observed heterozygosity exceeded expected heterozygosity, indicating heterozygote excess is common in *S. filiforme*. Population genetic analyses revealed that *S. filiforme* exhibits high clonality at the spatial scales sampled (1.5 – 5 m between ramets) where the ratio of unique genotypes to samples was always less than R = 0.62. In fact, there were only 6 unique multilocus genotypes detected among 123 shoots sampled in the Florida Bay population.

Across all populations, moderate yet significant genetic differentiation was observed (F_{ST} = 0.15, p = 0.001). The Gulf- and Atlantic-side populations exhibited relatively low within-group differentiation and consistently clustered separately, suggesting the Florida Keys archipelago presents a barrier to gene flow. The Bahamas population was most differentiated from all other populations (F_{ST} = 0.19 – 0.53), while the Bermuda population did not exhibit as great differentiation (F_{ST} = 0.14 – 0.28) though it is geographically most distant from all other populations.

The findings from this study suggest *S. filiforme* maintains moderate to high levels of heterozygosity and higher than expected genetic diversity even though it is a highly clonal seagrass. Population structure clearly develops across the complex topography and hydrology of the Florida Keys, in which Gulf and Atlantic populations represent distinct genetic clusters. Gulf and Atlantic genetic disjunctions have been observed for a number of other marine organisms in the southeastern U.S., suggesting the Florida peninsula represents a phylogeographic break for *S. filiforme* and several other taxa. In addition to geologic history, contemporary isolating mechanisms such as limited propagule exchange mediated by ocean currents may contribute to lower genetic connectivity between Florida, Bermuda and the Bahamas populations.

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Introduction

Syringodium filiforme (Cymodoceaceae), commonly known as manatee grass, is one of three dominant seagrasses found throughout the coastal tropical Atlantic (Green and Short, 2003). Throughout its range, *S. filiforme* forms meadows that sustain benthic primary production and serve as critical habitat for juvenile fisheries. These meadows also provide ecosystem services such as sediment stabilization and improved water quality. Seagrasses are currently experiencing worldwide decline due to a combination of several stressors, but local impacts related to water quality such as sedimentation and nutrient over-enrichment have been attributed as the most significant cause of contemporary seagrass loss (Orth et al., 2006, Waycott et al., 2009). Evaluating seagrass ecosystem health using genetic indicators are integral to the success of broader conservation strategies.

Given seagrasses are considered foundational species, and communities are dominated by few species, the next hierarchical level of diversity, within-species diversity is pertinent to biodiversity conservation and ecosystem resilience. Genetic diversity in dominant plant species affects community-level dynamics by influencing species and faunal community diversity as well as biomass production (Boothe and Grime, 2003). Biodiversity conservation includes genetic diversity for the fundamental reason that genetic variation allows for adaptation via natural selection to occur, thereby enhancing long-term population viability (Booy et al., 2000). Low levels of diversity generally decrease population fitness through deleterious effects caused by genetic drift and inbreeding. In the well-studied temperate seagrass, *Zostera marina,* genetic diversity confers greater resistance to disturbance and enhances recovery rates following disturbance events (Hughes and Stachowicz, 2004; 2011).

In mixed mating systems, diversity is quantified by genotypic diversity (clonality), the relative proportion of genetically distinct individuals, and genetic or allelic diversity, the number of different gene copies (alleles) at each locus. Similar to other seagrasses, *S. filiforme* reproduces both through vegetative propagation and seed production. The relative rates of each reproductive mode influence diversity. Asexual reproduction does not introduce novel genotypes or alleles except through somatic mutation. Sexual reproduction may introduce novel genotypes and combinations of alleles through meiotic recombination, increasing both genotypic and allelic diversity. As in many other seagrass species, factors controlling the phenology of flowering, pollination success and seedling establishment in *S. filiforme* are poorly understood, therefore reliance on sexual reproduction in this species remains unknown.

If populations become isolated due to geographic or hydrologic barriers, mutation, genetic drift and selection will cause allele frequencies to diverge. Dispersal of sexual propagules enhances genetic exchange between populations, thereby increasing gene flow or connectivity. The balance between isolation and connectivity over several generations can be detected using population genetic analyses.

Populations, defined as groups of interbreeding individuals that exist together at the same time, are difficult to discern in sessile marine organisms. Genetic assignment tests can be used to determine how individuals cluster based on allele frequencies. Population structure analysis detects patterns of gene flow and delineates genetically distinct populations, sometimes suggesting population boundaries counter to expectations based on geographic proximity alone (Paetkau et al., 1995; Davies et al., 1999). This thesis reports on the development of microsatellite markers for population genetic analysis in *S. filiforme*, explores how population

genetic structure develops in *S. filiforme* across the Florida Keys, and evaluates connectivity of South Florida populations with other populations found throughout its wider geographic range.

In the first chapter, I describe the sequences of 17 microsatellite markers developed for *S. filiforme*. I also provide methods for optimized amplification. I found all markers to be polymorphic for the samples tested, rendering them useful for population genetic analyses. The sequence data for the 17 microsatellite markers are now available and easily accessible in the National Institute of Health genetic sequence database, GenBank. These markers provide the genetic tools to identify clonal genotypes and to determine genetic relatedness across individuals.

In the second chapter, I utilize the microsatellite markers to investigate genetic diversity and population connectivity in *S. filiforme* across the Florida Keys and Florida Bay, Tampa Bay, and with more remote populations in Bermuda and the Bahamas. I found moderate to high genetic diversity and heterozygote excess in all populations, suggesting that proclivity for clonal reproduction in *S. filiforme* does not necessarily depress genetic diversity. The data suggest that *S. filiforme* in the Florida Keys exists as two genetically distinct groups, the Gulf and Atlantic demes. The Bahamas population exhibits high differentiation from all other populations, perhaps due to isolation over time. While the Bermuda population is more geographically distant from the South Florida sites, it exhibits greater gene flow with these sites than does the Bahamas population. These findings suggest geographic history and contemporary oceanic circulation, but not necessarily distance, strongly influence the genetic connectivity of *S. filiforme* populations.

This thesis required the development of microsatellite markers and multiplex panels for *S. filiforme*, which creates opportunity for future survey-based and experimental research on this species. This project includes the first analysis of *S.filiforme* population genetics, providing 'baseline' data on genotypic and genetic diversity across varied sampling scales, as well as

overall population structure and connectivity in the northeastern portion of its distributional range. Assessing the genetic population structure of *S. filiforme* throughout its northeastern range limits in Bermuda, and a geographically remote area in the Bahamas, provide information relevant to species conservation and management programs. Managers may use findings presented here to guide further work in identifying appropriate source populations for restoration efforts and the prioritizing specific populations for protection.

Literature Cited

Booth, R., and Grime, J. (2003). Effects of genetic impoverishment on plant community diversity. *Journal of Ecology, 91*(5), 721-730.

Booy, G., Hendriks, R., Smulders, M., Van Groenendael, J., and Vosman, B. (2000). Genetic diversity and the survival of populations. *Plant Biology, 2*(4), 379-395.

Davies, N., Villablanca, F. X., and Roderick, G. K. (1999). Determining the source of individuals: Multilocus genotyping in nonequilibrium population genetics. *Trends in Ecology and Evolution, 14*(1), 17-21.

Green, E. P., and Short, F. T. (Eds.). (2003). *World atlas of seagrasses*. Berkeley: University of California Press.

Hughes, A. R., and Stachowicz, J. J. (2011). Seagrass genotypic diversity increases disturbance response via complementarity and dominance. *Journal of Ecology, 99*(2), 445-453.

Hughes, A., and Stachowicz, J. (2004). Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. *Proceedings of the National Academy of Sciences of the United States of America, 101*(24), 8998-9002.

Orth, R. J., Carruthers, T. J. B., Dennison, W. C., Duarte, C. M., Fourqurean, J. W., Heck, K. L., Jr., Hughes, R. A., Kendrick, G. A., Kenworthy, J. W., Olyarnik, S., Short, F. T., Waycott, M., and Williams, S. L. (2006). A global crisis for seagrass ecosystems. *Bioscience, 56*(12), 987-996.

Paetkau, D., Calvert, W., Stirling, I., and Strobeck, C. (1995). Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology, 4*, 347-354.

Waycott, M., Duarte, C. M., Carruthers, T. J. B., 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., and Williams, S. L. (2009). Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences, USA*, *106*(30), 12377-12381.

Chapter 1

Development of microsatellite markers for a tropical seagrass, *Syringodium filiforme* (Cymodoceaceae)[1]

[1] Published in *Applications in Plant Sciences* in 2014 with co-authors Kor van Dijk and Michelle Waycott.

Abstract

- *Premise of the study*: A total of 17 polymorphic microsatellite markers were developed for the tropical Atlantic seagrass, *Syringodium filiforme*, enabling analysis of population genetic structure in this species for the first time.
- *Methods and Results*: The 17 primers amplified di- and tri-nucleotide repeats revealing 2–8 alleles per locus among the South Florida populations tested. In the analysis of two populations from the Florida Keys (Florida, USA), observed heterozygosity ranged from 0.063 to 0.875, although sampling was from relatively closely located populations so heterozygosity is expected to be higher across larger spatial scales. Multiplex PCRs consisting of two 6-plex and one 5-plex reactions were developed to maximize genotyping efficiency.
- *Conclusions*: We present here 17 polymorphic markers that will be useful for the study of clonality and population structure of *Syringodium filiforme*, a marine plant that forms extensive habitat throughout the tropical Atlantic and Caribbean.

Key words: Caribbean; clonality; population genetics; seagrass; *Syringodium filiforme*

Introduction

Syringodium filiforme Kütz. is one of three dominant seagrasses native to the tropical Atlantic. It is widely distributed and habitat-forming, often growing intermixed with other species and sometimes in dense, monospecific meadows (Green and Short, 2003). *Syringodium filiforme* is capable of both recruitment from seed and clonal propagation through rhizome expansion (Kendrick et al., 2012). The relative contribution of each strategy is difficult to determine, but may be resolved using polymorphic microsatellites to accurately identify clones and examine genetic structure of populations (Arnaud-Haond et al., 2005). Primers for microsatellite loci have been developed for the only other congener, *Syringodium isoetifolium* (Asch.) Dandy (Matsuki et al., 2013; Wainwright et al., 2013). The evolutionary distance between species of *Syringodium* Kütz. (Les et al., 1997) render these markers unlikely to be suitable for use in *S. filiforme*, thus new markers were isolated. The microsatellites markers developed here will enable assessment of genetic diversity and population structure of *S. filiforme* throughout its range.

Methods and Results

Genomic DNA was extracted from leaf tissue of *Syringodium filiforme* from 8 samples collected in the Indian River Lagoon, Florida, USA (27.19298ºN, -80.18067ºW) using a DNeasy™ Plant Kit (QIAGEN Pty Ltd, Valencia, California, USA) according to manufacturer's protocols. About 250 ng of DNA from each sample were pooled to create a final solution containing 2 µg of total genomic DNA. Microsatellite loci were isolated by GenoScreen in Lille, France (www.genoscreen.fr) following the methods described by Malausa et al. (2011). Libraries were prepared by mechanically fragmenting genomic DNA and probing for the following microsatellite repeats: TG, TC, AAC, AAG, AGG, ACG, ACAT and ACTC. Enriched fragments

were amplified, purified and quantified. Sequencing using 454 GS-FLX® Titanium chemistry (Roche Diagnostics) returned a total of 4,410 distinct sequences containing microsatellite motifs. QDD software (Meglécz et al., 2010) was used to design 51 primer pairs. Primers were synthesized (Invitrogen, Carlsbad, California, USA) with M13-tails (5'-TGTAAAACGACGGCCAGT-3') preceding the 5' end of the forward primer sequences to facilitate cost-efficient fluorescent labeling of PCR products (Schuelke, 2000). To improve genotyping accuracy, 'pig-tails' (5'-GTTTCT-3') were added to the 5' end of the reverse primer sequences (Brownstein et al., 1996).

Primers were initially tested using DNA from 8 samples from two populations, Florida Bay (vouchers deposited at the State Herbarium of South Australia (AD), AD267896, AD267897, geo-reference, 25.12575°N, -080.78830°W) and Tampa Bay (27.65007ºN, -82.67941ºW). Given the close proximity of locations and unambiguous taxonomy, vouchers were only collected from one Florida Bay sampling site representing the entire South Florida region. DNA was extracted as above and gradient PCR performed containing the following reagent amounts in each 15 µL reaction: 1.5 mM $MgCl_2$, 1.3 mM dNTPs, 6.7 µg/µL BSA, 0.5 units Bioline Immolase^{TM} DNA polymerase and 1.50 µL Bioline ImmoBuffer^{TM} (Bioline, Taunton, MA, USA). Primers quantities included 0.6 ρmol of the M13-labeled forward primer, 2.4 ρmol each reverse primer and the fluoro-labeled M13 primer (5'-FAM, 5'-NED, or 5'VIC; Invitrogen, Carlsbad, California, USA) and 1.0–3.0 ng μL^{-1} of DNA template. PCR cycling conditions were 95ºC for 10 minutes, 35 cycles of 45 s at 95ºC, 30 s at an annealing temperature gradient of 52–58ºC, and 30 s at 72ºC, with final extension at 72ºC held for 5 minutes. PCR products were visualized on a 1.5% agarose gel where a single band of approximate expected size according to locus primer design signified successful amplification. To determine

polymorphism, PCR products were analyzed by capillary electrophoresis on a MegaBACE™ 1000 (GE Biosciences) with an internal ET-ROX 400 size standard (GE Biosciences). A final set of 17 microsatellite loci were found to be polymorphic in *S. filiforme*.

The high number of polymorphic loci enabled the development of multiplex PCR panels in which several loci are amplified in a single reaction, increasing genotyping efficiency while minimizing costs. Using the software Multiplex Manager v1.2 (Holleley and Geerts, 2009), two 6-plex and one 5-plex multiplexes were designed, and new fluorescently labeled forward primers synthesized (Invitrogen, Carlsbad, California, USA; Table 3). Multiplex PCRs were conducted using a Type-it® Microsatellite Multiplex PCR Kit (QIAGEN Pty Ltd, Valencia, California, USA) in 10 μL reactions containing 5 μL of Type-it® PCR Multiplex Master Mix, 0.5 μL of 2 μM primer mix (Table 3) and 1–5 ng of template DNA. PCR conditions were set to the manufacturer's optimized cycling protocol (Type-it® Microsatellite Multiplex PCR Kit; QIAGEN Pty Ltd, Valencia, California, USA).

To assess the genetic diversity of selected loci, two populations of *S. filiforme* were screened from the Florida Keys: the Elbow (25.12960°N, 80.26537°W) in the Upper Keys and the Sluiceway (24.82836°N, 80.87138°W) in the Middle Keys in Florida, USA (n = 36 and n =34, respectively). Samples were collected at least 2 m apart from an area of approximately 50 x 50 m. Fragment analysis of PCR products was performed on a capillary-based 3730xl DNA Analyzer (Applied Biosystems) with internal ET-ROX 500 size standard at the Georgia Genomics Facility (University of Georgia, Georgia, USA).

A total of 70 individual shoots sampled from two populations in the Florida Keys were screened and 37 unique multi-locus genotypes were identified. The probability individuals sharing the same genotype were derived via separate sexual events, P_{gen}, and the probability of

clonal identity, P_{sex}, were calculated in GenClone2.0 (Arnaud-Haond and Belkhir, 2007). The highest values observed for P_{gen} and P_{sex} were $1.07 \cdot 10^{-5}$ and $1.27 \cdot 10^{-4}$, respectively. The loci Syf-16, Syf-40 and Syf-51 were fixed for single alleles although they were polymorphic in the initial screening runs. All of the other 14 loci were polymorphic and allelic diversity was $A = 2–8$ (Table 2). Heterozygosity was only analyzed for genets, the presumptive genetic individuals based on unique multi-locus genotypes, to avoid bias due to overrepresentation of a single clone. Observed heterozygosity ranged from 0.063 to 0.875 and unbiased expected heterozygosity ranged from 0.063 to 0.792. Deviation from Hardy-Weinberg equilibrium was not observed for any locus ($p > 0.05$). Linkage disequilibrium among loci was tested using Genepop web version 4.2, using Fisher's method (Raymond and Rousset, 1995; Rousset, 2008), and was detected between loci pairs Syf-2 and Syf-6, Syf-6 and Syf-20, and Syf-2 and Syf-14 ($p < 0.05$), but is most likely an artifact of sampling relatively closely related populations.

Conclusions

The 17 polymorphic microsatellite loci and multiplex PCR panels developed in this study will facilitate population genetic analyses of the extensive meadows of *S. filiforme* found throughout tropical Atlantic marine ecosystems. The allelic diversity detected will allow assessment of clonality, genetic diversity and population genetic structure. Fixed alleles observed in the populations in the Florida Keys are probably due to the relatively close proximity of the sampling locations, as all loci were found to be polymorphic when comparing fewer samples from the Tampa Bay and Florida Bay populations. Clonal plant populations existing within geographical extremes of their range may exhibit higher clonality and less diversity, which may be true for *S. filiforme* in sub-tropical latitudes (Billingham et al., 2003). Therefore it is expected that sampling more broadly across the species range will increase the number of alleles detected.

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Literature Cited

Arnaud-Haond, S., Alberto, F., Teixeira, S., Procaccini, G., Serrao, E., and Duarte, C. (2005). Assessing genetic diversity in clonal organisms: Low diversity or low resolution? Combining power and cost efficiency in selecting markers. *Journal of Heredity* 96: 434-440.

Arnaud-Haond, S., and Belkhir, K. (2007). Genclone: A computer program to analyse genotypic data, test for clonality and describe spatial clonal organization. *Molecular Ecology Notes* 7: 15-17.

Billingham, M., Reusch, T., Alberto, F., and Serrao, E. (2003). Is asexual reproduction more important at geographical limits? A genetic study of the seagrass *Zostera marina* in the Ria Formosa, Portugal. *Marine Ecology Progress Series* 265: 77-83.

Brownstein, M., Carpten, J., and Smith, J. (1996). Modulation of non-templated nucleotide addition by *Taq* DNA polymerase: Primer modifications that facilitate genotyping. *Biotechniques* 20: 1004-1110.

Green, E. P., and Short, F. T. (Eds.). (2003). *World Atlas of Seagrasses*. Berkeley: University of California Press.

Holleley, C. E., and Geerts, P. G. (2009). Multiplex Manager 1.0: A cross-platform computer program that plans and optimizes multiplex PCR. *Biotechniques* 46: 511-517.

Kendrick, G. A., Waycott, M., Carruthers, T. J. B., Cambridge, M. L., Hovey, R., Krauss, S. L., Lavery, P.S., et al. (2012). The central role of dispersal in the maintenance and persistence of seagrass populations. *Bioscience* 62: 56-65.

Les, D. H., Cleland, M. A., Waycott, M. (1997) Phylogenetic studies in Alismatidae, II: evolution of marine angiosperms (seagrasses) and hydrophily. *Systematic Botany* 22: 443-463.

Malausa, T., Gilles, A., Meglécz, E., Blanquart, H., Duthoy, S., Costedoat, C., Dubut V., et al. (2011). High-throughput microsatellite isolation through 454 GS-FLX Titanium pyrosequencing of enriched DNA libraries. *Molecular Ecology Resources* 11: 638-644.

Matsuki, Y., Takahashi, A., Nakajima, Y., Lian, C., Fortes, M. D., Uy, W. H., Campos W.L., et al. (2013). Development of microsatellite markers in a tropical seagrass *Syringodium isoetifolium* (Cymodoceaceae). *Conservation Genetics Resources* 5: 715-717.

Meglécz, E., Costedoat, C., Dubut, V., Gilles, A., Malausa, T., Pech, N., and Martin, J. (2010). QDD: A user-friendly program to select microsatellite markers and design primers from large sequencing projects. *Bioinformatics* 26: 403-404.

Raymond, M., and Rousset, F. (1995). Genepop (version-1.2) - population-genetics software for exact tests and ecumenicism. *Journal of Heredity* 86: 248-249.

Rousset, F. (2008). GENEPOP'007: A complete re-implementation of the GENEPOP software for windows and linux. *Molecular Ecology Resources* 8: 103-106.

Schuelke, M. (2000). An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology* 18: 233-234.

Wainwright, B. J., Arlyza, I. S., and Karl, S. A. (2013). Isolation and characterization of eighteen polymorphic microsatellite loci for the topical seagrass, *Syringodium isoetifolium*. *Conservation Genetics Resources* 5: 943-945.

Tables

Table 1.1: Characteristics of 17 microsatellite loci developed for *Syringodium filiforme*. Reverse primer sequences, and therefore also size range, do not include the optional 'pig-tail' (5'-GTTTCT-3') sequence.

Locus	Primer sequences (5' - 3')	Repeat motif	Size range (bp)	T_a (°C)	GenBank accession no.
SYF-02	F: ACGAAGAAGAGGAGGAAGAGG	$(GAG)_{10}$	108 - 117	60	KM359149
	R: GTGTGGAATTGCAAGGAGGT				
SYF-03	F: ACAAATGATGACTAAGGAGGGG	$(TC)_{12}$	244 - 258	60	KM359150
	R: CTTATTAAGAATTAGTGCTCTTATGGC				
SYF-04	F: GGTGGTCGTACTTTTCAGGC	$(GTT)_{11}$	139 - 169	60	KM359151
	R: CAAAGCCAAGCAAACCTCAT				
SYF-06	F: ATCAATTCTTTGCCGATCC	$(AG)_{10}$	196 - 236	60	KM359152
	R: GAGGAGGATGATCTTGACCG				
SYF-12	F: TGAAACTGTTGAAGTGGAGGTG	$(TC)_{10}$	206 - 218	60	KM359153
	R: GCTGCAATTGTAAACCCCATT				
SYF-14	F: CCCTTGCATCGACACTATCC	$(TTC)_{11}$	134 - 166	60	KM359154
	R: CCCACTAAAAGAACATACGTACTGAA				
SYF-15	F: TTAGTTTCACTTTTCTGGCCG	$(CTT)_{17}$	281 - 293	60	KM359155
	R: GAACAGAAGCATGCAGTCTAGC				
SYF-16	F: CAAACATGGGCAGAGAAACA	$(TC)_{10}$	155 - 157	60	KM359156
	R: TGATCATGGACCTCAGGGA				
SYF-19	F: AAATCTCAAATCATCCTACGCA	$(TC)_{12}$	139 - 147	60	KM359157
	R: CGTCTGAAGGGTAGGTGTGG				
SYF-20	F: CCAACTCTGGCTGGCTGT	$(CT)_{10}$	180 - 198	60	KM359158
	R: GTTTCTGTCGGTATGTTCGAGCCATATT				
SYF-22	F: GAAAGGCCTCCATTCCATTT	$(AGG)_9$	146 - 161	60	KM359159
	R: TCGATTCTCCTCTTTCTTCGAC				
SYF-25	F: CCGTCGTAAGGACAAGGAAG	$(GAC)_9$	94 - 106	60	KM359160
	R: AGCAAAACAGCGTTCATGG				
SYF-29	F: GGACGAAATGAATGGTACGC	$(GA)_9$	215 - 223	60	KM359161
	R: GATTCTCACCTCGGAGCAAC				
SYF-37	F: TAGTCATTGCTCTATTGGGGTG	$(TTC)_8$	240 - 246	60	KM359162
	R: TGTGATTGTTAAGCCGAAAGG				
SYF-40	F: TCGTTGATCATAGAAGTCTCATCTC	$(TC)_8$	114 - 116	60	KM359163
	R: CCATAAAGGTAGCCCACACC				
SYF-49	F: TAAGAGGAACGACCCCATCA	$(GAC)_8$	140 - 143	60	KM359164
	R: CCTTGTCCTCCTCGTCTTCA				
SYF-51	F: TAGGGTTGTAGGAGCCAGGA	$(GA)_8$	164 - 174	60	KM359165
	R: GGGTGACTAGAAGCATTGCC				

Table 1.2: Summary genetic statistics for two populations of *Syringodium filiforme* screened with the newly developed microsatellites. Number of alleles (*A*), observed heterozygosity (H_o), unbiased expected heterozygosity (H_e), and p-values (*P*) for deviation from Hardy-Weinberg equilibrium are provided for each locus. The number of alleles (*A*) observed in the Tampa Bay and Florida Bay populations has also been provided to show that additional alleles are detected across larger spatial scales among the small number of preliminary screening samples.

	Elbow Reef (n = 36)				Sluiceway (n = 34)				Tampa Bay and Florida Bay (n = 8)
Locus	***A***	***H_o***	***H_e***	***P***	***A***	***H_o***	***H_e***	***P***	***A***
SYF-02	3	0.714	0.547	0.173	3	0.813	0.579	0.069	3
SYF-03	5	0.762	0.722	0.800	3	0.875	0.675	0.478	3
SYF-04	5	0.762	0.754	0.759	2	0.688	0.514	0.309	2
SYF-06	6	0.905	0.792	0.627	3	0.625	0.685	0.609	3
SYF-12	3	0.714	0.675	0.772	2	0.125	0.121	1.000	3
SYF-14	8	0.810	0.808	0.470	3	0.875	0.595	0.042	3
SYF-15	3	0.381	0.361	0.381	3	0.625	0.675	0.277	2
SYF-16	1	---	---	---	1	---	---	---	2
SYF-19	4	0.714	0.618	0.192	2	0.467	0.370	0.528	2
SYF-20	4	0.571	0.632	0.186	3	0.750	0.548	0.215	2
SYF-22	3	0.619	0.577	1.000	2	0.375	0.315	1.000	2
SYF-25	3	0.619	0.633	0.579	3	0.250	0.232	1.000	2
SYF-29	2	0.095	0.093	1.000	2	0.563	0.417	0.256	2
SYF-37	2	0.095	0.093	1.000	2	0.375	0.387	1.000	2
SYF-40	1	---	---	---	1	---	---	---	2
SYF-49	2	0.143	0.136	1.000	2	0.063	0.063	---	2
SYF-51	1	---	---	---	1	---	---	---	3

Table 1.3: Suggested multiplex PCR panels for *Syringodium filiforme* using fluorescent labels FAM, NED, and VIC, and primer stock concentration of 100 μM. The volume listed is for both forward and reverse primers to be added to each panel with water to a total volume of 500 μL.

Locus	Label	Volume (μl)	Locus	Label	Volume (μl)	Locus	Label	Volume (μl)
Panel Syf-A			**Panel Syf-B**			**Panel Syf-C**		
SYF-25	VIC	5	SYF-40	FAM	10	SYF-29	FAM	10
SYF-19	FAM	12	SYF-51	FAM	10	SYF-14	VIC	6
SYF-02	NED	7.5	SYF-16	NED	10	SYF-49	NED	5
SYF-20	NED	7.5	SYF-04	VIC	7.5	SYF-22	NED	7
SYF-15	NED	7.5	SYF-12	VIC	7.5	SYF-06	NED	7
			SYF-03	VIC	7.5	SYF-37	NED	8

Chapter 2

Gene flow and population structure of the tropical seagrass, *Syringodium filiformе*, in the Florida Keys and northeastern subtropical Atlantic region[2]

[2] To be submitted for publication with co-authors Kor van Dijk and Michelle Waycott.

Abstract

Evaluating genetic diversity of seagrasses provides insight into reproductive mode and adaptation potential, and is therefore integral to broader conservation strategies for coastal ecosystems. In this study, we assessed genetic diversity, population structure and gene flow in *Syringodium filiforme* in the Florida Keys and northeastern subtropical Atlantic. We used microsatellite markers to analyze 20 populations throughout the Florida Keys, South Florida, Bermuda and the Bahamas. We measured diversity through allelic- and heterozygosity-based calculations, and evaluated population structure using F-statistics and model-based methods. Gene flow was assessed through pairwise F_{ST}-based migration rates and direction of differentiation calculations. There was moderate to high diversity within populations, as we detected 35 – 106 alleles across all populations, and in some instances very high clonality (R = 0.04 – 0.62). Observed and expected heterozygosity ranged from 0.21 to 0.51 and 0.18 to 0.44, respectively. There was significant among-population differentiation (F_{ST} = 0.15, p = 0.001) and evidence of population structure, dividing the region into four genetic population clusters. The greatest genetic differentiation was found between the Bahamas and all other populations (F_{ST} = 0.19 – 0.53), bolstering support for four population clusters consisting of South Florida Atlantic and Gulf groups, and the Bahamas and Bermuda. Though the Bermuda population is the most geographically distant population from all others, it was not the most genetically isolated. We observed asymmetric patterns in gene flow, with a few instances in which there was higher than expected gene flow from Atlantic to Gulf populations. In South Florida, clustering into Gulf and Atlantic groups indicate dispersal in *S. filiforme* may be limited by geographic and hydrologic barriers, but admixture between populations indicates genetic exchange may sometimes occur between narrow channels in the Florida Keys, maintaining regional connectivity.

Introduction

Seagrass beds are the dominant benthic habitat in southern coastal Florida (Fourqurean et al., 2001). These extensive beds support marine food webs including epiphytic algae to large grazers, deliver ecosystem services through stabilizing coastal sediments and improving local water quality, and have recently been recognized for their role in carbon storage (Costanza et al., 1997; Fourqurean et al., 2012). As is true globally, seagrasses in South Florida are threatened by local impacts related to water quality such as sedimentation and nutrient over-enrichment (Orth et al., 2006). In order to understand the full impact of environmental decline and perturbations in seagrass ecosystems, evaluating 'baseline' diversity, population structure and gene flow is essential.

Capable of both vegetative propagation and reproduction by seed, seagrasses have been considered predominantly clonal, therefore harboring low genetic diversity (Barrett et al., 1993; Kendrick et al., 2005). The recent development of high-resolution markers has led to studies that have countered this expectation by revealing higher than previously reported genetic diversity in several seagrass species (Arnaud-Haond et al., 2005). Demographic processes including rates of sexual reproduction, dispersal of propagules and successful establishment of seedlings influence genetic diversity and connectivity of populations in marine environs, but gene flow is ultimately mediated by ocean hydrology and limited by geographic barriers. Environmental conditions, such as water quality, and prevailing winds and local water movement, contribute to fine-scale population genetic structure (Oliva et al., 2014; Sinclair et al., 2014). Geographic history, including glaciation and continental drift, in conjunction with modern gene flow patterns influenced by oceanic hydrology, determine regional genetic connectivity (van Dijk et al., 2009;

Serra et al., 2010). In this study, we assessed the genetic diversity and population structure of the tropical Atlantic seagrass, *Syringodium filiforme* in a complex hydrologic setting.

S. filiforme populates expansive meadows, contributing to the vast extent of seagrasses in South Florida covering more than 14,000 km^2 of shallow coastline (Fourqurean et al., 2002). This species is sometimes dominant in early successional meadows because it has relatively high horizontal rhizome elongation rates (Marbà & Duarte, 1998) and tolerates sediment conditions that are less favorable to another dominant seagrass, *Thalassia testudinum*, yet is also found in the climax state of seagrass bed development (Williams, 1990). Given these traits, *S. filiforme* is able to quickly colonize bare areas through clonal propagation, but also through seed and vegetative fragment dispersal, especially following disturbance (Kendall et al., 2004). How this growth strategy of rapid clonal growth and presumed limited sexual reproduction impacts genetic diversity and gene flow in *S. filiforme* in the Florida Keys remains unknown.

South Florida coastal waters are characterized by complex hydrology and well studied biogeochemical gradients (Briceño et al., 2013). The Florida Keys archipelago extends 350 km from the South Florida mainland to Key West, separating the Gulf of Mexico and Florida Bay from the Atlantic Ocean. The Gulf of Mexico Loop Current and Florida Current promote high region-wide hydrologic connectivity, while tidal currents promote local mixing and dispersion (Lee and Williams, 1999). The distribution of *S. filiforme* across the Florida Keys ranges from marginal and patchy in Florida Bay, to sparse in offshore intermixed beds on the Atlantic side, to dense, monospecific stands along the Middle and Lower Keys on the Gulf of Mexico side (Fourqurean et al., 2003; 2001). The Florida Keys archipelago separates geographically proximal *S. filiforme* populations in the Atlantic and Gulf basins, leading us to predict these basins host

genetically distinct populations due to limited opportunity for propagule exchange and gene flow.

In this study, we used species-specific microsatellite loci to assess genetic diversity, population genetic structure and connectivity in *Syringodium filiforme* across the Florida Keys and northeastern subtropical Atlantic. The additional populations throughout the range of *S. filiforme* were included in the analysis to investigate connectivity across regional boundaries. We set out to answer the following questions: (1) How does clonality vary across 500 m^2 and 2500 m^2 spatial scales? (2) How much genetic diversity is present within and between populations of *S. filiforme*? (3) Does genetic differentiation among populations result in population structure? (4) Are there patterns in the degree and direction of gene flow between populations?

Methods

Sample collection

A total of 18 meadows, hereafter termed populations, were sampled in South Florida (Figures 2.1 & 2.2). Eight populations in the Upper and Middle Keys on the Atlantic side, two populations on the Gulf side (Sprigger and Sluiceway) and a single population in Tampa Bay were sampled in 2014. Six populations in the Middle and Lower Keys on the Gulf side and a single population in Graham's Harbor on the Bahamian island of San Salvador were sampled in 2015. Additionally in 2015, single sites in Blackwater Sound, Florida Bay and Bailey's Bay, Bermuda were also sampled following a slightly different sampling design (in order to also accommodate for research questions in a separate study). Leaves of at least 50 individuals were randomly collected within a ~2,500 m^2 sampling area, spaced 5 m apart, for the 2014 collection, and within a ~500 m^2 sampling area, spaced 1.5 m apart, for the 2015 collection. In Florida Bay and Bermuda, six and five smaller areas (spaced < 1 km and < 280 m apart) were sampled,

respectively. Within each area, 24 leaves were collected from ramets (spaced ~1.5 m apart) in a ~ 70 m^2 sampling area.

DNA extraction, PCR and genotyping

Total genomic DNA was extracted from the samples collected in 2014 using a DNeasy™ Plant Kit (QIAGEN) according to the manufacturer's instructions. Extracted DNA was quantified on a Qubit® 2.0 Fluorometer (Invitrogen). Samples collected in 2015 were sent to the University of Wisconsin Biotechnology (University of Wisconsin, Wisconsin, USA) for extraction and quantification. DNA was extracted from 40-50 mg of dried leaf tissue using the CTAB method as described in Saghai-Maroof et al. with minimal modification (1984). Following elution, a final DNA cleaning step was performed using a 1.5:1 by volume ratio of Axygen Clean-Seq beads (Corning Life Sciences, Corning, NY, USA) to extracted DNA to remove any remaining inhibitory compounds in the sample. DNA was quantified using Quant-IT PicoGreen fluorescent dye (Thermo Fisher, Waltham, MA, USA). All extracted DNA was diluted to a concentration of ~5ng μL^{-1}.

A total of 17 microsatellite loci were amplified using fluorescently labeled primers (Bijak et al., 2014). PCR was conducted in three multiplex panels using a Type-it® Microsatellite Multiplex PCR Kit (QIAGEN) in 10 µL reactions, consisting of 8.5 µL of Type-it® PCR Multiplex Master Mix, 0.5 µL of 2 µM primer mix and 1 µL of diluted template DNA. PCR conditions were set to the manufacturer's optimized cycling conditions (QIAGEN). PCR products were sequenced on a capillary-based 3730xl DNA Analyzer (Applied Biosystems) with an internal ET-ROX 500 size standard at the Georgia Genomics Facility (University of Georgia, Georgia, USA). Fragment lengths for each locus were determined using Geneious V7.1.7 (Biomatters Ltd.).

Genet identity and genotypic richness

The number of unique multi-locus genotypes (MLGs), *G*, present in each population calculated by the P_{gen} estimation method of Parks and Werth (1993), and the probability individuals sharing the same genotype were derived via separate sexual events (P_{sex}) were both estimated using GENCLONE version 2.0 (Arnaud-Haond and Belkhir, 2007a). Genotypic richness, *R*, the proportion of genets in the population, was calculated as $R = (G-1)/(N-1)$.

Within-population genetic variability

For the remainder of population genetic analyses, replicate MLGs were removed from the dataset to avoid allele frequency bias due to the presence of clones. The total number of alleles, *A*, average number of alleles per locus, N_A, and average allelic richness per locus standardized by smallest sample size, A_R, were calculated using the 'diveRsity' package in R (Keenan et al., 2013). For each population, observed heterozygosity (H_o), expected heterozygosity (H_e), and deviation from Hardy-Weinberg equilibrium as measured by the inbreeding coefficient, F_{IS}, were calculated in GENALEX version 6.5 (Peakall and Smouse, 2006; 2012). Linkage disequilibrium using a sequential Bonferroni correction to account for multiple comparisons was tested for each population using log-likelihood tests in GENEPOP version 4.2 (Raymond and Rousset, 1995; Rousset, 2008).

Genetic differentiation

An analysis of molecular variance (AMOVA) was performed to assess overall genetic differentiation under the assumptions of the Infinite Allele Model in GENODIVE (Mierman and Tienderen, 2004). Standard deviations for AMOVA F-statistics were calculated by jackknife resampling over loci, and permutation tests were used to assess significance. Fixation indices F_{ST} (Weir & Cockerham, 1984), G_{ST} (Nei, 1973), G'_{ST} (Hedrick, 2005) and Jost's *D* (Jost, 2008;

2009) were calculated for all possible pairwise population combinations using the 'diveRsity' package in R. Principal component analysis (PCA) was performed in GENODIVE using a covariance matrix based on individual allele frequencies to determine whether geographically proximal samples exhibit similar allele frequencies, but without the assumption of hierarchical genetic structure.

Population structure and migration

To determine the most likely number of population clusters, K, population assignment utilizing a Bayesian approach was performed in the genetic software program STRUCTURE (Pritchard et al., 2000). Model parameters were set to K = 1 – 20, with an initial burn-in period of 100,000 iterations followed by 1,000,000 Markov Chain Monte Carlo repetitions. The most likely number of population clusters was determined by the ad hoc quantity, ΔK (Evanno et al., 2005). Complementary software programs, CLUMPAK (Kopelman et al., 2015) were used for downstream processing, and DISTRUCT was used for visual representation (Rosenburg, 2004). Average total migration, N_m, was estimated using F_{ST} (Wright, 1951) and rare allele methods (Barton and Slatkin, 1986) in GENALEX (Peakall and Smouse, 2006; 2012) and GENEPOP, respectively. Additionally, pairwise relative migration rates were estimated using Alcala's *Nm* (Alcala et al., 2014) and directionality of differentiation was estimated according to novel methods developed by Sundqvist et al. (manuscript in review) using 'diveRsity' in R, and then visualized using another R package, 'qgraph' (Epskamp et al., 2012).

Results

Genet identity and genotypic richness

For most populations, P_{gen} ranged from 3.3×10^{-8} to 7.0×10^{-3} and P_{sex} ranged from 1.7×10^{-7} to 2.0×10^{-3}. However, there were higher values for P_{sex} in the following populations: Crane

(0.094), Key West (0.15), Tampa Bay (0.063), and Florida Bay (0.19). Out of 2720 total pairwise comparisons, there were 37 instances in which linkage disequilibrium was significant after the Bonferroni correction was applied (p-value < 0.003). Genotypic richness ranged from 0.04 to 0.62 (Table 2.1).

Within-population genetic variability

Total number of alleles ranged from 35 to 106 and the average number of alleles ranged from 2.06 to 6.24. Once adjusted for sample size, allelic richness was similar across all populations, ranging from 2.03 to 2.56 (Table 2.1). Observed heterozygosity ranged from 0.21 to 0.51, and expected heterozygosity ranged from 0.18 to 0.44. Deviation from Hardy-Weinberg conditions was detected in nine populations ($p < 0.05$), most of which exhibited negative inbreeding coefficients.

Genetic differentiation

AMOVA revealed significant genetic differentiation between populations ($F_{ST} = 0.149 \pm 0.017$, *P-value* = 0.001; Table 2.2). The results of pairwise population differentiation were consistent across all statistics, F_{ST}, G_{ST}, G'_{ST} and Jost's D (Figures 2.3, 2.4, 2.5 & 2.6), with maximum values calculated as 0.53, 0.38, 0.64 and 0.30, respectively. Since similar patterns in relative differentiation between populations were observed, and differences were primarily found in magnitude of pairwise values, only F_{ST} will be discussed in detail. Pairwise differentiation values were low within Atlantic populations ($F_{ST} = 0 – 0.09$), and low to moderate within Gulf ($F_{ST} = 0.01 – 0.24$) populations, with maximum differentiation found between Marathon and Crane (Figure 2.1). Pairwise differentiation values between Atlantic and Gulf populations ranged from 0.05 to 0.33, with Gulf sites Marathon and Crane exhibiting the minimum and maximum values between Atlantic sites Davis and Conch, respectively. Florida Bay exhibited similar levels

of differentiation between Gulf (F_{ST} = 0.14 – 0.24) and Atlantic (F_{ST} = 0.18 – 0.26) sites. Tampa Bay exhibited high differentiation between Atlantic populations (F_{ST} = 0.24 – 0.37) and moderate to high differentiation between Gulf populations (F_{ST} = 0.10 – 0.28). The highest overall pairwise differentiation was found between the Bahamas and Tampa Bay, where F_{ST} = 0.53. The next greatest values were found between the Bahamas and Gulf populations (F_{ST} = 0.26 – 0.47), and values were moderate between the Bahamas and Atlantic populations (F_{ST} = 0.19 – 0.26). For Bermuda, F_{ST} = 0.37 with Tampa Bay, F_{ST} = 0.27 with Florida Bay, and F_{ST} = 0.36 with the Bahamas. Bermuda pairwise differentiation with Atlantic and Gulf sites was moderate to high, with F_{ST} values ranging from 0.14 to 0.22 and 0.18 to 0.28, respectively.

In the PCA, the first two principal component axes contained 18.3% and 7.7% of total variance (Figure 2.7). The Atlantic and Gulf sites clustered separately, with some overlap occurring, namely between Gulf sites Marathon, Pigeon and Sprigger and Atlantic sites. Tampa Bay clustered with the Gulf sites, while Bermuda clustered between Gulf and Atlantic sites. The Bahamas clustered separately from all other sites.

Population structure and migration

Population structure was present, with greatest statistical support for K = 2 (ΔK = 297.26), followed by K = 4 (ΔK = 20.84) number of population clusters (Figure 2.8). For K = 2, Atlantic and Gulf sides cluster separately, with Tampa Bay, Florida Bay, the Bahamas and Bermuda included in the Gulf cluster (Figure 2.9). For K = 4, Bahamas and Bermuda form their own clusters (Figure 2.10). In both population structures, Gulf sites Sprigger, Marathon and Pigeon exhibit admixture with Atlantic populations. Average migration between all populations was 1.7 and 2.6 migrants per generation, following the F_{ST} method and private alleles method, respectively. Relative pairwise migration was highest among Atlantic populations, ranging from

0.17 to 1, with lowest genetic exchange occurring from Conch to Elbow, and the highest from Davis to Carysfort (Figure 2.11). Exchange within the Gulf populations ranged from 0.02 to 0.80, with the greatest exchange occurring between Sluiceway and Sprigger. Exchange to and from Key West was particularly low, not exceeding 0.08. The Gulf site Marathon actually exhibited greater exchange with Atlantic populations than with those in the Gulf. There was also greater relative migration from Atlantic sites to Marathon, Pigeon and Sprigger than there was from within the Gulf. Florida Bay exhibited relative migration rates lower than 0.13 with greatest outgoing migration to the Atlantic site Davis. Tampa Bay exhibited migration rates lower than 0.15 with highest migration coming from Gulf sites. The Bahamas exhibited negligible migration rates, not exceeding 0.08. Incoming relative migration was 0.07 in Bermuda, while outgoing migration ranged from 0.01 to 0.23, with the highest exchange occurring with Atlantic populations.

Discussion

Our results further affirm that tendency toward clonal reproduction in seagrasses does not preclude high levels of genetic diversity (e.g. Reusch, 2001; Coyer et al., 2004; Arnaud-Haond et al., 2005). The predominance of clonality, determined by shared multilocus genotypes, was variable among populations of *S. filiforme*. Moderate to high allelic diversity and heterozygote excess was detected in almost every population. We found evidence of genetic differentiation and population structure, in which the region can be divided into two or four major demes. Overall migration rates were low, and asymmetric gene flow patterns were observed.

For all genotypes, values for P_{gen} were less than 0.01, indicating there is a low probability of generating the genotypes observed in this study by chance. While for most genotypes values for P_{sex} were also less than 0.01, there were a few cases in which P_{sex} exceeded 0.05 in Crane,

Tampa Bay, Florida Bay and Key West, suggesting somatic mutation or scoring error may have led to overestimation of the number of unique MLGs sampled. This may be in part be due to clonal dominance and small sample size at the sites in question, leading to low statistical power in P_{sex} estimation (Arnaud-Haond et al., 2007b). High values for P_{sex} might also suggest genotypes in these populations are highly similar due to shared ancestry, and may therefore represent sibling recruits. Though this finding may affect assessment of clonality in these populations, the few instances in which $P_{sex} > 0.05$ were unlikely to have greatly impacted the accuracy of heterozygosity estimates and other statistical analyses performed. The loci used in this study were not linked, as only about 1% of pairwise comparisons revealed significant linkage disequilibrium.

Genotypic richness varied widely across sampling sites, but cannot be proportionately compared across all populations due to disparities in sampling area dimensions. Sample collections in 2014 were from within an area of ~2500 m^2, in which genotypic richness ranged from 0.37 to 0.62. Sample collections in 2015 were from within an area of ~500 m^2, in which genotypic richness ranged from 0.05 to 0.43. In the larger areas sampled in 2014, genets exhibited both 'phalanx' (clonal aggregation) and 'guerilla' (extreme intermingling of genotypes) growth strategies, with some genets extending over one hundred meters. For the 2015 collection sites, it is likely that sampling in a smaller total area with shorter distances between each shoot sampled led to a decrease in detection efficiency of total genets and number of alleles present in the population (Leberg, 2002). Allelic richness standardized by smallest sample size was consistent across all sites, suggesting the observed higher number of alleles and unique MLGs in 2014 collection populations may be related to the spatial scale of sampling.

In Blackwater Sound, the northeastern region of Florida Bay, only 6 genotypes were detected out of 123 shoots sampled, with the same clone found to span across distances of at least one kilometer. While in Bailey's Bay, Bermuda, 10 unique genotypes out of 24 samples were detected within a single ~ 70 m^2 sample patch, exhibiting very high genotypic diversity within a much smaller area. Greater clonality observed in Florida Bay may accurately reflect decreased access to propagules and diminished gene flow due to the enclosed, isolated nature of this area, consistent with the notion that asexual reproduction is more prevalent in peripheral populations (Billingham et al., 2003). To further investigate the spatial genetic structure of *S. filiforme* in the South Florida, Bermuda and Bahamas populations, we can use the genetic data presented here and accompanying GPS data (unpublished) to identify the geographic scale at which autocorrelation develops between clones.

Nine populations were not in Hardy-Weinberg equilibrium, eight of which exhibited significant outbreeding, while Bermuda exhibited slight but significant inbreeding. The general excess of heterozygosity found in almost all sites suggests greater gene diversity is present in *S. filiforme* than would otherwise be expected under random mating conditions. Even if sexual reproduction is nominal in the populations studied, clonality can maintain (or increase) heterozygosity over generations via somatic mutation and through selection for heterozygotes, under the presumption heterozygosity confers fitness advantages (Stoeckel et al., 2006). Low genotypic richness was detected in some populations, likely a result of local population processes such as limited gene flow and high clonality, but overall, *S. filiforme* maintains moderate to high allelic diversity at the individual and population level.

Significant genetic differentiation among populations was detected. While similar patterns of population pairwise differentiation were observed for F_{ST}, G_{ST}, G'_{ST} and Jost's *D*,

magnitude of differentiation varied across these statistics. Pairwise values of Jost's *D* suggest low overall allelic differentiation between populations, but because this statistic is based on the distribution of effective number of alleles across populations, it is not helpful in evaluating how demographic processes affect population structure. Whereas G_{ST} is based on the comparison of expected heterozygosity, or gene diversity, within and among populations, and therefore provides more information regarding genetic drift and migration (G'_{ST} is standardized by the maximum possible value of G_{ST}). F_{ST} presented in this study was calculated using an ANOVA approach to estimate within-population and among-population variance components, which are then used to calculate F_{ST}. For all F-statistics, the greatest differentiation occurred between the Bahamas and Florida sites. Though Bermuda is the furthest distance from all other populations, this population did not exhibit the highest pairwise differentiation values. Our analyses suggest *S. filiforme* populations in Bermuda and especially in the Bahamas have been isolated from Florida populations in recent evolutionary history, leading to genetic drift and greater differentiation from populations closer to the core of *S. filiforme's* range (Slatkin, 1985; Billingham et al., 2003).

Results of the PCA show that populations do group according to patterns in how loci covary across sites, with Atlantic and Gulf populations separated into distinct groups. The Bermuda population appeared to be closely related to both Atlantic and Gulf populations, while the Bahamas population clustered separately from all other populations. The STRUCTURE model runs evaluated the genotypic data using a Bayesian approach to assign individuals to population clusters irrespective of geographic origin, yet produced similar results to those generated by F_{ST}-based and variance-based analyses. There was greatest statistical support for two population clusters according to Evanno's ΔK, with another level of population structure also supported,

consisting of four population clusters in which Bermuda and the Bahamas populations are treated as genetically distinct populations. The strongest population structure clearly develops in South Florida, where the Florida Keys archipelago acts as a modern and/or historical barrier to gene flow between Gulf and Atlantic demes. Though Tampa Bay is hundreds of kilometers away from the Florida Keys, it still groups with Gulf populations, suggesting shared ancestry or propagule sources between these populations. These findings are consistent with the presence of phylogeographic breaks between Gulf and Atlantic populations for a number of other marine organisms, possibly resulting from changes in connectivity due to glacial advances and retreats that occurred throughout the Pleistocene (e.g. Avise, 1992 Felder and Staton, 1994; Young et al., 2002; Lee and Foighil, 2004; Mathews, 2006). Relative migration and direction of differentiation calculations revealed asymmetric patterns in the magnitude and direction of genetic exchange mostly in congruence with findings in the previously discussed analyses in this study. Interestingly, there are exceptions to the Atlantic-Gulf divide, in which relatively high gene flow was found to occur from Atlantic sites to Gulf sites Marathon, Pigeon and Sprigger.

As contemporary surface ocean currents are implicated in the movement of propagules, and therefore indirectly affect genetic exchange between populations (Kendrick et al., 2012; McMahon et al., 2014), the relatively high genetic exchange within Atlantic populations may be explained by hydrologic connectivity along the Florida Keys Atlantic coastline. These gene flow patterns agree with the modeled and observed movement of spiny lobster (*Panulines argus*) larvae along a 'recruitment conveyor' in the Florida Keys, in which spawning larvae near the Yucatán Peninsula have been identified as source populations (Yeung and Lee, 2002). The net eastward and northward movement of the Florida current along the Florida Shelf and the intermittent formation of small eddys could facilitate local movement and entrainment of

propagules (Lee & Williams, 1999). Less genetic exchange within the Gulf populations is perhaps related to the isolating topography of the Lower Keys, in which several small key islands and narrow channels separate the seagrass meadows, hindering the movement of propagules. Though mean transport is from Gulf to Atlantic, westward tidal flow sometimes occurs from Atlantic waters through channels in the Keys (Lee and Smith, 2002; Smith, 1994) and could promote movement of propagules of Atlantic origin through to Gulf side populations Marathon, Pigeon and Sprigger. These findings are consistent with a study on the only congener of *S. filiforme, Syringodium isoetifolium*, for which population genetic structure in the western North Pacific was strongly influenced by the Kuroshio Current (Kurokochi et al., 2015). Though modern oceanic hydrology may control dispersal trajectories in *S. filiforme,* it is likely that the gene flow patterns observed here are also reflect evolutionarily historical population dynamics under past continent arrangements and sea levels.

Conclusions

Despite its status as an early colonizer species and tendency toward clonal propagation, we detected high genetic diversity in most populations of *S. filiforme* sampled in this study. Low differentiation was observed between more proximal populations in the Atlantic and Gulf, while highest differentiation was observed between the Florida Keys and the Bahamas populations. However, the Bahamas population sampled in this study is not representative of the entire archipelago. In fact, the sampling location was in a particularly remote and eastern island, San Salvador. We expect further sampling along more proximal Bahamian islands to show greater connectivity with Atlantic Florida populations. Population structure developed most strongly along the Florida Keys, suggesting the archipelago presents the greatest barrier to gene flow in *S. filiforme* identified in the northeastern subtropical Atlantic, consistent with similar

phylogeographic breaks identified for several other marine taxa. These findings provide coastal managers with 'baseline' population genetic data for *S. filiforme*, and give impetus for investigation of the drivers of diversity in this species.

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Literature Cited

Alcala, N., Goudet, J., and Vuilleumier, S. (2014). On the transition of genetic differentiation from isolation to panmixia: What we can learn from G_{ST} and *D*. *Theoretical Population Biology*, *93*, 75–84.

Arnaud-Haond, S., Alberto, F., Teixeira, S., Procaccini, G., Serrão, E. A., and Duarte, C. M. (2005). Assessing genetic diversity in clonal organisms: Low diversity or low resolution? Combining power and cost efficiency in selecting markers. *Journal of Heredity*, *96*(4), 434–440.

Arnaud-Haond, S., and Belkhir, K. (2007a). Genclone: A computer program to analyse genotypic data, test for clonality and describe spatial clonal organization. *Molecular Ecology Notes*, *7*, 15–17.

Arnaud-Haond, S., Duarte, C. M., Alberto, F., and Serrão, E. A. (2007b). Standardizing methods to address clonality in population studies. *Molecular Ecology*, *16*(24), 5115–5139.

Avise, J. C. (1992). Molecular population structure and the biogeographic history of a regional fauna: A case history with lessons for conservation biology. *Oikos*, *63*, 62–76.

Barrett, S. C. H., Eckert, C. G., and Husband, B. C. (1993). Evolutionary processes in aquatic plant populations. *Aquatic Botany*, *44*, 105–145.

Barton, N. H., and Slatkin, M. (1986). A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. *Heredity*, *56*, 409–415.

Billingham, M. R., Reusch, T. B. H., Alberto, F., and Serrão, E. A. (2003). Is asexual reproduction more important at geographical limits? A genetic study of the seagrass *Zostera marina* in the Ria Formosa, Portugal. *Marine Ecology Progress Series*, *265*, 77–83.

Briceño, H. O., Boyer, J. N., Castro, J., and Harlem, P. (2013). Biogeochemical classification of

South Florida's estuarine and coastal waters. *Marine Pollution Bulletin, 75*, 187–204.

Costanza, R., d'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R. V., Paruelo, J., Raskin, R. G., Sutton, P., and van den Belt, M. (1997). The value of the world's ecosystem services and natural capital. *Nature, 387*, 253–260.

Coyer, J. A., Diekmann, O. E., Serrão, E. A., Procaccini, G., Milchakova, N., Pearson, G. A., Stam, W. T., and Olsen, J. L. (2004). Population genetics of dwarf eelgrass *Zostera noltii* throughout its biogeographic range. *Marine Ecology Progress Series, 281*, 51–62.

Epskamp, S., Cramer, A.O.J., Waldorp, L.J., Schmittmann, V.D., and Borsboom, D. (2012). qgraph: Network visualizations of relationships in psychometric data. *Journal of Statistical Software, 48*(4), 1–18.

Evanno, G., Regnaut, S., and Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, *14*(8), 2611–2620.

Felder, D. L., Staton, J. L., Journal, S., May, N., Felder, D. L., and Staton, J. L. (1994). Genetic differentiation in trans-Floridian species complexes of *Sesarma* and *Uca* (Decapoda: Brachyura). *Journal of Crustacean Biology*, *14*(2), 191–209.

Fourqurean, J. W., Willsie, A., Rose, C. D., and Rutten, L. M. (2001). Spatial and temporal pattern in seagrass community composition and productivity in south Florida. *Marine Biology*, *138*(2), 341–354.

Fourqurean, J. W., Durako, M. J., Hall, M. O., and Hefty, L. N. (2002). Seagrass distribution in South Florida: a multi-agency coordinated monitoring program. In J. W. Porter and K. G. Porter (Eds.), *The Everglades, Florida Bay, and Coral Reefs of the Florida Keys: An Ecosystem Source Book* (497–522). Boca Raton, Florida: CRC Press.

Fourqurean, J. W., Duarte, C. M., Kennedy, H., Marba, N., Holmer, M., Angel Mateo, M., Apostolaki, E. T., Kendrick, G. A., Krause-Jensen, D., McGlathery, K. J., and Serrano, O. (2012). Seagrass ecosystems as a globally significant carbon stock. *Nature Geoscience*, *5*(7), 505–509.

Hedrick, P. W. (2005). A standardized genetic differentiation measure. *Evolution; International Journal of Organic Evolution*, *59*(8), 1633–1638.

Jost, L. (2008). GST and its relatives do not measure differentiation. *Molecular Ecology*, *17*(18), 4015–4026.

Jost, L. (2009). D vs. GST: Response to Heller and Siegismund (2009) and Ryman and Leimar (2009). *Molecular Ecology*, *18*(10), 2088–2091.

Keenan, K., McGinnity, P., Cross, T. F., Crozier, W. W., and Prodöhl, P. A. (2013). diveRsity: An R package for the estimation of population genetics parameters and their associated errors. *Methods in Ecology and Evolution*, *4*, 782–788.

Kendall, M. S., Battista, T., and Hillis-Starr, Z. (2004). Long term expansion of a deep *Syringodium filiforme* meadow in St. Croix, US Virgin Islands: The potential role of hurricanes in the dispersal of seeds. *Aquatic Botany*, *78*(1), 15–25.

Kendrick, G. A., Duarte, C. M., and Marbà, N. (2005). Clonality in seagrasses, emergent properties and seagrass landscapes. *Marine Ecology Progress Series*, *290*, 291–296.

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., Mascaro I Vidol, O., Ooi, J. L. S., Orth, R. J., Rivers, D. O., Ruiz -Montoya, L., Sinclair, E. A., Stattin, J., van Dijk, K., and Verduin, J. J. (2012). The central role ofdispersal in the maintenance and persistence of seagrass populations. *Bioscience*, *62*(1), 56–65.

Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., and Mayrose, I. (2015). CLUMPAK: A program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources*.

Kurokochi, H., Matsuki, Y., Nakajima, Y., Fortes, M. D., Uy, W. H., Campos, W. L., Nadaoka, K., and Lian, C. (2015). A baseline for the genetic conservation of tropical seagrasses in the western North Pacific under the influence of the Kuroshio Current: the case of *Syringodium isoetifolium*. *Conservation Genetics*.

Leberg, P. L. (2002). Estimating allelic richnes: Effects of sample size and bottlenecks. *Molecular Ecology*, *11*, 2445–2449.

Lee, T., and Foighil, D. Ó. (2004). Hidden Floridian biodiversity: Mitochondrial and nuclear gene trees reveal four cryptic species within the scorched mussel, *Brachidontes exustus*, species complex. *Molecular Ecology*, *13*(11), 3527–3542.

Lee, T. N., and Smith, N. (2002). Volume transport variability through the Florida Keys tidal channels. *Continental Shelf Research*, *22*(9), 1361–1377.

Lee, T. N., and Williams, E. (1999). Mean distribution and seasonal variability of coastal currents and temperature in the Florida Keys with implications for larval recruitment. *Bulletin of Marine Science*, *64*(1), 35–56.

Mathews, L. M. (2006). Cryptic biodiversity and phylogeographical patterns in a snapping shrimp species complex. *Molecular Ecology*, *15*(13), 4049–4063.

Marbà, N., and Duarte, C. M. (1998). Rhizome elongation and seagrass clonal growth. *Marine Ecology Progress Series*, *174*, 269–280.

McMahon, K., van Dijk, K.-J., Ruiz-Montoya, L., Kendrick, G. A., Krauss, S. L., Waycott, M., Verduin, J., Lowe, R., Statton, J., Brown E., and Duarte, C. (2014). The movement ecology

of seagrasses. *Proceedings of the Royal Society B, 281*(1795), 20140878.

Meirmans, P. G., and Van Tienderen, P. H. (2004). GENOTYPE and GENODIVE: Two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes, 4*, 792–794.

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences of the United States of America, 70*(12), 3321–3323.

Orth, R. J., Carruthers, T. J. B., Dennison, W. C., Duarte, C. M., Fourqurean, J. W., Heck, K. L., Jr., Hughes, R. A., Kendrick, G. A., Kenworthy, J. W., Olyarnik, S., Short, F. T., Waycott, M., and Williams, S. L. (2006). A global crisis for seagrass ecosystems. *Bioscience*, *56*(12), 987-996.

Parks, J. C., and Werth, C. R. (1993). A study of spatial features of clones in a population of Bracken fern, *Pteridium aquilinum* (Dennstaedtiaceae). *American Journal of Botany, 80*(5), 537–544.

Peakall, R., and Smouse, P. E. (2006). GenAlEx 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes. 6*, 288–295.

Peakall, R., and Smouse, P. E. (2012). GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research–an update. *Bioinformatics, 28*, 2537–2539.

Pritchard, J. K., Stephens, M., and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*(2), 945–959.

Raymond, M., and Rousset, F. (1995). Genepop (version-1.2): Population-genetics software for exact tests and ecumenicism. *Journal of Heredity, 86*, 248–249.

Reusch, T. (2001). New markers-old questions: population genetics of seagrasses. *Marine Ecology Progress Series*, *211*(1), 261–274.

Rosenberg, N. A. (2004). DISTRUCT: A program for the graphical display of population structure. *Molecular Ecology Notes*, *4*(1), 137–138.

Rousset, F. (2008). GENEPOP'007: A complete re-implementation of the GENEPOP software for windows and linux. *Molecular Ecology Resources, 8*, 103-106.

Saghai-Maroof, M. A., Soliman, K. M., Jorgensen, R. A., and Allard R. W. (1984). Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. *Proceedings of the National Academy of Sciences of the United States of America*, *81*, 8014–8018.

Slatkin, M. (1985). Gene flow in natural populations. *Annual Review of Ecology, Evolution, and Systematics, 16,* 393–430.

Smith, N. P. (1994). Long-term Gulf-to-Atlantic transport through tidal channels in the Florida Keys. *Bulletin of Marine Science*, *54*(3), 602–609.

Stoeckel, S., Grange, J., Fernández-Manjarres, J. F., Bilger, I., Frascaria-Lacoste, N., and Mariette, S. (2006). Heterozygote excess in a self-incompatible and partially clonal forest tree species - *Prunus avium* L. *Molecular Ecology*, *15*, 2109–2118.

Sundqvist, L., Zackrisson, M., and Kleinhans, D. (2013). Directional genetic differentiation and asymmetric migration. Manuscript submitted for publication.

Weir, B. S., and Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution*, *38*(6), 1358–1370.

Williams, S. L. (1990). Experimental studies of Caribbean seagrass bed development. *Ecological Monographs*, *60*(4), 449–469.

Wright, S. (1951). The genetical structure of populations. *Annals of Eugenics, 15*, 323-354.

Yeung, C., and Lee, T. (2002). Larval transport and retention of the spiney lobster, *Panulirus argus*, in the coastal zone of the Florida Keys, USA. *Fisheries Oceanography*, *11*(5), 286–309.

Young, A. M., Torres, C., Mack, J. E., and Cunningham, C. W. (2002). Morphological and genetic evidence for vicariance and refugium in Atlantic and Gulf of Mexico populations of the hermit crab *Pagurus longicarpus*. *Marine Biology*, *140*(5), 1059–1066.

Tables

Table 2.1: Summary genetic statistics for all populations. Numeric codes are provided for each population. Sample size (*N*), number of unique multilocus genotypes (*G*), genotypic richness (*R*), total number of alleles (*A*), average number of alleles per locus (N_A), allelic richness per locus (A_R), observed heterozygosity (H_o), expected heterozygosity (H_e) and inbreeding coefficient (F_{IS}) are reported for each population. Standard error is included for H_o, H_e and F_{IS}. Values in bold indicate significant deviation from Hardy-Weinberg equilibrium at $p < 0.05$.

Population	**N**	**G**	**R**	**A**	**N_A**	**A_R**	**H_o**	**H_e**	**F_{IS}**
1 Carysfort	48	19	0.38	98	5.76	2.53	0.51 ± 0.08	0.43 ± 0.07	**-0.16 ± 0.04**
2 Elbow	45	28	0.61	106	6.24	2.53	0.47 ± 0.08	0.43 ± 0.07	**-0.08 ± 0.02**
3 Dixie	50	20	0.39	88	5.18	2.48	0.44 ± 0.08	0.39 ± 0.07	**-0.10 ± 0.05**
4 Conch	47	18	0.37	85	5.00	2.47	0.51 ± 0.09	0.40 ± 0.06	**-0.23 ± 0.05**
5 Davis	47	22	0.46	102	6.00	2.56	0.48 ± 0.07	0.44 ± 0.07	-0.09 ± 0.02
6 Molasses	48	22	0.45	93	5.47	2.55	0.49 ± 0.07	0.43 ± 0.07	**-0.13 ± 0.01**
7 Alligator	45	19	0.41	90	5.29	2.55	0.45 ± 0.07	0.42 ± 0.06	-0.08 ± 0.05
8 Tennessee	46	29	0.62	102	6.00	2.51	0.41 ± 0.07	0.40 ± 0.07	-0.04 ± 0.04
9 Sprigger	32	18	0.55	73	4.29	2.49	0.44 ± 0.07	0.38 ± 0.06	**-0.17 ± 0.03**
10 Sluiceway	48	22	0.45	66	3.88	2.43	0.42 ± 0.07	0.34 ± 0.06	**-0.20 ± 0.04**
11 Marathon	22	10	0.43	67	3.94	2.45	0.44 ± 0.09	0.42 ± 0.05	0.08 ± 0.14
12 Pigeon	43	17	0.38	75	4.41	2.44	0.38 ± 0.06	0.33 ± 0.05	**-0.12 ± 0.05**
13 Bahia Honda	48	15	0.30	67	3.94	2.43	0.39 ± 0.08	0.35 ± 0.06	-0.09 ± 0.07
14 Water	38	12	0.30	67	3.94	2.47	0.42 ± 0.07	0.38 ± 0.05	-0.10 ± 0.08
15 Crane	31	12	0.37	59	3.47	2.37	0.29 ± 0.06	0.28 ± 0.05	-0.05 ± 0.08
16 Key West	23	2	0.05	35	2.06	2.03	0.41 ± 0.12	0.21 ± 0.06	-0.94 ± 0.04
17 Tampa Bay	33	6	0.16	47	2.76	2.24	0.21 ± 0.07	0.18 ± 0.05	-0.09 ± 0.08
18 Florida Bay	123	6	0.04	54	3.18	2.36	0.41 ± 0.08	0.34 ± 0.05	-0.19 ± 0.10
19 Bahamas	44	19	0.42	69	4.06	2.37	0.32 ± 0.08	0.29 ± 0.07	-0.08 ± 0.05
20 Bermuda	107	20	0.18	67	3.94	2.39	0.26 ± 0.06	0.29 ± 0.06	**0.12 ± 0.07**

Table 2.2: Analysis of Molecular Variance (AMOVA) results. Standard deviation is reported for F-statistic values.

Source of Variation	**Nested in**	**Variance (%)**	**F-statistic**	**F-value**	**P-value**
Within Individual		0.924	F_{IT}	0.076 ± 0.023	
Among Individual	Population	-0.073	F_{IS}	-0.085 ± 0.02	1
Among Population		0.149	F_{ST}	0.149 ± 0.017	0.001

Figures

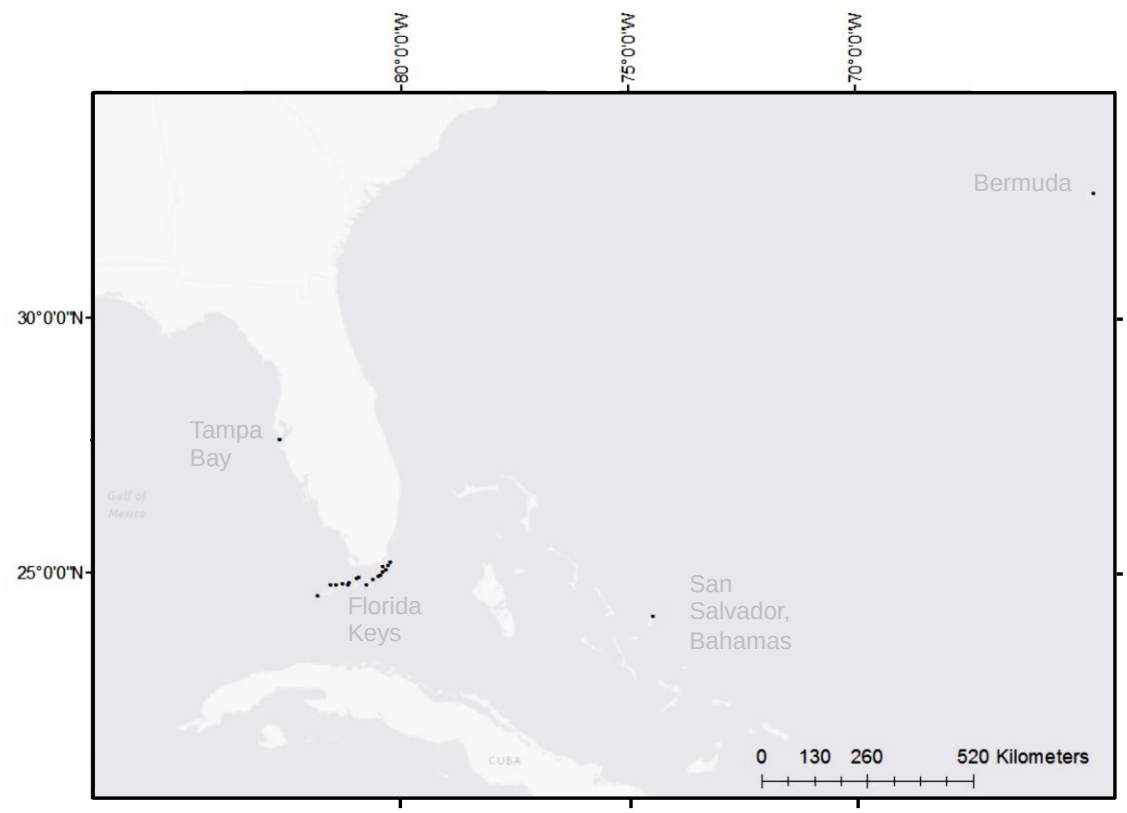


Figure 2.1: Site map of sampling areas.

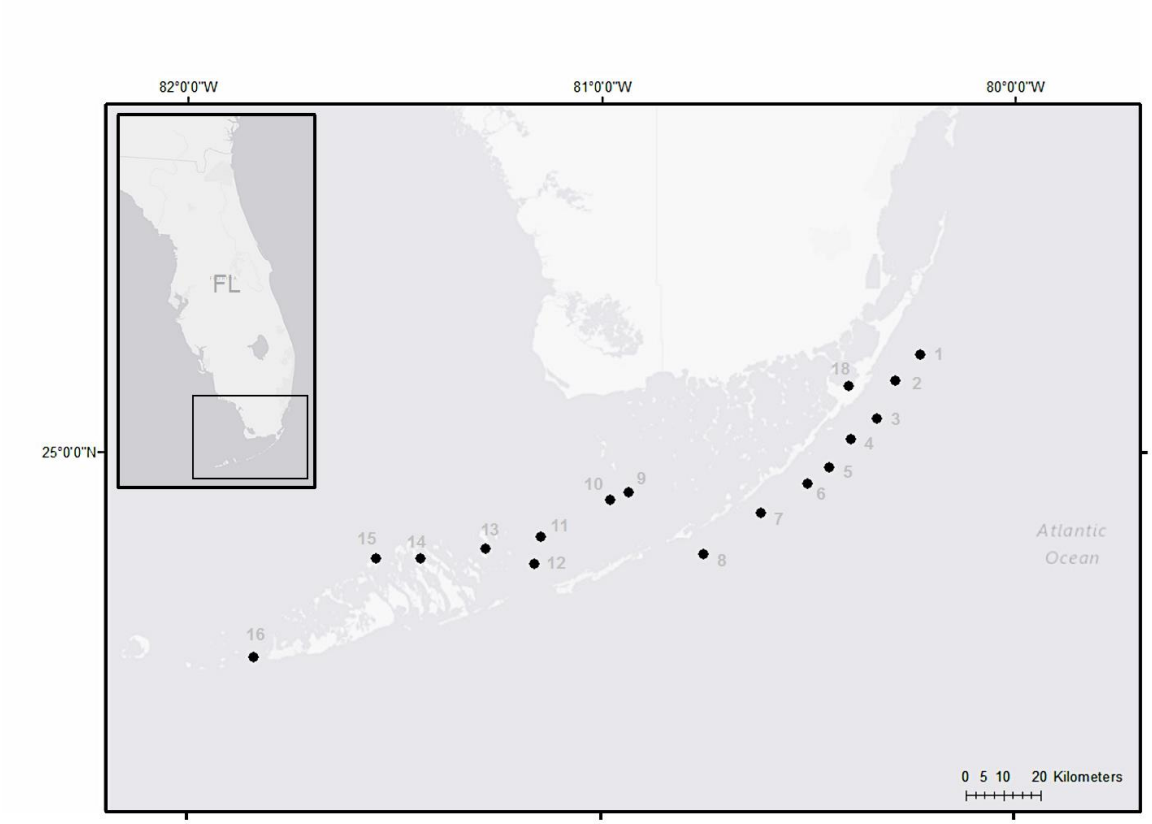


Figure 2.2 Site map of Florida Keys sample sites. Refer to Table 2.1 for population codes.

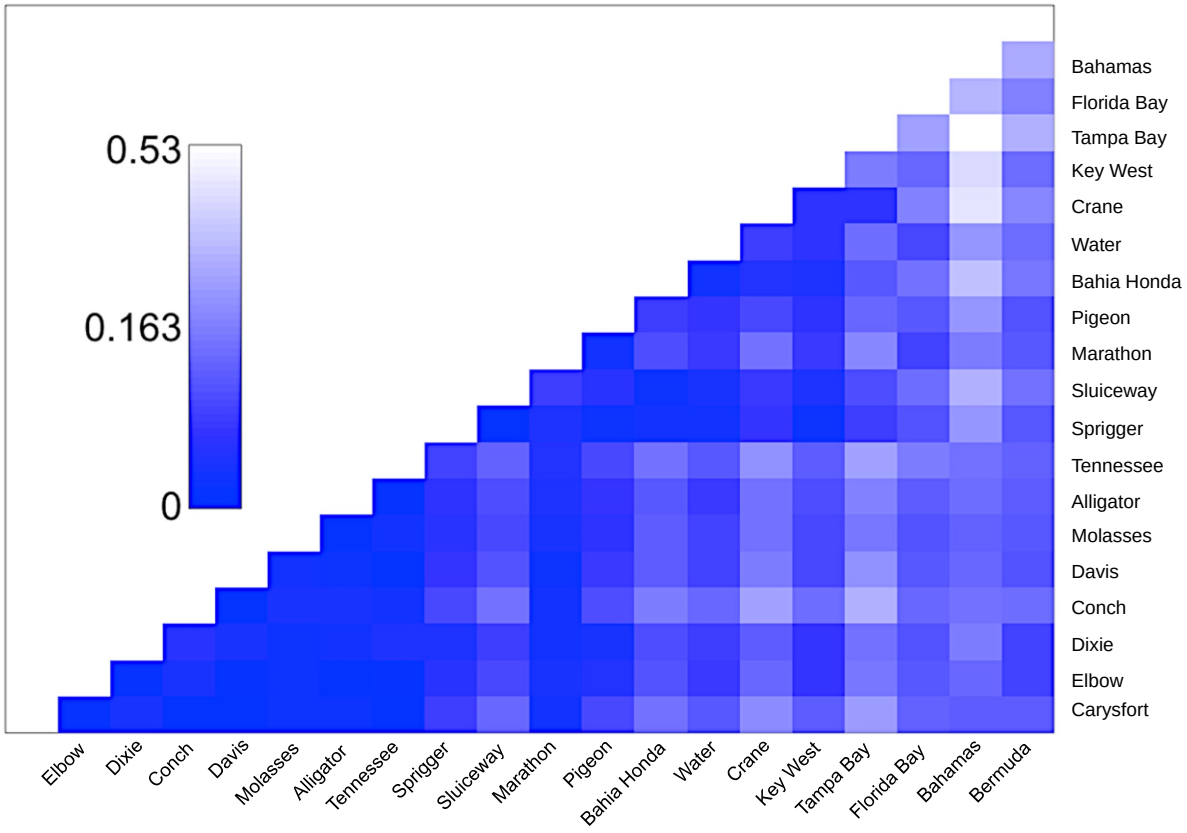


Figure 2.3: Pairwise F_{ST} values for all possible population combinations.

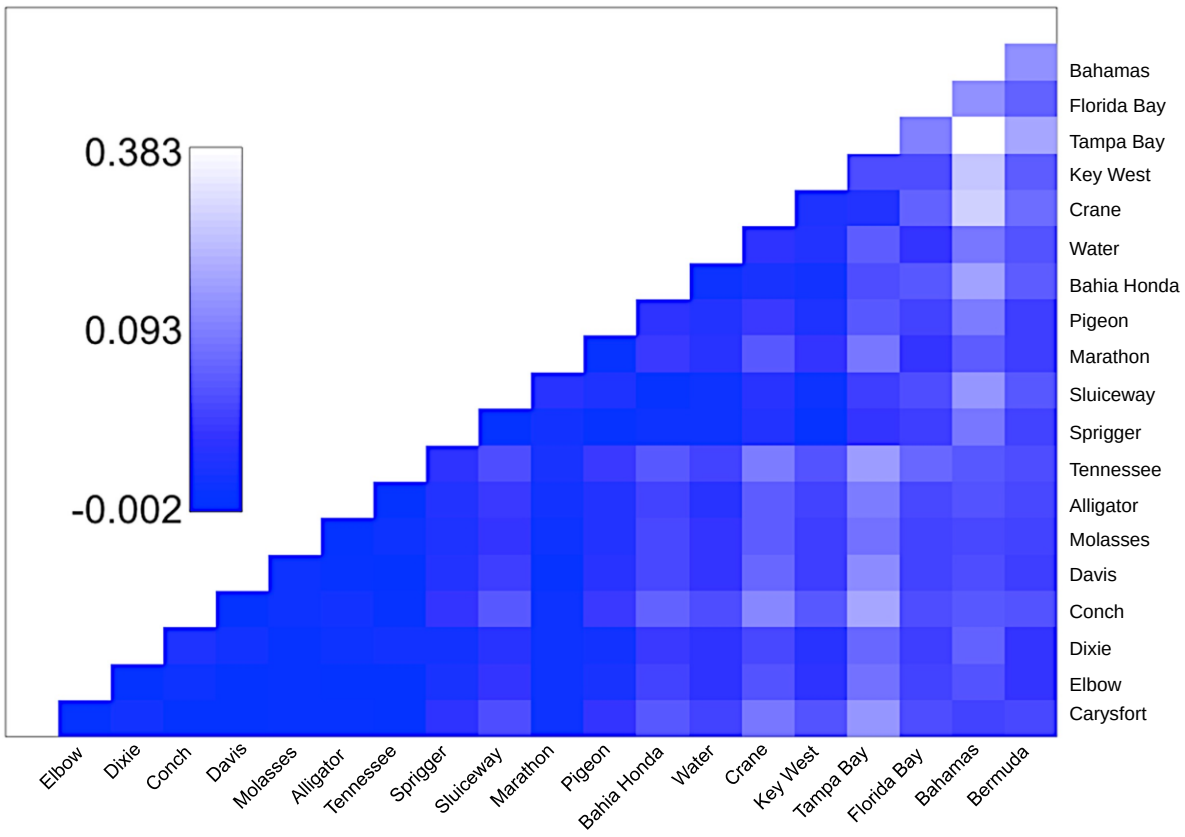


Figure 2.4: Pairwise G_{ST} values for all possible population combinations.

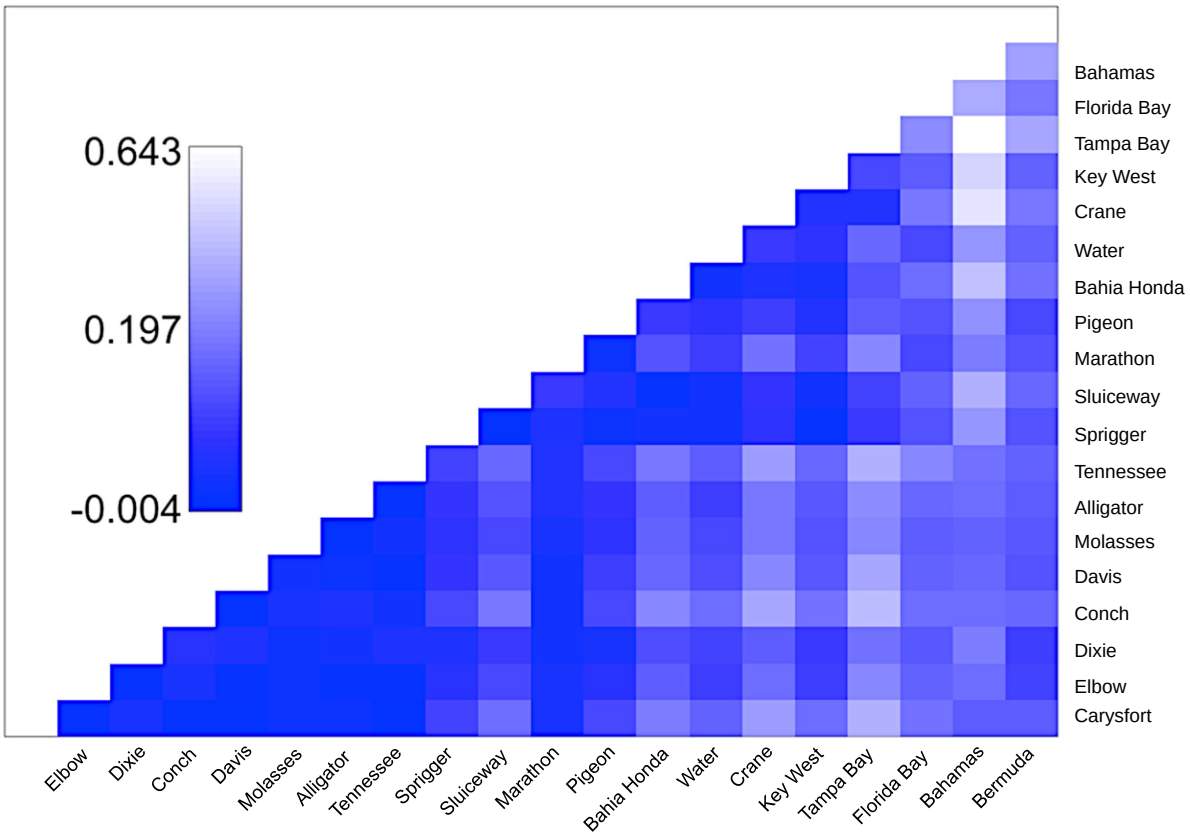


Figure 2.5: Pairwise G'_{ST} values for all possible population combinations.

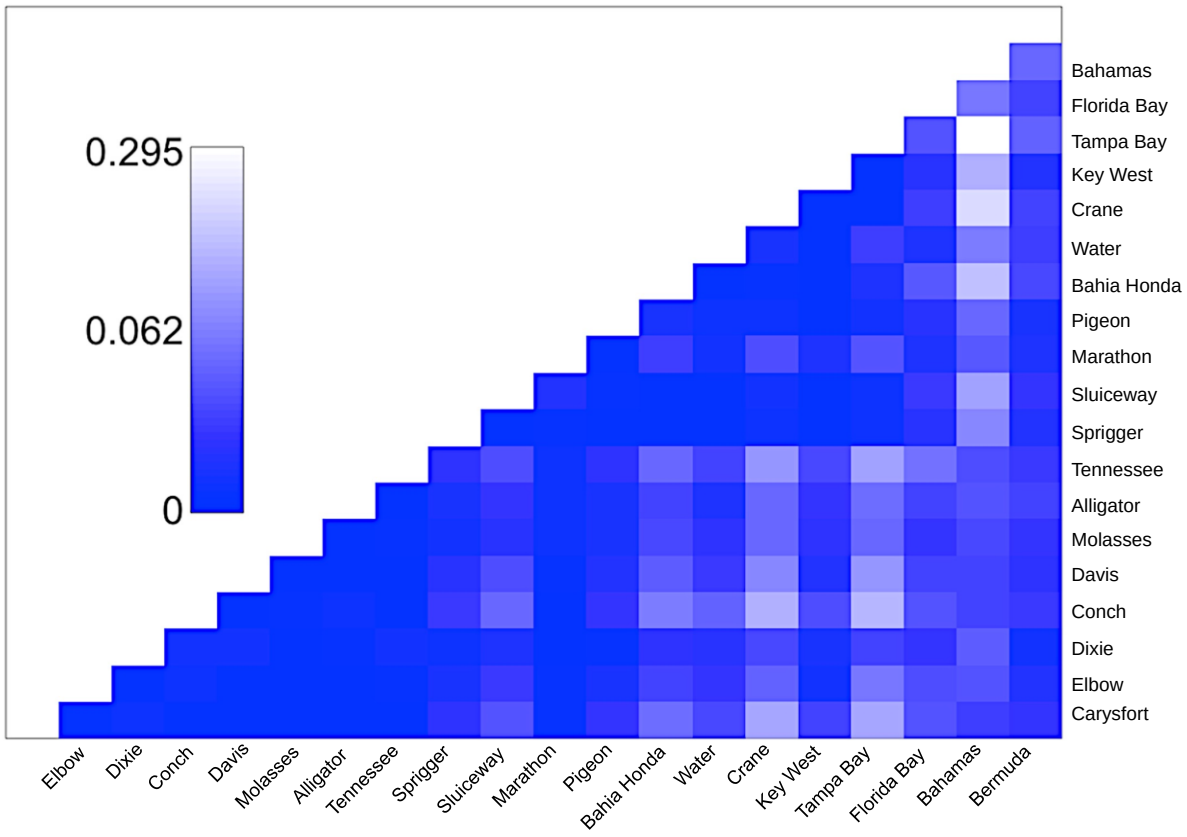


Figure 2.6: Pairwise Jost's *D* values for all possible population combinations.

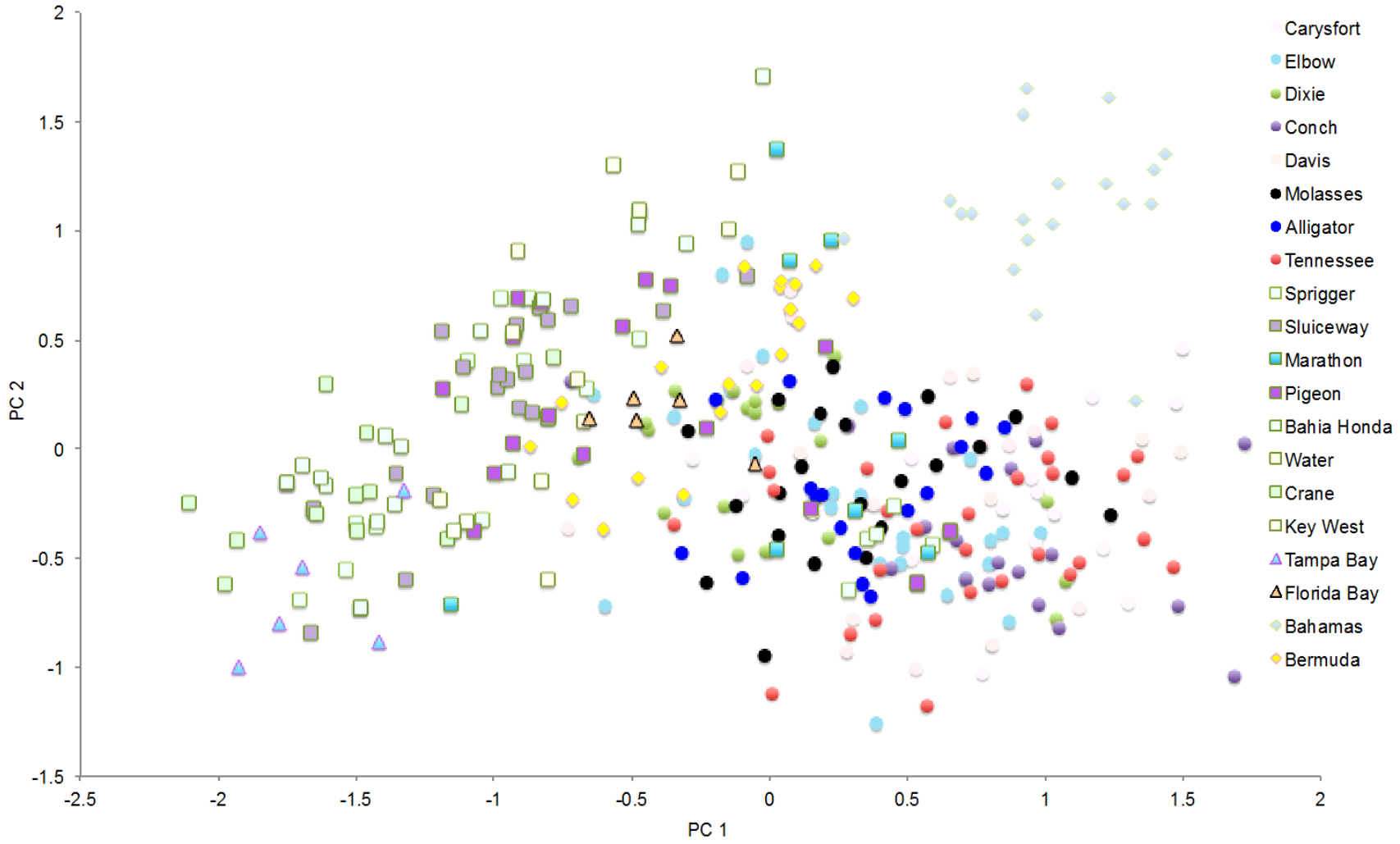


Figure 2.7: Results from the principal component analysis (PCA) plotted as axis loading values for the two principal component axes containing the greatest amount of variance, PC 1 (18.3% variance) and PC 2 (7.7% variance).

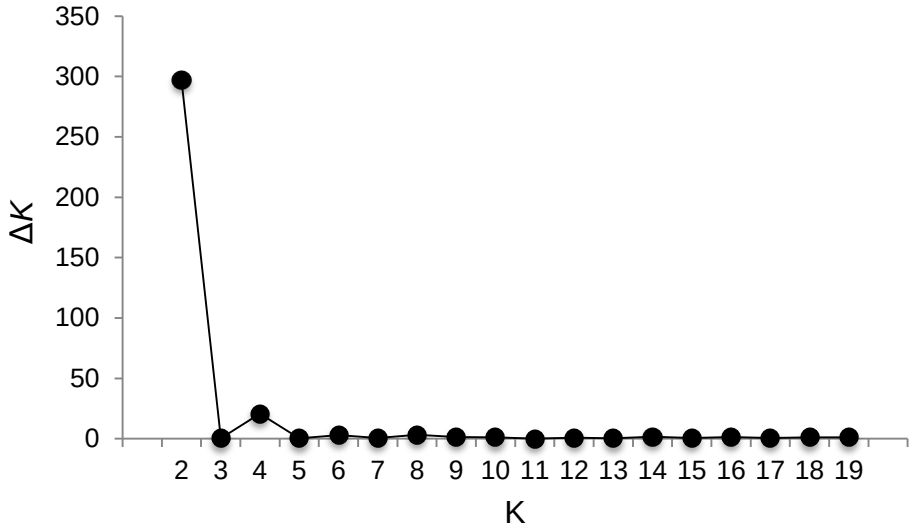


Figure 2.8: Δ*K* plot of STRUCTURE results.

The 20 sampled populations grouped into 2 (and possibly 4) distinct clusters based on 20 model runs following Evanno et al. (2005).

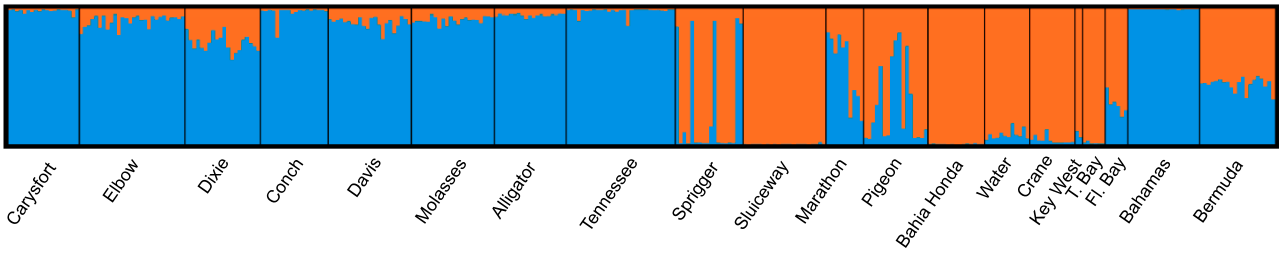


Figure 2.9: Results for STRUCTURE K = 2 cluster assignment. Populations are on the x-axis, and individual genotypes are represented as vertical bars within each population. Subgroups are depicted by color, and individual membership to a cluster is represented by color. Admixture was specified in the model, therefore membership to more than one cluster may occur.

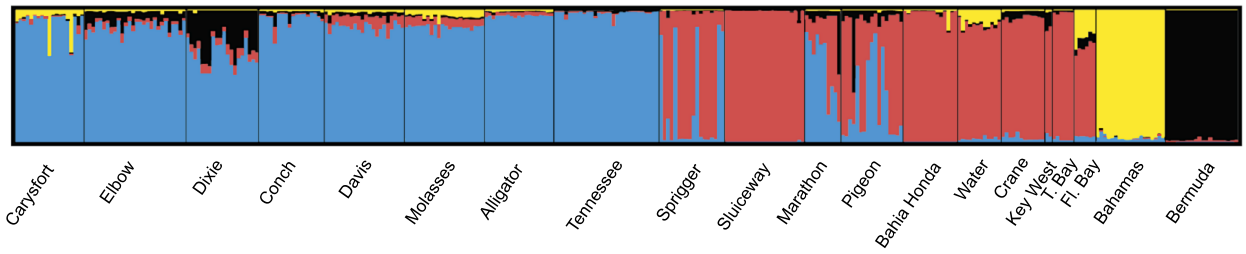


Figure 2.10: Results for STRUCTURE K = 4 cluster assignment. Populations are on the x-axis, and individual genotypes are represented as vertical bars within each population. Subgroups are depicted by color, and individual membership to a cluster is represented by color. Admixture was specified in the model, therefore membership to more than one cluster may occur.

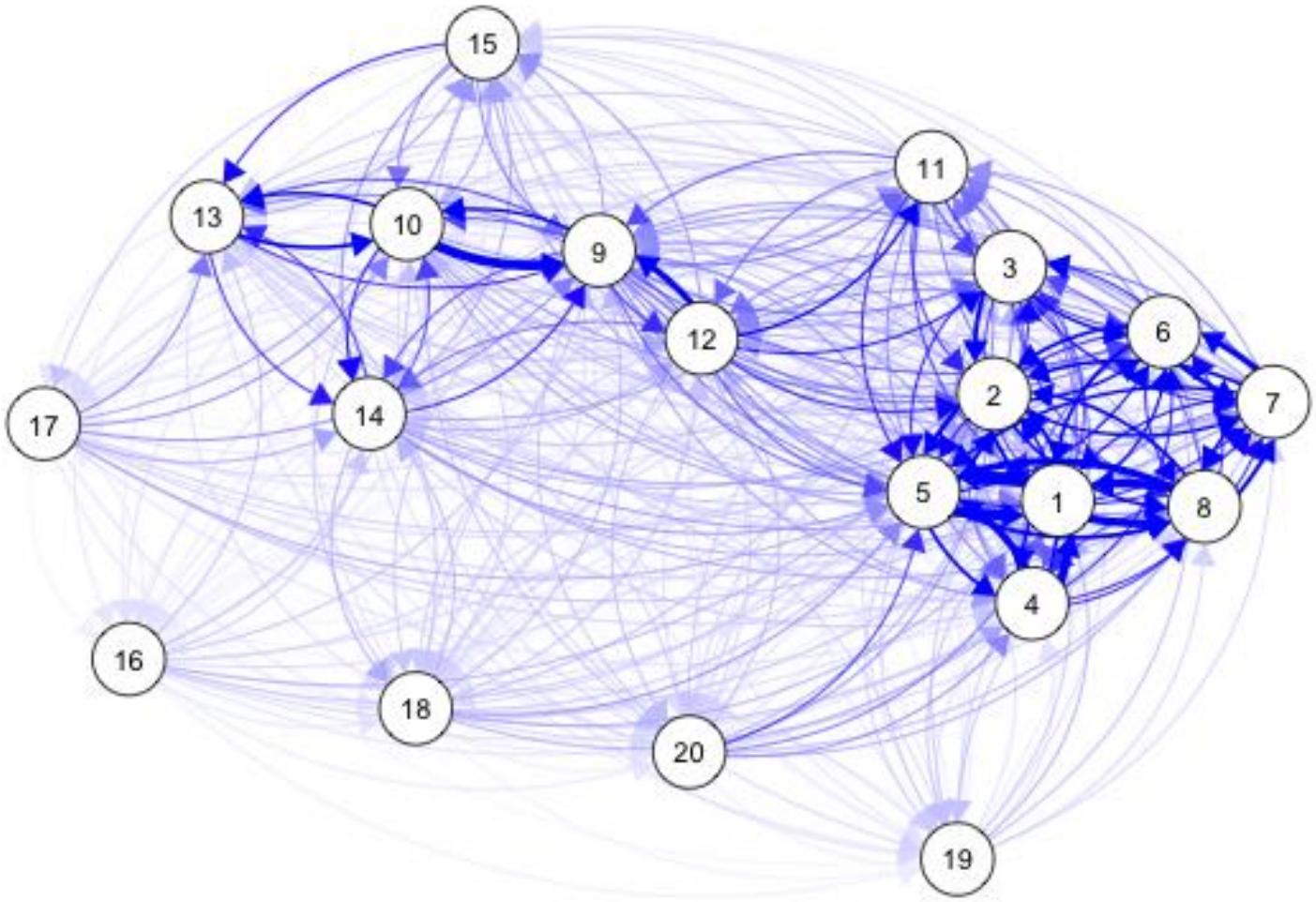


Figure 2.11: Relative amounts of gene flow between populations based on Alcala's N_m (2014) and Sundqvist's estimated direction of differentiation (manuscript in review). Nodes represent each population, and arrows indicate the direction of gene flow. Arrows are weighted according to values for N_m. Refer to Table 2.1 for population codes.

Summary and prospects for future research

In this thesis, 17 polymorphic microsatellite markers were developed for *Syringodifum filiforme* and are now available to interested parties via GenBank, the NIH genetic sequence database. These markers were used for the first time in the work reported here to analyze genetic diversity in *S. filiforme* in the Florida Keys and additional sites in South Florida and the greater northeastern subtropical Atlantic. Throughout the region sampled, moderate to high allelic variation and high levels of heterozygosity across most populations suggest *S. filiforme* maintains higher than expected genetic diversity at the individual and population levels. Population structure is clearly present, in which Gulf and Atlantic populations along the Florida Keys are genetically distinct. Gene flow patterns agree with this population structure and show that Atlantic populations are the most highly connected, which may reflect propagule movement via prevailing surface ocean currents.

These results help guide coastal managers in seagrass conservation efforts by illuminating genetic population boundaries. Though the Gulf and Atlantic populations are proximal, they represent separate evolutionary units. Managers may use this information to identify appropriate source material for restoration efforts. Depending on program goals, they may either avoid transplanting material across the established Gulf-Atlantic boundary to preserve regional genetic identity, or they may transplant across this boundary to enhance genetic diversity. And now that 'baseline' measurements of genetic diversity in *S. filiforme* have been established, future studies may attempt to understand the effects of disturbance (both natural and anthropogenic) and environmental variables such as pollution and habitat degradation on diversity in this seagrass through experimental and/or field surveys.

Appendix

Appendix.1: Exploratory analysis on the effect of seagrass bed nutrient status and distance from shore on clonality in Florida Bay, USA and Bailey's Bay, Bermuda

Motivation for study

In this subset of analyses, I attempted to investigate how environmental factors, specifically nutrient availability, impact the relative contribution of vegetative propagation and sexual reproduction, hereafter referred to as clonality, in *Syringodium filiforme* through genetic analysis. I chose to focus on nutrients because coastal eutrophication is a relevant environmental stressor in the tropical Atlantic. With all other environmental variables held constant, seagrass responses to nutrient enrichment range from enhanced growth in nutrient-limited environments to negative physiological responses resulting in growth inhibition (Burkholder et al., 2007). Suboptimal growth conditions related to high nutrients levels may favor the expansion and maintenance of more 'hearty' clones, i.e. older and larger clones, or clones with better-suited genotypes to local conditions (Honnay and Bossuyt, 2005). In a temperate seagrass, *Cymodocea nodosa,* Oliva et al. found reduced flowering in eutrophic waters, indicating poor water quality inhibits sexual reproduction (2014). Alternatively, stressful conditions enhanced seed production in a clonal herb, suggesting sexual reproduction can provide escape from less inhabitable environments (Kleunen et al., 2001). To test whether seagrass populations are dominated by fewer clones and less genetically diverse in areas with higher sediment and leaf nutrient contents, I proposed to study variation in clonality of *Syringodium filiforme* across nutrients gradients in two shallow bays: Florida Bay, USA, and Bailey's Bay, Bermuda.

Methods

In Florida Bay (25° 8' 45.0132" N, 80° 24' 31.1076" W), three sites were selected along the shoreline and three sites were selected approximately 1 km offshore in the bay interior (Site map A.1). In Bailey's Bay (32° 20' 57.588" N, 64° 43' 26.9754" W), three sites were selected along the shoreline and two sites were selected approximately 200 m offshore (Site map A.2). At each site, 24 leaves were collected from ramets (spaced ~1.5 m apart) in a ~ 70 m^2 sampling area, depending on meadow morphology and seagrass distribution, for genetic analysis. Genotypic richness for each site was determined following methods described in Chapter 2. Several leaves were collected throughout the sampling area and then pooled into a single sample per site for nutrients analysis. Within this same area, 6 sediment cores were collected in each Florida Bay site using a 30 cc syringe (volume = 33 cm^3) for sediment nutrient analyses.

Sediment organic matter content was determined using the loss on ignition method. Carbon (C) and nitrogen (N) contents were determined on a Carlo Erba Elemental Analyzer. For sediment C content, a correction for inorganic carbon was applied. Total phosphorus (P) was measured by flow injection analysis on QuickChem® 8500 Automated Ion Analyzer following a dry oxidation, hot acid digestion method described in Fourqurean et al., 1992.

To analyze differences in sediment nutrients between shoreline and bay interior sites, and between individual sites, nested analyses of variance (ANOVA) were performed separately for percent organic matter, carbon, nitrogen and phosphorus.

Results and lessons learned

In Florida Bay, a gradient was successfully established between shoreline and bay interior sites for sediment organic matter, carbon and phosphorus content (Table A.1 and A.2). Sediment organic matter and carbon content were significantly higher in interior bay sites, while

phosphorus content was significantly higher in shoreline sediments (Figures A.3, A.4 and A.6). For these sediment measurements, there were also significant interactions between position and site, indicating that though there are nutrient differences between shoreline and bay interior environments, sediment nutrients are highly variable within each site, even at small spatial scales. Sediment nitrogen content did not vary significantly between bay interior and shoreline sites (Figure A.5).

Leaf N and P content in Florida Bay ranged from 1.52 to 2.07 %, and 0.09 to 0.11%, respectively, which are consistent with median values reported for seagrass species globally, and mean values reported for *S. filiforme* in South Florida (Duarte, 1990; Campbell and Fourqurean, 2009). Leaf tissue N:P was generally higher in the bay interior seagrass patches than in the shoreline patches, indicating the bay interior beds were more phosphorus-limited (Figure A.7). Genotypic richness was low overall, with only 5 genetically unique individuals detected among 123 individual genotypes. Two shoreline patches and one bay interior patch were comprised of single clones. Most of the clones were found in multiple patches, and one clone was found in both the bay interior and along the shoreline, spanning a distance of at least 1 km. The dominance of a small number of clones across a wide area might indicate there is limited gene flow and recruitment capability from external populations in this small, enclosed bay.

In Bailey's Bay, leaf N and P content ranged from 1.31 to 1.58 %, and 0.10 to 0.17%, respectively, which are slightly below median values reported for seagrass species globally (Duarte, 1990). Leaf N:P ratios were slightly lower in shoreline seagrass patches than in offshore patches (Figure A.8). Genotypic richness was highly variable between sites, with two shoreline patches and one offshore patch being dominated by a single clone, whereas several genetically distinct individuals were present in the remaining shoreline and offshore patches. Interestingly,

no genotypes were shared across sites, even though the maximum distance between patches was only ~ 280 m. Bailey's Bay is only partially enclosed by small islands at its mouth, and is otherwise open to the ocean, perhaps enhancing potential for propagule exchange and gene flow with adjacent *S. filiforme* meadows.

This study did not serve its original intention to determine the impact of nutrient levels on seagrass reproductive mode. In Florida Bay, the shoreline sediments were indeed more enriched with phosphorus, and leaf N:P in *S. filiforme* along the shore was lower, suggesting phosphorus was more available at these sites due to water column nutrient levels or sediment phosphate-binding characteristics (Short et al., 1990; Long et al., 2008). However, there were no clear patterns in clonality revealed between shoreline and bay interior habitats because there was such low genotypic diversity in the entire area sampled. In Bailey's Bay, there was evidence of slight phosphorus enrichment along the shoreline, but also no clear patterns in genotypic diversity. The dominance of single clones in certain patches adjacent to patches with high numbers of genetically individuals suggests that recruitment processes may be highly specific to patch-level environmental conditions and demographic stochasticity in flowering rates, pollination success and propagule establishment. Even if patterns in reproductive strategy had emerged between the shoreline and bay interior sites, nutrients might not have been the only factor affecting seagrass growth conditions, as light levels varied between the two environments in Florida Bay. Also, disturbance from boat activity was not accounted for in either study area.

In order to satisfactorily understand how seagrass beds respond to elevated nutrient levels in isolation, investigators should manipulate fertilization rates in situ and then compare changes in clone composition annually, as seagrass meadow responses to nutrient addition have been observed on inter-annual to decadal time scales (Ferdie and Fourqurean, 2004). Sites should be

selected to ensure equal access to propagules and similar environmental conditions; therefore I recommend selecting sites within the same sub-basin so that biogeochemical and hydrological properties are relatively constant across treatments. In order to account for variation in meadow clone composition even at small spatial scales, sampling should be conducted across several replicate seagrass patches within each treatment area. To determine the spatial scale of sampling, I suggest sampling initially within 50 m x 50 m areas (n = 50, with ramets spaced at least 5 m apart) to gauge the extent of clonality and to adjust accordingly to develop the most appropriate sampling design. I recommend avoiding basins dominated by few clones, as investigators will need to sample over larger areas, while smaller sampling areas will be sufficient in basins with greater initial variation in clonality.

The work done here is preliminary in nature, but sampling on multiple spatial scales did produce the finding that genotypic diversity was very low (compared to other populations studied in Chapter 2) in the enclosed, northeastern region of Florida Bay, similar to findings that isolated or peripheral seagrass populations exhibit lower genetic diversity (Billingham et al., 2003). This study also provides 'baseline' data on how, but not yet why, clonality in *S. filiforme* varies across basins.

Appendix.1 Literature Cited:

Billingham, M. R., Reusch, T. B. H., Alberto, F., and Serrão, E. A. (2003). Is asexual reproduction more important at geographical limits? A genetic study of the seagrass *Zostera marina* in the Ria Formosa, Portugal. *Marine Ecology Progress Series*, *265*, 77–83.

Burkholder, J. M., Tomasko, D. A., and Touchette, B. W. (2007). Seagrasses and eutrophication. *Journal of Experimental Marine Biology and Ecology*, *350*, 46–72.

Campbell, J. E., and Fourqurean, J. W. (2009). Interspecific variation in the elemental and stable

isotope content of seagrasses in South Florida. *Marine Ecology Progress Series*, *387*, 109–123.

Duarte, C. (1990). Seagrass nutrient content . *Marine Ecology Progress Series*, *67*, 201–207.

Ferdie, M., and Fourqurean, J. W. (2004). Responses of seagrass communities to fertilization along a gradient of relative availability of nitrogen and phosphorus in a carbonate environment. *Limnology and Oceanography*, *49*(6), 2082–2094.

Fourqurean, J. W., and Zieman, J. C. (1992). Phosphorus limitation of primary production in Florida Bay: Evidence from C: N: P ratios of the dominant seagrass *Thalassia testudinum*. *Limnology and Oceanography*, *37*(1), 162–171.

Honnay, O., and Bossuyt, B. (2005). Prolonged clonal growth: Escape route or route to extinction? *Oikos*, *108*, 427–432.

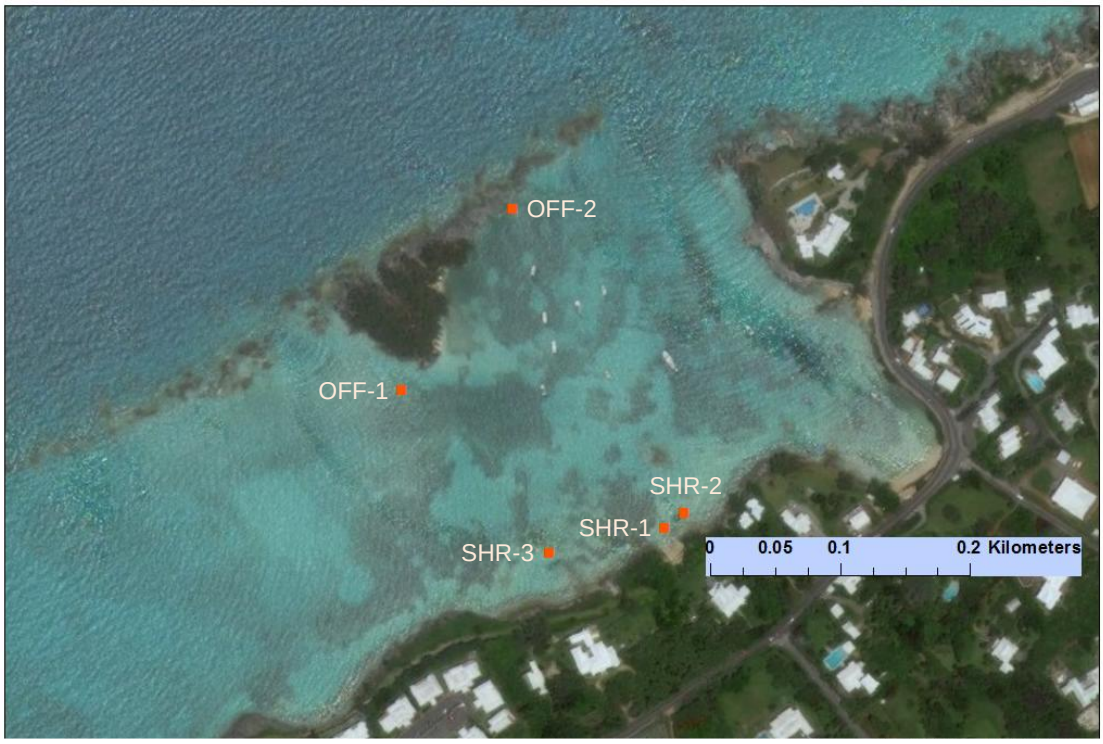
Kleunen, M. Van, Fischer, M., and Schmid, B. (2001). Effects of intraspecific competition on size variation and reproductive allocation in a clonal plant. *Oikos*, *94*, 515–524.

Long, M. H., McGlathery, K. J., Zieman, J. C., and Berg, P. (2008). The role of organic acid exudates in liberating phosphorus from seagrass-vegetated carbonate sediments. *Limnology and Oceanography*, *53*(6), 2616–2626.

Oliva, S., Romero, J., and Marta, P. E. (2014). Reproductive strategies and isolation-by-demography in a marine clonal plant along an eutrophication gradient. *Molecular Ecology*, *23*, 5698–5711.

Short, F., Dennison, W., and Capone, D. (1990). Phosphorus-limited growth of the tropical seagrass *Syringodium filiforme* in carbonate sediments. *Marine Ecology Progress Series*, *62*, 169–174.

Site map A.1: Blackwater Sound, Florida Bay.



Site map A.2: Bailey's Bay, Bermuda.

Table A.1: *N*, the number of observations or cores, mean organic matter, carbon, nitrogen and phosphorus contents, and standard error values reported for Florida Bay sediments with sites grouped by location.

Organic Matter	**N**	**Mean (%)**	**Standard Error**
Interior	18	10.15	0.65
Shoreline	17	5.11	0.49
Carbon	**N**	**Mean (%)**	**Standard Error**
Interior	16	6.53	0.59
Shoreline	18	4.30	0.66
Nitrogen	**N**	**Mean (%)**	**Standard Error**
Interior	16	0.44	0.04
Shoreline	18	0.28	0.07
Phosphorus	**N**	**Mean (%)**	**Standard Error**
Interior	16	0.01	0.001
Shoreline	18	0.11	0.014

Table A.2: Nested analysis of variance results for Florida Bay sediment organic matter, carbon, nitrogen and phosphorus with individual sites nested with position relative to shore (interior or shoreline).

Organic Matter	**d*f***	**MS**	***F***	***P***
Position	1	221.4	44.8	<0.001
Position*Site	4	14.1	2.8	0.042
Error	29	5.0		
Carbon	**d*f***	**MS**	***F***	***P***
Position	1	42.2	10.5	0.003
Position*Site	4	26.6	6.6	<0.001
Error	28	4.0		
Nitrogen	**d*f***	**MS**	***F***	***P***
Position	1	0.2	3.4	0.075
Position*Site	4	0.1	1.7	0.176
Error	28	0.1		
Phosphorus	**d*f***	**MS**	***F***	***P***
Position	1	0.1	117.6	<0.001
Position*Site	4	0.0	16.6	<0.001
Error	28	0.0		

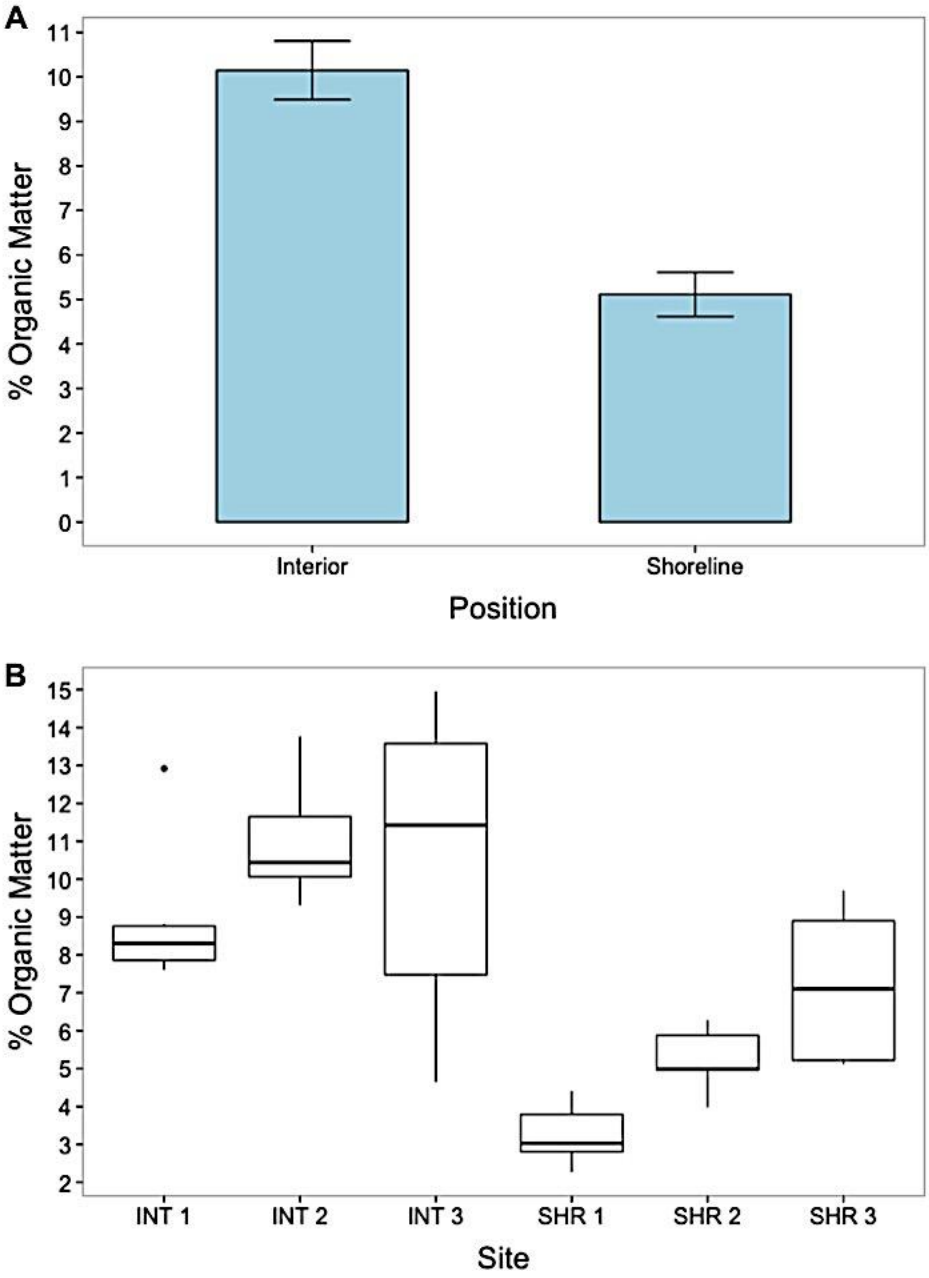


Figure A.3: Organic matter content A) by position (error bars = standard error of the mean) and B) by individual site.

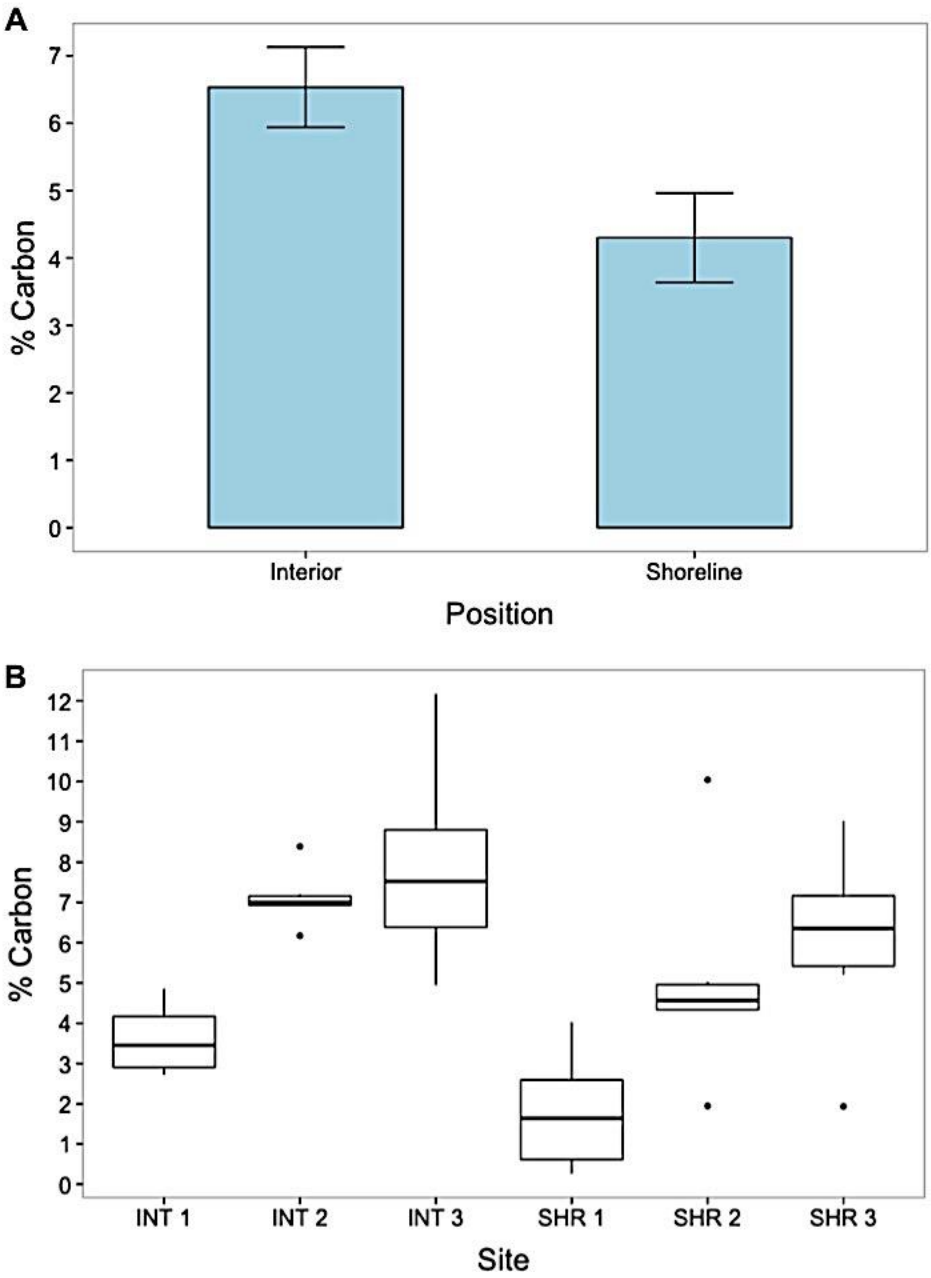


Figure A.4: Carbon content A) by position (error bars = standard error of the mean) and B) by individual sites.

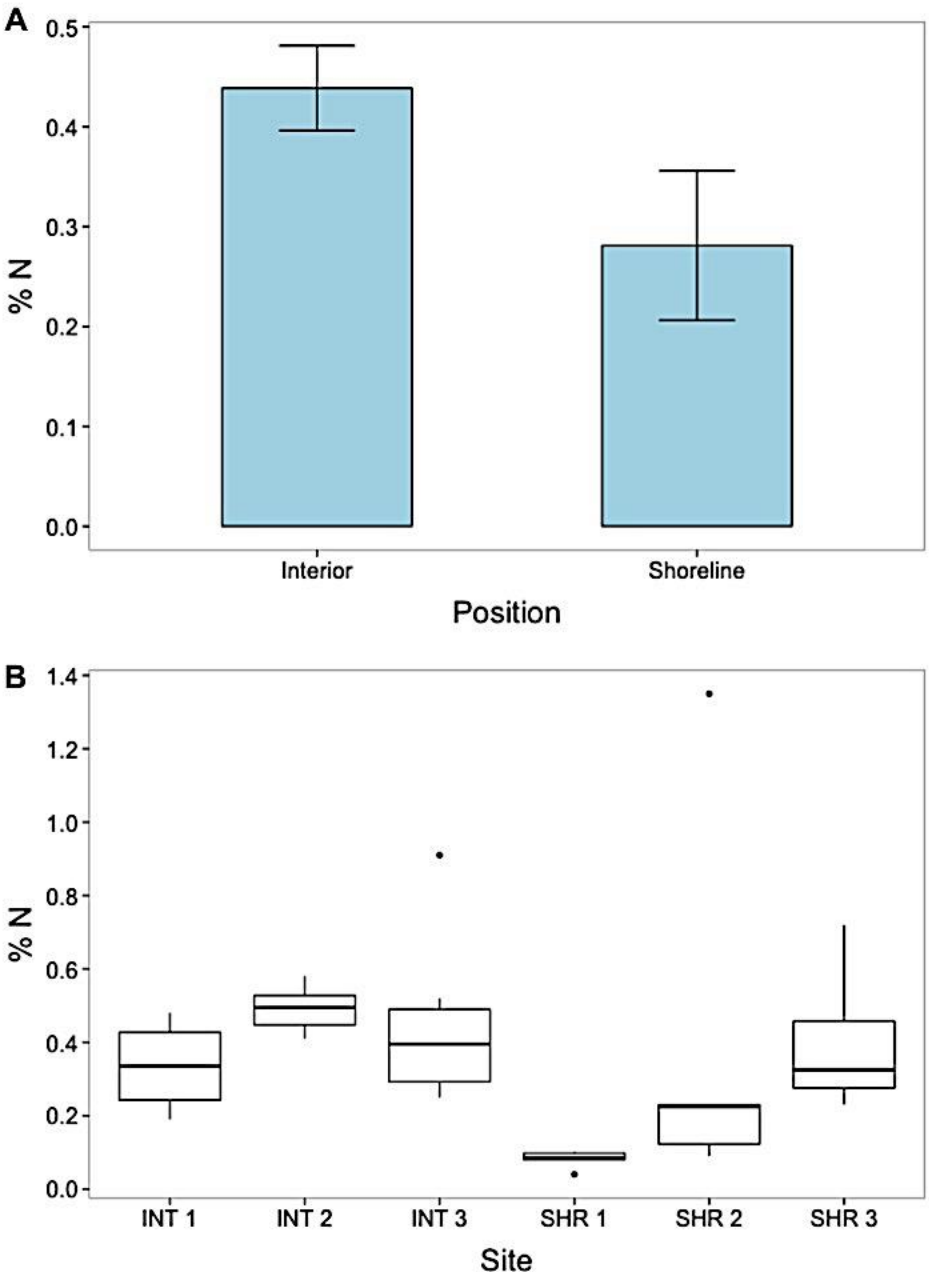


Figure A.5: Nitrogen content A) by position (error bars = standard error of the mean) and B) by individual sites.

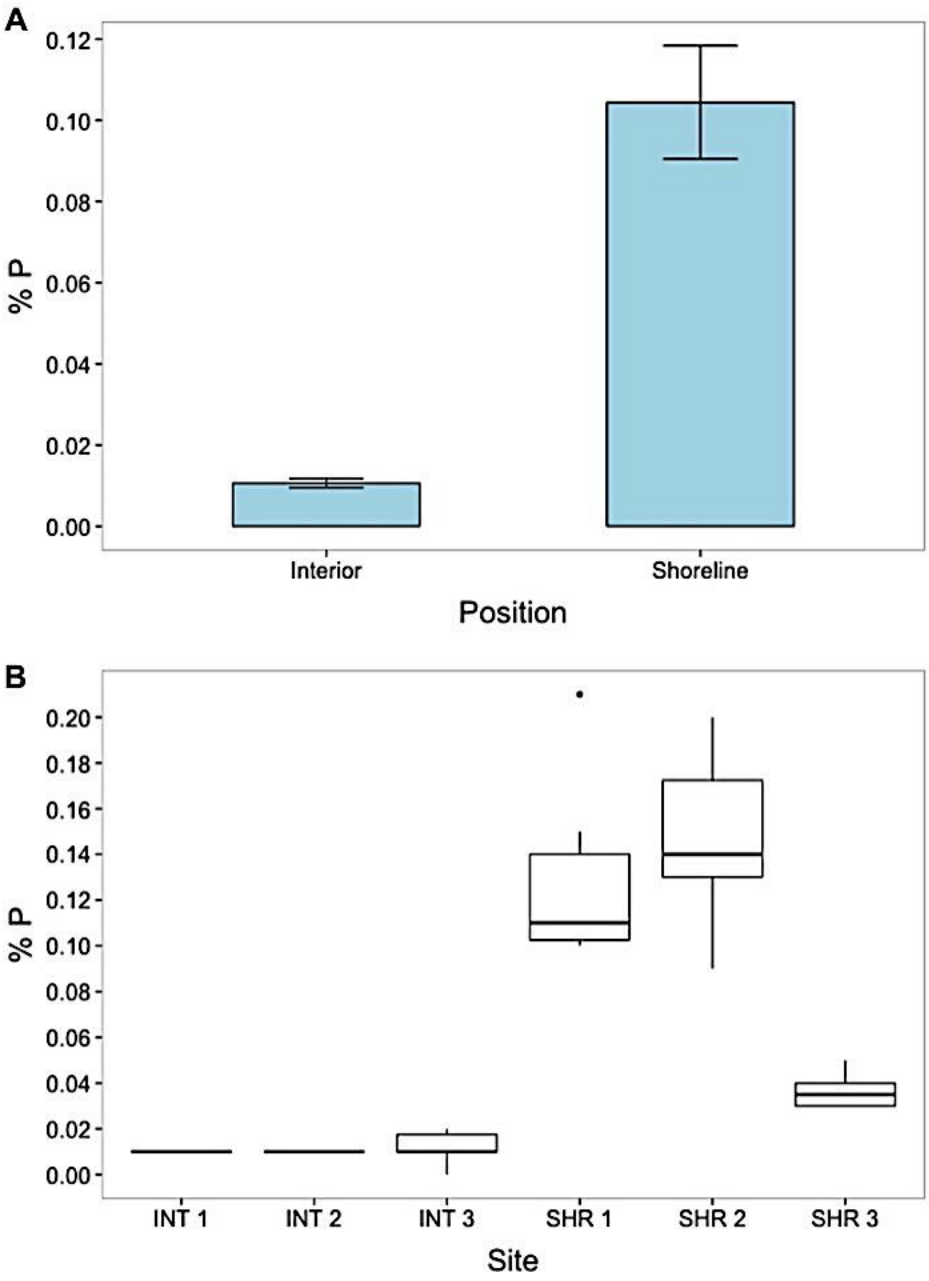


Figure A.6: Phosphorus content A) by position (error bars = standard error of the mean) and B) by individual sites.

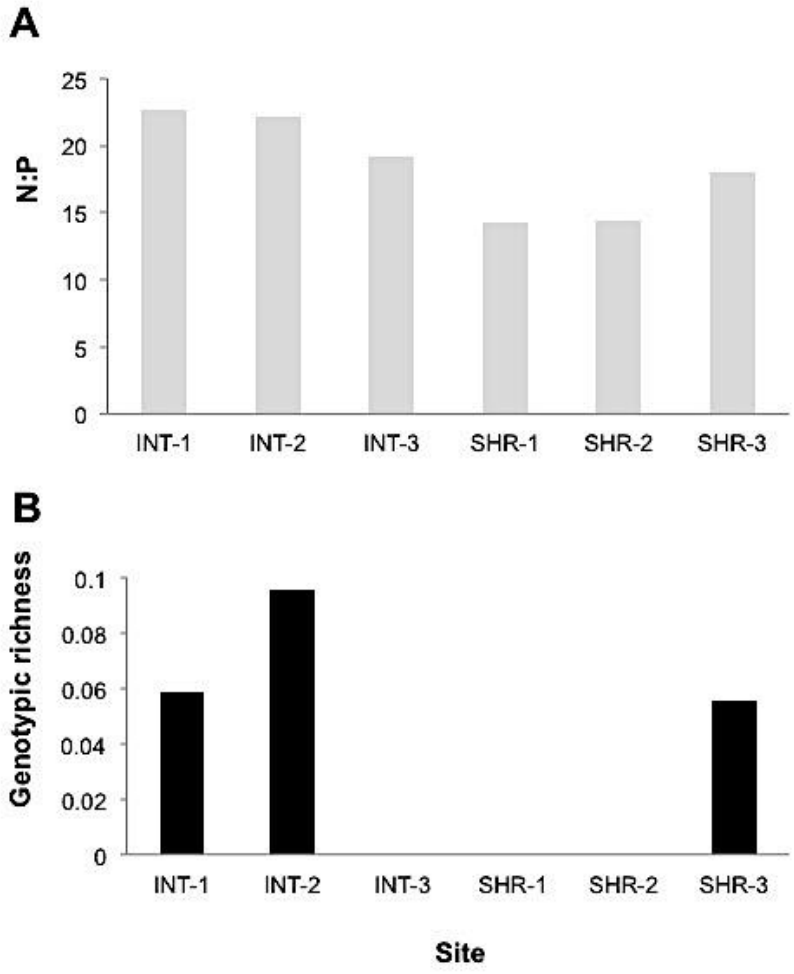


Figure A.7: Florida Bay A) leaf N:P and B) genotypic richness by site.

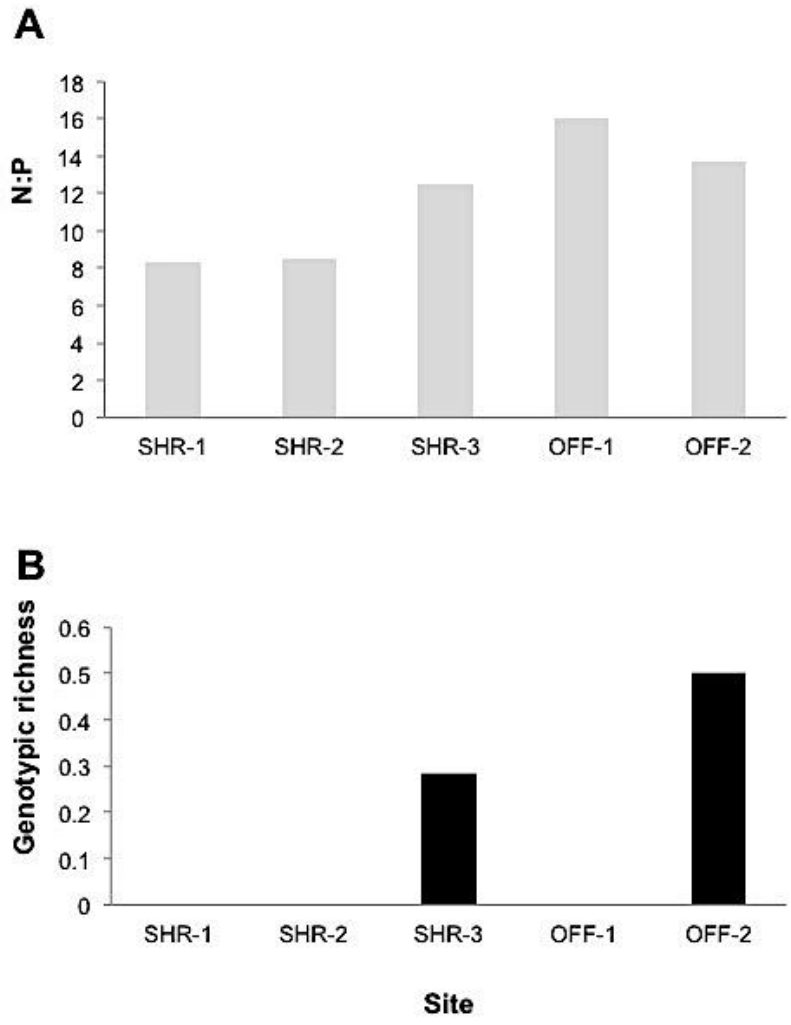


Figure A.8: Bailey's Bay A) leaf N:P and B) genotypic richness by site.