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Spatiotemporal variation in patterns of genetic diversity, genetic structure, and life history across *Zostera marina* meadows in North Carolina, USA

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ABSTRACT: Seagrass meadows are some of the most productive marine plant ecosystems in the world, yet their loss continues on a global scale. *Zostera marina*, an ecologically important foundation species, reproduces both sexually and asexually, yielding different levels of genetic diversity throughout its range, which in turn can influence resistance to, and resilience from, environmental disturbances. Understanding the genetic structure and diversity of these populations, and how they fluctuate over space and time, will aid in the conservation and management of seagrasses in an environment where the effects of climate change are likely to be chronic. Using microsatellite data, we examined spatiotemporal genetic structure and genetic diversity of *Z. marina* over a 10 yr period at 2 sites in North Carolina (USA), the southern limit of its geographic range in the Western Atlantic. Both meadows were genetically diverse, with very little spatial genetic structure existing within and between sites, and relative temporal stability between decadal time points. Within-site kin structure was more pronounced in the earlier years, and allelic richness increased over time at both sites, suggesting an increase in sexual reproduction, potentially in response to thermal stress. Despite the genetic similarities between sites, life history strategies showed phenotypic plasticity, and several metrics of genetic diversity were associated with meadow health. These findings point to the adaptive potential of *Z. marina* and provide promising insight into how the species will perform as the effects of climate change continue to amplify over the next century.

KEY WORDS: Genetic diversity · Temporal stability · Climate change · *Zostera marina*

1. INTRODUCTION

Zostera marina is a clonal species of seagrass that forms extensive meadows in subtidal and intertidal coastal regions (Moore & Short 2007). It is commonly found in both estuarine and coastal areas throughout the temperate Northern Hemisphere where it is at the base of diverse food webs (Duffy 2006, Moore & Short 2007, Ehlers et al. 2008, Zimmerman et al. 2017). As a dominant foundation species, *Z. marina* forms some of the most productive marine plant ecosystems in the world (Thayer et al. 1984) and provides numerous critical ecological services such as nutrient cycling, carbon sequestration, and sediment stabilization (Orth et al. 2006, Ehlers et al. 2008, Waycott et al. 2009). However, anthropogenically induced climate change is negatively impacting ecosystems around the globe, and threats associated with climate change are expected to amplify throughout the next century (IPCC 2014). Threats to *Z. marina* meadows are particularly critical at their southern limit in North Carolina (USA), where water temperatures are already approaching

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physiological thresholds (Jarvis et al. 2012) and competition with subtropical species, such as *Halodule wrightii*, is present (Thayer et al. 1984, Micheli et al. 2008). Understanding how to mitigate losses and protect these meadows against disturbances is therefore crucial.

Several studies have shown that genetic and genotypic diversity can increase the resistance and resilience of seagrass species to biotic or abiotic environmental disturbances, as well as promote population growth, abundance, and the diversity of other species within an ecosystem (Duffy 2006, Procaccini et al. 2007, Massa et al. 2013, Evans et al. 2017). As a clonal species, *Z. marina* meadows can produce varying levels of genetic diversity over time and space, and the ability of seagrass species to persist and thrive in future environmental conditions depends heavily on their genetic adaptability (Ehlers et al. 2008, Salo & Gustafsson 2016). For example, genetically distinct individuals differ in important morphological traits related to valuable ecosystem functions, such as nutrient uptake rates and biomass (Hughes et al. 2009a); thus, more genetically diverse seagrass meadows may have a wider range of these traits enabling them to persist in the face of, and adapt to, climate change (Salo et al. 2015).

Spatiotemporal variability in the genetic structure of *Z. marina* meadows can be caused by changes in life history, seed dispersal mechanisms, habitat characteristics, or competitive interactions (Kamel et al. 2012, Reynolds et al. 2017). Seed dispersal, in particular, is a major driver of gene flow between populations, mainly through the rafting of seeds (Hosokawa et al. 2015). Although the dispersal potential of *Z. marina* can be high, with distances of 20–300 km reported (Kendrick et al. 2012, Reynolds et al. 2013, Hosokawa et al. 2015), realized dispersal distances of seeds and pollen can be relatively short (within the range of ≤10 m) as they are negatively buoyant and sink upon release (Reusch et al. 2000, Kendrick et al. 2012). Nevertheless, seed dispersal is instrumental in preserving the genetic diversity of meadows by introducing novel genotypes into the population (Kendrick et al. 2012, Hays et al. 2021a).

Life history strategies can also play an important role in shaping genetic diversity, as *Z. marina* can reproduce both sexually and asexually. When the predominant mode of reproduction is asexual, within-population genetic diversity diminishes and among-population substructure is enhanced (Reusch et al. 2000). Although the perennial form is the most common life history strategy in *Z. marina,* a shift to mixed-annual and annual life history strategies is also observed in seagrass populations and is often indicative of a stressful environment (Jarvis et al. 2012, Johnson et al. 2020). In environments where stress is intense, such as the high water temperatures observed in North Carolina, sexual reproduction may be critical for meadow maintenance, as the majority of adult shoots die off during the height of summer, and sexually recruited seedlings replace the empty meadow patches between remnant surviving vegetative shoots (Jarvis et al. 2012). If sexual reproduction prevails over asexual reproduction in stressful habitats, then genetic diversity will consequently increase as new genotypes are produced.

The distribution of genetic diversity in eelgrass is highly variable: connectivity can occur between populations separated by up to 150 km (Olsen et al. 2004, Muñiz-Salazar et al. 2005, Källström et al. 2008). Conversely, differentiation has been found at the scale of meters, such as between quadrats, tidal heights, and bays (Ruckelshaus 1998, Becheler et al. 2010, Kamel et al. 2012), suggesting that the spatial scale of genetic connectivity between *Z. marina* meadows can be unpredictable. Importantly, how these patterns of diversity persist over time can have important ecological consequences; if they too are highly variable, management decisions based on a single timepoint may not accurately represent the longer-term dynamics of the system (Reynolds et al. 2017). Here, we used microsatellite markers to assess spatiotemporal patterns of genetic diversity, population structure, and kinship within and across decades in 2 *Z. marina* meadows in North Carolina. Studies investigating the genetic diversity and structure of *Z. marina* meadows over long periods of time are rare, and demonstrating temporal stability in these populations may prove useful in future conservation studies with long-term seagrass restoration objectives. We also discuss these genetic patterns in relation to the life history strategies and seed dispersal potential in these meadows. Finally, we ask whether metrics of genetic diversity are significant predictors of meadow health and resilience and consider these implications for future conservation and management of *Z. marina* meadows.

2. MATERIALS AND METHODS

2.1. Sample collection and genotyping

Two *Zostera marina* meadows, located in southeastern North Carolina, were sampled to quantify meadow-level patterns of genetic diversity and struc-

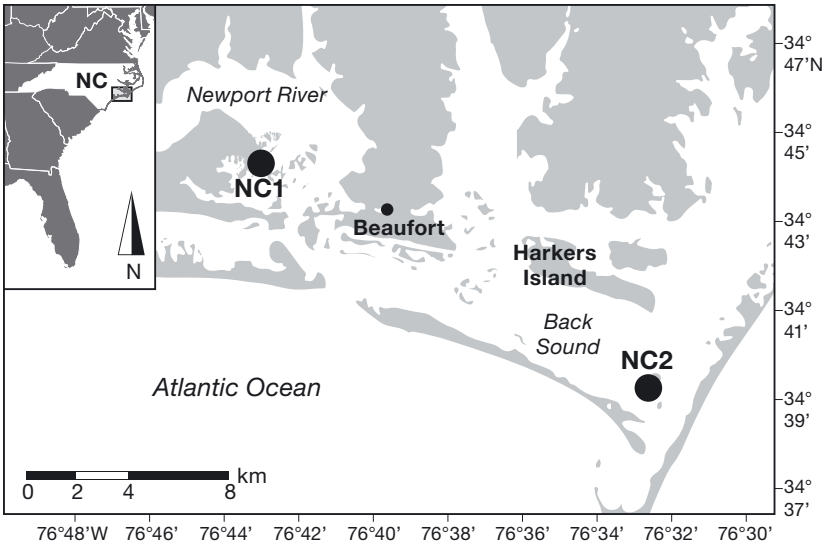

Fig. 1. Study sites in southeastern North Carolina (USA): Phillips Island (NC1) is in the Newport River Estuary approximately 15 km from Morgan Island (NC2), located in Back Sound

ture. Morgan Island (34° 66′ N, 76° 52′ W) is located on a shallow open shelf in the Back Sound, and Phillips Island (34° 43′ N, 76° 41′ W) is located on a semi-enclosed shelf in the Newport River Estuary, approximately 15 km away (Fig. 1). Phillips Island (NC1) is a mixed bed of mixed-annual *Z. marina* and *Halodule wrightii*, an opportunistic species abundant in North Carolina, while Morgan Island (NC2) seagrass beds are dominated by perennial *Z. marina* with small patches of *Ruppia maritima* (Field et al. 2021). Both sites are shallow, with mean lower low water depths of <0.25 to 0.5 m (Penhale 1977, Thayer et al. 1977). Between 10 and 50 shoots were haphazardly collected roughly 5 m apart, in the same 30 m × 30 m area at each site over a span of 10 yr. Samples were collected in May 2007, May 2008, February 2016, March 2017, and June 2017. Additional samples were collected from NC2 in May 2016 (total n = 293; Table 1). Each sample was also categorized by season. Early samples were those collected in February and March and correspond to young seedlings and, at NC2, potentially remnant rhizomes from the previous reproductive period. Late samples were collected in May and June and represent those seedlings that have survived to peak meadow biomass and will contribute to the next generation.

Shoots were dried and cleaned in the field by wiping both sides of the leaves with paper towels until no epiphytes remained, placed in labeled ~120 ml (4 oz) Whirlpak bags, filled with approximately 28 g (1 oz) of silica gel desiccant, placed on ice, and transported to the Center for Marine Science at the University of North Carolina Wilmington. Samples were removed from the bags, placed in a buffer solution, and frozen prior to analysis. DNA was extracted using a PowerPlant® Pro DNA Isolation Kit. Nine microsatellite loci previously characterized for *Z. marina* (see Kamel et al. 2012) were amplified in 2 multi-

Table 1. Summary statistics for each *Zostera marina* location averaged over all 9 microsatellites. Observations include: sample size (n), mean number of alleles per locus (*A*), mean allelic richness, rarified to 10 individuals (*A*r), observed heterozygosity (H_o), expected heterozygosity (H_e), mean inbreeding coefficient (F_{IS}), clonal richness (*R*), mean kinship coefficient (*k*), percentage of loci pairs in linkage disequilibrium (LD), and percentage of loci out of Hardy-Weinberg equilibrium (HWE). Values in **bold** are significant at $p < 0.05$

Year	Month	n	*A*	*A*r	H_o	H_e	F_{IS}	*R*	*k*	LD	HWE
Phillips (NC1)											
2007	May	10	3.8	3.8	0.622	0.547	**−0.354**	1	0.067	11	33
2008	May	11	3.1	3.1	0.556	0.496	**−0.325**	1	0.097	14	33
2016	February	49	6.4	3.8	0.628	0.590	−0.065	0.98	0.013	6	11
2017	March	36	5.9	3.8	0.654	0.616	−0.074	1	0.031	3	11
2017	June	48	6.7	3.8	0.593	0.612	0.031	1	0.014	25	22
Morgan (NC2)											
2007	May	11	4.0	3.9	0.466	0.575	0.041	1	0.069	3	11
2008	May	10	3.5	3.5	0.538	0.575	−0.086	1	0.048	8	44
2016	February	48	7.2	3.7	0.549	0.562	0.025	1	0.011	6	22
2016	May	25	4.6	3.3	0.480	0.548	**0.127**	1	0.002	11	11
2017	March	28	5.6	3.6	0.627	0.574	−0.095	1	0.005	0	11
2017	June	17	4.4	3.4	0.477	0.545	**0.129**	1	0.015	6	11

plex PCR reactions. Individual primer working stocks contained 1 µl of 10 µM fluorescently labeled forward primer, and 10 µl each of 50 µM unlabeled forward and reverse primers diluted in 80 µl of ddH_2O. Primers were subsequently combined into 2 primer mixes containing 4 or 5 different primers. PCR conditions for all multiplex reactions were as follows: 95.0°C for 15 min; followed by 2 cycles of 15 s at 94.0°C, 30 s at 60.0°C, and 45 s at 72.0°C; then 2 cycles of 15 s at 94.0°C, 30 s at 59.0°C, and 45 s at 72.0°C; then 2 cycles of 15 s at 94.0°C, 30 s at 58.0°C, and 45 s at 72.0°C; then 2 cycles of 15 s at 84.0°C, 30 s at 57.0°C, and 45 s at 72.0°C; and finally 28 cycles of 94.0°C for 15 s, 30 s at 56.0°C, and 45 s at 72.0°C; ending with a 2 min extension at 72.0°C. Following PCR, 2 separate reactions were prepared, containing 0.5 µl of PCR product from each of the 2 multiplex mixes. PCR products were added to 9 µl of highly deionized formamide (Hi-Di) and 0.4 µl of GeneScan-600 (LIZ) size standard (Applied Biosystems) for genotyping on an ABI Prism 3130XL Genetic Analyzer. Fragments were scored using the software Strand version 2.3.69 (Toonen & Hughes 2001).

2.2. Data analyses

2.2.1. Genetic diversity

Because seagrasses can spread clonally via rhizome extension, a genetic individual (genet) may consist of many shoots (ramets) covering several meters. Even though a sampling distance of 1–1.5 m is generally adequate to avoid repeated sampling of clones in *Z. marina* (Olsen et al. 2004), it is possible that the same genet might be sampled more than once if large clones are present. Clones were identified using GenClone (Arnaud-Haond & Belkhir 2007), and duplicate multilocus genotypes (MLGs) were removed before further analyses. The probability of identical MLGs arising from different sexual reproduction events was assessed by calculating the probability of identity (P_{ID}) for each site across all loci using GenAlex v.6 (Peakall & Smouse 2012). P_{ID} calculates the probability that 2 individuals drawn at random within a population will have the same MLG. The calculated P_{ID} for each site was low, ranging from 4.1×10^{-5} at NC1 to 7.3×10^{-6} at NC2, indicating that the marker system had a high degree of power to identify unique MLGs.

Microchecker version 2.2.3 was used to determine whether any deviations from Hardy-Weinberg equilibrium (HWE) were due to null alleles or large allele drop-out, as well as to check for stuttering (Van Oosterhout et al. 2004). All data were tested for departure from HWE within each sample by locus and over all loci using Genepop version 1.2 (Raymond & Rousset 1995). Mean number of alleles and observed and expected heterozygosity (H_o and H_e) were calculated using Arlequin version 3.5 (Excoffier & Lischer 2010), and allelic richness, rarefied to 10 individuals, was calculated using the 'PopGenReport' package in R version 3.6.3 (Gruber & Adamack 2015, R Core Team 2020). Inbreeding coefficents (F_{IS}) were calculated using FSTAT version 2.9.3.2 (Goudet 2001). Genotypic diversity (R) was calculated in GenClone as the number of unique MLGs relative to the number of shoots collected (Arnaud-Haond & Belkhir 2007). All statistical models were run in R version 3.6.3 (R Core Team 2020). Data were examined for outliers, collinearity, and variance inflation factors in R prior to analysis (Zuur et al. 2007). Regression analyses were used to test for differences in diversity metrics between sites, across years, and across season.

2.2.2. Spatiotemporal genetic structure

To assess the degree of genetic structure among samples, all pairwise values of population genetic differentiation were determined by calculating Weir & Cockerham's F-statistics (F_{IS}, F_{IT}, F_{ST}) using Arlequin version 3.5, and tested for significance by 10 000 permutations of the data (Excoffier & Lischer 2010). Hierarchical analyses of molecular variance (AMOVA) were also implemented in Arlequin to test for both spatial (between sites) and temporal (between years) genetic structure.

Additionally, Structure version 2.3.4 was used to detect genetic structure by pooling all individuals and assigning them to population clusters (Pritchard et al. 2000). The number of genetic clusters (K) within *Z. marina* was assessed for values of K ranging from 1 to 11 (the number of samples) using the admixture model with allelic frequencies correlated among populations and including prior population information. Ten Bayesian Markov chain Monte Carlo (MCMC) searches of 1 000 000 steps with a 100 000 step burn-in were run, and the maximal values of ΔK were used based on the rate of change in the log probability of data between successive K values (Evanno et al. 2005) to find the values of K that best fit the observed distribution of genotypes. Because Structure elucidates the highest level of genetic structure, it is possible to test for hierarchical structure by running each identified cluster separately. All clusters (K) identified in the initial analysis

were evaluated again separately to search for substructure. To further partition genetic variance within and among clusters identified by Structure, AMOVA was again implemented in Arlequin. A discriminant analysis of principal components (DAPC) was performed using the 'adgenet' package in R to visually infer clusters of genetically related individuals (Jombart et al. 2010).

A subset of the individuals for which GPS coordinates were available (from samples collected at NC1 and NC2 in February 2016) was used to test for a correlation between genetic and geographic distance. A Mantel test was used to characterize the relationship between inferred levels of genetic relatedness and geographic distance between individuals within sites, as well as between individuals between sites. Significance of correlations in all Mantel tests was assessed with 999 matrix randomizations using the 'adegenet' package in R (Jombart 2008). A Mantel correlogram was also used to determine if and at what scale spatial autocorrelation occurred within sites and was conducted using 'ecodist' in R (Goslee & Urban 2007).

Finally, to investigate spatial patterns of relatedness among individuals, kinship coefficients (Loiselle et al. 1995), which are based on the relative probability of 2 alleles being identical by descent between each pair of individuals, were calculated in Genodive (Meirmans & Van Tienderen 2004). Following the approach of Iacchei et al. (2013), individuals were binned according to the following levels of kinship (k): 'nearly identical', $0.57 > k > 0.375$; 'full siblings', $0.375 > k > 0.1875$; 'half siblings', $0.1875 > k > 0.09375$; and 'quarter siblings', $0.09375 > k > 0.047$. Here, the bounds represent the midpoints between coancestry coefficients (Loiselle et al. 1995, Iacchei et al. 2013). A permutation test was also conducted in Genodive (Meirmans & Van Tienderen 2004), where the observed number of closely related individuals within sampling sites was compared to a null distribution of kinship coefficients generated by randomly assigning individuals to sites (Iacchei et al. 2013).

2.2.3. Meadow properties

In addition to the genetic data, a subset of samples contained measures of seagrass biomass, a metric of meadow health. Data were collected from both sites in May 2007, May 2008, and May 2016. Biomass was quantified from cores taken haphazardly at a distance of ≥5 m at each location with a 22 cm diameter corer (n = 5 per site). All cores were sieved in the field (1 cm mesh) and plant and root/rhizome material were stored at 4–6°C until analysis. Shoots were separated from the rhizome directly below the leaf sheath into above-ground (AG) and below-ground (BG) biomass (Setchell 1929). AG biomass included both vegetative and flowering shoots. All biomass samples were dried at 60°C to a constant weight (dry weight, DW) and reported as g DW m^{-2}. Total biomass (sum of all AG and BG material), AG biomass (vegetative plus flowering), BG biomass, and AG:BG biomass ratio were calculated.

ANCOVA was used to compare the relationship between AG and BG biomass to determine whether allocation patterns (i.e. phenotypes) differed between sites. Regression analysis was used to determine whether the AG:BG ratio changed over time. Multiple linear regression with stepwise forward elimination was used to test which of the genetic diversity metrics (Table 1) influenced plant biomass measures. Goodness-of-fit for regression analyses was assessed by examining residuals for patterns and calculating the predicted r^2. However, given the small number of observations, caution should be used when interpreting results.

3. RESULTS

3.1. Genetic diversity

A total of 293 seagrass shoots were amplified at 9 microsatellite loci; all samples were polymorphic. Genclone revealed only 1 clone at NC1 in February 2016, with all other individuals being identified as genetically distinct individuals, reflected in high genotypic richness values (Table 1). The clone was removed from all subsequent analyses. Microchecker found no evidence of null alleles, stuttering, large allele dropout, or scoring errors. Linkage disequilibrium (%) was relatively low across all samples, and no specific patterns were found across loci or samples; all samples were in HWE. Due to the unbalanced sample size, we ran all analyses on the full dataset as well as on a reduced dataset with 10 individuals randomly selected per sample (n = 110). Given that the results of these 2 approaches differed minimally between datasets, we present the results of the analyses on the full dataset (see the Supplement at www.int-res.com/articles/suppl/m683p053_supp.pdf for additional analyses on the reduced dataset).

There was no significant effect of site on rarified allelic richness ($F_{1,9}$ = 0.11, p = 0.76; Table 1), but NC1 had significantly higher H_o ($F_{1,9}$ = 7.71, p = 0.02;

Table 1). Significantly negative F_{IS} values were found at 2 sites in NC1, while there was significant evidence of inbreeding in 2 of the sites at NC2 (Table 1). Indeed, NC2 had significantly higher F_{IS} values than NC1 ($F_{1,9}$ = 9.04, p = 0.01). Rarified allelic richness differed over time, with earlier years having lower values ($F_{3,9}$ = 13.63, p = 0.005; Fig. 2). Season also had a significant effect on rarified allelic richness ($F_{1,9}$ = 8.39, p = 0.02) and H_o ($F_{1,9}$ = 5.28, p = 0.04), with samples from earlier in the year having higher values. Genotypic diversity was high over both space and time (Table 1).

3.2. Spatiotemporal genetic structure

Analysis of pairwise genetic differences (F_{ST}) revealed significant genetic variation among the 11 samples, with 46 of 55 (83.6%) F_{ST} values being significantly different from zero (Fig. 3; Table S1). F_{ST} values ranged from 0.005 to 0.097, indicating low to moderate genetic differentiation (Table S1). Overall, mean F_{ST} values within ($\bar{x}$ = 0.03) vs. between ($\bar{x}$ = 0.04) sites were not significantly different ($F_{1,53}$ = 2.29, p = 0.14), indicating that spatial structuring is unlikely to be strong. Indeed, the AMOVA revealed extremely slight partitioning of genetic variation both between sites and among years, with most of the genetic variation (>97%) occurring among individuals within samples (Table 2a,b). Pairwise genetic differentiation between samples collected in the same year was significant (Fig. 3; Table S1), showing that small-scale changes in allele frequencies can occur over short periods of time.

The broader pattern of limited differentiation between sites and years was reinforced by the clustering analysis which identified a peak at K = 2, suggesting 2 genetic clusters. The substructure analysis showed no further population structure. Cluster 1 contained individuals sampled in 2007 and 2008, while cluster 2 contained individuals sampled in 2016 and 2017 (Fig. S1). When individuals were partitioned into these decadal clusters, the AMOVA was able to explain slightly more of the observed genetic variation (3.5 vs. <1%; Table 2c). Indeed, mean F_{ST} values within ($\bar{x}$ = 0.02) vs. between ($\bar{x}$ = 0.05) decades were significantly different ($F_{1,53}$ = 39.81, p < 0.0001), though still in the range of low genetic differentiation. Results from the DAPC showed similar decadal clusters with a clear overlap (Fig. 4).

Within the February 2016 samples, geographic distance was not significantly correlated with genetic distance at scales between 5 and 120 m (NC1: r^2 =

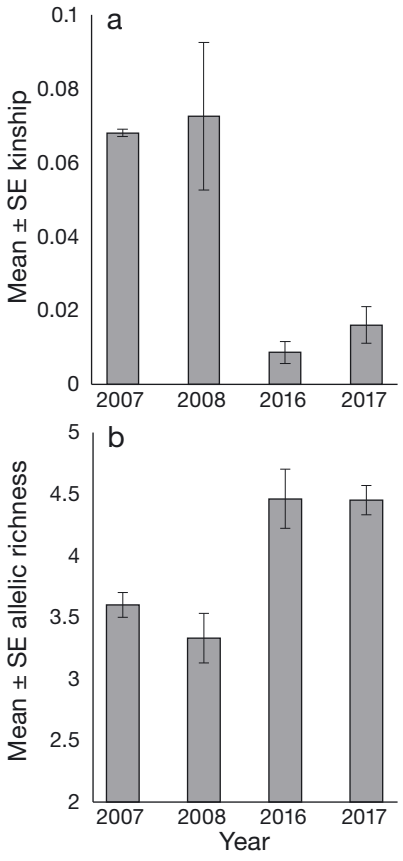

Fig. 2. Changes in *Zostera marina* diversity metrics over time: (a) mean kinship, (b) allelic richness, rarified to 10 individuals. Both relationships were significant (p < 0.05)

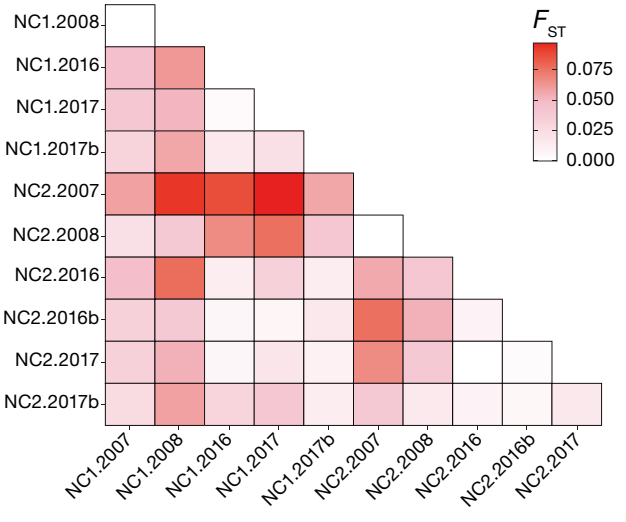

Fig. 3. Heat map of pairwise F_{ST} values for the 11 *Zostera marina* samples from Phillips Island (NC1) and Morgan Island (NC2), North Carolina. Dark red cells denote higher pairwise F_{ST} estimates (and greater genetic divergence) among samples; lighter cells denote lower estimates (and greater genetic similarity)

Table 2. Hierarchical analysis of molecular variance (AMOVA) as a weighted average over all loci. (a) Between sites (Phillips Island and Morgan Island, North Carolina); (b) among years (2007, 2008, 2016, and 2017); (c) using clusters returned by Structure version 2.3.4 (2007/08 and 2016/17)

Source of variation	df	Variance components	Percentage of variation	p
(a)				
Between sites	1	0.02	0.56	0.07
Among samples within sites	9	0.06	2.35	<0.001
Within samples	575	2.58	97.09	<0.001
(b)				
Among years	3	0.03	0.90	0.04
Among samples within years	7	0.05	2.05	<0.001
Within samples	575	2.58	97.05	<0.001
(c)				
Between clusters	1	0.09	3.54	0.006
Among samples within clusters	9	0.04	1.61	<0.001
Within samples	575	2.58	94.85	<0.001

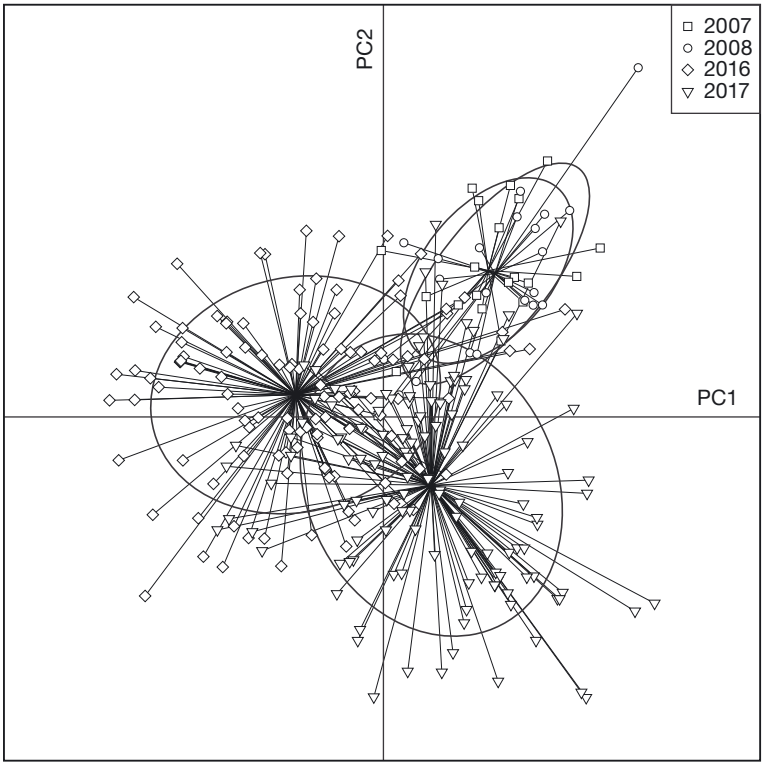

Fig. 4. Discriminant analysis of principal components, visualizing the modest genetic substructuring in *Zostera marina* over time. Each oval represents 1 of 4 sampling years

0.0004, p = 0.66; NC2: r^2 = 0.002, p = 0.24; Fig. 5). Between the sites which were separated by 15 km, geographic distance was only very weakly correlated with genetic distance (r^2 = 0.03, p = 0.03). The Mantel correlogram further confirmed that at large distances (i.e. between sites), spatial autocorrelation was negative, although the low values of Mantel r (<0.06) suggested this correlation was very weak. Within sites, no significant spatial autocorrelation was found.

Overall, kinship coefficients among individuals ranged from −0.113 to 0.277, with an overall mean kinship of 0.00002. However, the mean kinship among individuals within samples (n = 11) was 0.033 ± 0.032, which was significantly higher than the mean kinship among individuals located in different samples (k = −0.002 ± 0.103; $F_{1,292}$ = 14.92, p = 0.001). There were no significant differences in kinship between sites ($F_{1,9}$ = 1.02, p = 0.34; Table 1) but kinship changed significantly over time ($F_{3,9}$ = 34.97, p = 0.0002), with the earlier years having higher kinship coefficients (Fig. 2; Table S2). There were significantly more kin groupings than expected by chance in 9 out of 11 samples, with the exception of February 2016 and March 2017 at NC2. Overall, this pattern was stronger in the earlier years: for example, all samples from 2007 and 2008 showed a greater proportion of half-siblings, as much as 25% more, than expected by chance (Fig. 6; Table S2). In addition to the high levels in kinship seen in the 9 samples, 6 samples also showed significantly fewer related individuals than would be expected by chance.

3.3. Meadow properties

Mean total biomass ranged from 82.9 to 266.2 g DW m^{-2} across samples. AG biomass ranged from 28 to 118.4 g DW m^{-2} and BG biomass from 40.4 to 201.6 g DW m^{-2} (Table 3). The results of the ANCOVA indicate that AG biomass was significantly correlated with BG biomass ($F_{1,4}$ = 527.62, p = 0.002) and site ($F_{1,4}$ = 472.31, p = 0.002), and the interaction of site × BG biomass was significant ($F_{1,4}$ = 285.88, p = 0.004). Indeed, plants in NC1 had greater total biomass and allocated relatively more to AG biomass than plants in NC2 (Fig. S2a), with AG:BG ratios at NC1 ≈1 and those at NC2 <1 (Table 3). While not significant, the temporal pattern also varied across sites: ratios at NC1 decreased

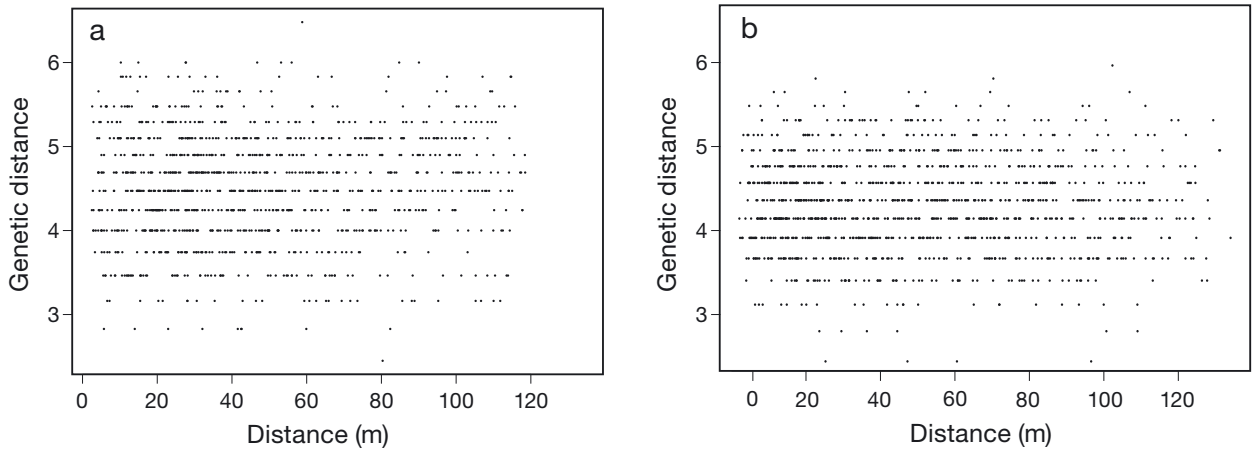

Fig. 5. Relationship between genetic and geographic distance for *Zostera marina* shoots sampled at (a) Phillips Island (NC1) and (b) Morgan Island (NC2) in February 2016. Neither relationship was significant

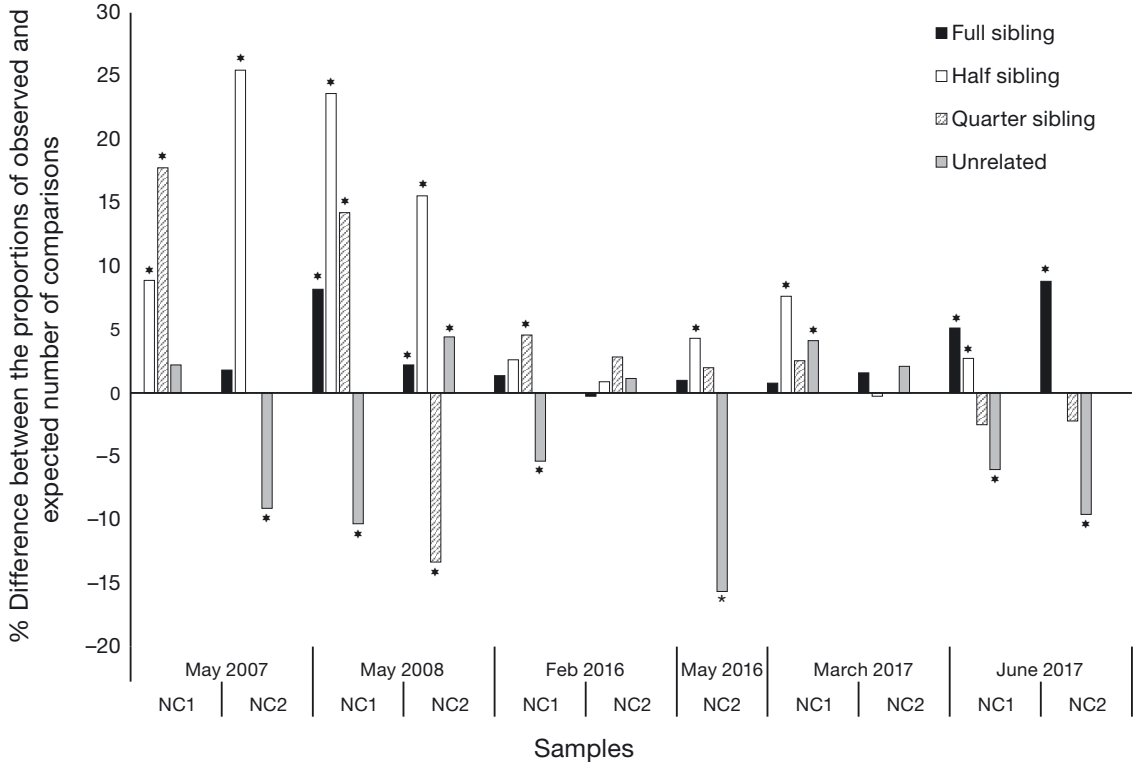

Fig. 6. Proportion of kinship observed for each sample that is in excess of expected levels due to chance in each of 4 kinship categories. *Significant differences at $p < 0.05$. See Table 1 for site information

over time but remained near 1; ratios at NC2 increased over time (Fig. S2b). Year, site, and rarified allelic richness were included in the multiple linear regression to explain variation in BG biomass, total biomass, and the AG:BG ratio. All 3 variables were significant predictors of BG biomass, while site and rarified richness were significant predictors of the AG:BG ratio, and none were significant predictors of total biomass (Table 4). Year, rarified allelic richness, and H_o were included in the regression analyses to explain variation in AG biomass and were all significant predictors (Table 4). Examination of residuals revealed no patterns, and the predicted r^2 values were similar to the actual values.

Table 3. *Zostera marina* total, above-ground (AG) and below-ground (BG) biomass, and AG:BG biomass ratios for 2 sites (NC1: Phillips Island; NC2: Morgan Island) in North Carolina in May 2007, 2008, and 2016. Biomass values are g dry weight m^{-2}. Means ± SE (n = 5)

Year	Month	Total biomass	AG biomass	BG biomass	AG:BG ratio
NC1					
2007	May	205.2 ± 10.3	118.4 ± 9.5	86.8 ± 6.1	1.4 ± 0.2
2008	May	82.9 ± 12.3	42.5 ± 7.9	40.4 ± 4.9	1.1 ± 0.1
2016	May	57.5 ± 17.3	24.2 ± 10.7	33.4 ± 10.4	0.8 ± 0.3
NC2					
2007	May	266.2 ± 56.5	64.6 ± 22.9	201.6 ± 39.2	0.3 ± 0.1
2008	May	174.6 ± 63.8	44.0 ± 14.2	130.6 ± 52.1	0.2 ± 0.1
2016	May	89.3 ± 14.2	28.0 ± 4.2	61.3 ± 10.9	0.5 ± 0.1

Table 4. Results from the multiple linear regression analysis with stepwise forward selection of explanatory variables. Significant predictors ($p < 0.05$) are in **bold**. AG: above-ground; BG: below-ground

Response	Predictors	df	Estimate	SE	*F*-ratio	p
AG biomass	Year	1	−19.67	0.69	811.35	**0.02**
	Allelic richness	1	16.99	2.168	61.40	**0.04**
	Heterozygosity	1	368.32	11.27	1068.19	**0.02**
BG biomass	Year	1	−70.35	0.94	5559.07	**0.01**
	Site	1	71.66	0.96	5565.88	**0.01**
	Allelic richness	1	136.43	3.18	1844.37	**0.01**
Total biomass	Year	1	−62.93	5.88	114.69	0.05
	Site	1	91.29	14.92	37.45	0.10
	Allelic richness	1	341.89	73.03	21.91	0.13
AG:BG ratio	Year	1	−0.03	0.01	7.97	0.22
	Site	1	−0.51	0.01	1541.66	**0.02**
	Allelic richness	1	0.54	0.04	125.72	**0.04**

4. DISCUSSION

Long-term investigations of genetic diversity of *Zostera marina* meadows are important, particularly in the face of climate change, as associations between low genetic diversity and long-term population declines are becoming increasingly apparent (Alotaibi et al. 2019). In monospecific seagrass meadows, genetically diverse populations are often more stable, productive, and can recover more quickly from disturbance than less diverse populations (Hughes & Stachowicz 2004, Reusch et al. 2005, Hughes et al. 2009b). This association has led to the use of spatial patterns of genetic diversity and kinship structure to infer variation in population resilience (e.g. Stachowicz et al. 2013, DuBois et al. 2021), resulting in both management and restoration practices that assume observed patterns of genetic diversity are stable over time (Reynolds et al. 2017). However, few studies have assessed spatial genetic structure of *Z. marina* meadows over a long-term (decadal) timescale, including the most recent time period where the impacts of climate change have significantly altered marine habitats (Cloern et al. 2016). Indeed, time series are revealing that many of the world's coastal ecosystems are in a continuing state of change and that these rates of environmental change are occurring faster than had been anticipated only decades ago (Petersen et al. 2008, Cloern et al. 2014, 2016). It is thus critical to gain a more complete understanding of the breadth of genetic diversity in impacted ecosystems and, importantly, to better understand how they have continued and how they will continue to respond to these rapid changes.

Overall, metrics of genetic diversity were high and not significantly different between the 2 *Z. marina* meadows sampled here (Table 1). Values for allelic richness were similar to or higher than those found in several populations sampled at a similar spatial scale in the USA (California: Ort et al. 2012, Reynolds et al. 2017; Virginia: Reynolds et al. 2013), in Sweden (Martínez-García et al. 2021), and in the UK (Alotaibi et al. 2019), despite the low rarified sample size of 10 individuals in this study. For those studies with a temporal component, genetic diversity either remained stable (Reynolds et al. 2017) or declined (Alotaibi et al. 2019) across a decadal time scale, in contrast to the increase in allelic richness seen here (Fig. 2). Populations with higher genetic diversity may have more adaptive potential because of higher levels of standing genetic variation, which makes them more robust to changing environmental conditions (Willi et al. 2006, but see Teixeira & Huber 2021). As such, the high and increasing allelic richness found at both sites might indicate the potential for greater resistance to and recovery from disturbances compared to other regions and has significant implications for the management of *Z. marina* at the species' biogeographic transition zone (Greenbaum et al. 2014, York et al. 2017, Statton et al. 2018). The location of the sites may even explain the patterns in genetic diversity observed here. Seagrass meadows in North Carolina are subject to frequent natural disturbance from tropical storms (Paerl et al. 2019), thermal stressors (Jarvis et al. 2012,

Bartenfelder 2019), and sedimentation (Mills & Fonseca 2003). This can favor increased sexual reproduction, which may lead to greater diversity in areas prone to moderate disturbance (Ferber et al. 2008, Cabaco & Santos 2012). Indeed, genotypic richness was high in all 11 samples (Table 1).

Interestingly, genetic diversity also varied within years, with early samples (from February and March) having higher allelic richness and H_o than later samples (from May and June). While this may represent undetected spatial or stochastic variation (Ort et al. 2012), many organisms, including seagrasses, experience extremely high mortality early in life (Alagna et al. 2013, Statton et al. 2017), and the differences across seasons may reflect changes in the genetic characteristics between seedlings and surviving adults due to the decrease in population size (Frankham 1996). Selection pressures that reduce population size are often assumed to act uniformly and randomly, but it is possible that selection differentially affects individuals. For example, strong purifying selection may erode genetic diversity leading to decreases across a season as seen here (Cvijović et al. 2018, Hays et al. 2021b). However, previous research in *Z. marina* found no relationship between seed genetic diversity and adult clonal richness; more work is thus needed to understand how sexual reproduction, mortality, and stochasticity influence standing genetic variation in seagrass populations (Hays et al. 2021a).

While richness did not differ between sites, inbreeding coefficients and heterozygosity did, with F_{IS} values being significantly lower and H_o being significantly higher at NC1. This pattern might be explained by differences in the timing of life history shifts in the region. Although observations of increased flowering effort and seed production occurred as early as 2004, the *Z. marina* meadow at NC1, previously documented to be perennial, had completely shifted to a mixed-annual life history strategy by 2007/08 (Jarvis et al. 2012). This strategy relies on increased seed production, successful seed germination, and seedling growth for meadow reestablishment on an annual basis and provides the mechanism necessary for *Z. marina* populations to persist, even during times of high abiotic stress (Jarvis et al. 2012). While the mixed-annual life history strategy has not been formally documented at NC2, shifts from perennial to the mixed-annual life history strategy were observed at nearby meadows in Back Sound around 2017 (Bartenfelder 2019). The longer perennial history observed at NC2 might thus explain the observations of higher F_{IS} and lower H_o as sexual reproduction was delayed.

Overall, the high genetic diversity within meadows was coupled with relatively weak spatial genetic structure. While most pairwise comparisons of genetic differentiation were significant (Fig. 3; Table S1), site only accounted for 0.56% of the observed genetic variation, and geographic distance was only a weak predictor of genetic distance (r^2 = 0.03) between meadows separated by 15 km. *Z. marina* has a large dispersal potential: although eelgrass seeds are negatively buoyant, and thus do not typically travel farther than a few meters, they are often carried longer distances by positively buoyant, flowering branches and can travel up to hundreds of kilometers on ocean currents (Orth et al. 2006, Kendrick et al. 2012, McMahon et al. 2014, Smith et al. 2018). Thus, the absence of strong spatial genetic structure may be due to unrestricted seed dispersal within and between the 2 meadows. Hydrological data show that water movement in the area depends strongly on winds. For example, northeastward-directed winds favor transport into Back Sound while northwestward blowing winds favor transport into the lower Newport River (NC1) (Logan et al. 2000). These currents provide a conduit through which seeds may disperse. Biotic dispersal may also play a role in enhancing gene flow in this system, as several studies have demonstrated waterfowl, turtles, and fish to be capable of biotic dispersal of *Z. marina* seeds over distances similar to those in this study (Sumoski & Orth 2012, Tulipani & Lipcius 2014). These organisms exist within the geographic range of the meadows, so biotic dispersal as a mechanism of gene flow between sites may play an important role in meadow connectivity.

Temporal genetic structure explained a larger, though still small, amount (3.5%) of the observed genetic variation (Table 2c). Both the Bayesian assignment test and DAPC returned 2 clusters, corresponding to the 2 decadal samples (Fig. 4; Fig. S1). As with the modest spatial genetic structure, the temporal analysis indicated relative consistency over a 10 yr period, with mean F_{ST} values pointing to low genetic differentiation (Hartl & Clark 2007). This is encouraging, as studies that have examined genetic structure and diversity in populations over time suggest that reductions to temporal genetic integrity are associated with a loss of local adaptive potential (Hansen et al. 2002, Bourret et al. 2011). Importantly, a key driver resulting in the observed genetic differentiation over decades appears to be a shift in kin structure. While kinship coefficients remained similar between sites, there was a striking decrease in mean kinship within sites over time (Fig. 2). For example, the proportion of observed half-siblings was

as much as 27% greater than expected in the earlier decades; this number decreased to less than 8% in the later years (Fig. 6; Table S2). The minimal spatial genetic structure does not point to important increases in gene flow: NC1 and NC2 were similarly differentiated in both the early and later decades, despite the differences in kinship. Interestingly, the decreased relatedness among individuals, coupled with higher allelic richness, does suggest an increase in the number of genotypes (i.e. seed output) over time. It is still unknown whether this represents increases in seed production at a regional scale or increases in sexual reproduction at a local scale.

However, allocation strategies did vary significantly between NC1 and NC2 across all years, resulting in the large amount of observed variability in shoot biomass, which appears to be a feature of this region (Jarvis et al. 2012, Combs et al. 2021). As a mixed-annual meadow, plants at NC1 allocated more to AG biomass, while plants at NC2, a perennial meadow, allocated more to BG biomass (Table 3). The difference in biomass allocation is a result of seasonal loss of *Z. marina* shoots in mixed-annual meadows. As shoots die back annually and reestablish from seedings, mixed-annual populations do not have as much time to build up BG biomass compared to perennial populations which maintain biomass year-round (Jarvis et al. 2012). These differences between primarily vegetative growth in perennial meadows to increased reliance upon sexual reproduction in mixed-annual meadows thus appear to be highly plastic in this species (Johnson et al. 2017), reinforced here by the finding of little genetic differentiation between sites. Phenotypic plasticity is one important mechanism by which plant populations can adapt to ongoing environmental changes (Chevin et al. 2010, Bertelli et al. 2021). For example, while the AG:BG ratio at NC1 has declined over time, the opposite pattern was observed at NC2 (Table 3). Variation was also observed in the magnitude of the change in the AG:BG ratio. The observed change in the NC1 AG:BG ratio was within the range of previous year estimates while NC2 increased steadily at each timestep, making this an emerging trend that might be reflecting a continued change in allocation patterns here. Identifying drivers, constraints, and thresholds of the highly plastic response in sexual reproduction will be key to more effective management and restoration of this foundation species.

In addition to these plastic responses, genetic diversity in *Z. marina* has a positive effect on numerous meadow properties, e.g. shoot densities (Ehlers et al. 2008) and biomass (DuBois et al. 2021), and seagrass meadows themselves positively influence sediment stability (Folmer et al. 2012), nutrient cycling (Flindt et al. 1999), and carbon sequestration (Fourqurean et al. 2012, Miyajima & Hamaguchi 2019). Despite the small dataset on meadow health in this study (Table 4), rarified allelic richness was correlated with AG and BG biomass, and with the AG:BG ratio, often used as a measure of resilience (Hemminga 1998, Lee et al. 2007, Barry et al. 2018). These results are consistent with the growing body of work on the ecological importance of intraspecific variation, and how genetic diversity positively affects *Z. marina* biomass, the abundance and diversity of associated species, as well as population, community, and ecosystem-level responses to disturbance (Williams 2001, Hughes & Stachowicz 2004, Reynolds et al. 2018).

Temperatures will continue to rise as climate change intensifies, resulting in concomitant widespread loss of seagrass meadows and loss of genetic diversity. Our results demonstrate that here, at the southern geographic limit of *Z. marina* in the Western Atlantic, decadal patterns of genetic structure and diversity remain relatively stable, despite genetic differentiation across seasons, changes in phenotype, and shifts in kin structure. This increases the utility of such genetic data for management actions and is key for improving the success of restoration and conservation efforts in this region. However, genetic monitoring across all regions is needed to understand the factors and processes underlying meadow health, which is critical for implementing appropriate management practices (Cook & Sgrò 2018). For example, genetic monitoring can inform whether the establishment of new recruits is the result of clonal spread or sexual reproduction and can also shed light on the genetic basis of many important traits (Mijangos et al. 2015, Jackson et al. 2021).

However, measuring genetic diversity is often complicated: patterns of diversity can change rapidly over space and time and can create patches that are genetically differentiated over both large and small areas (Becheler et al. 2010). Managers need long-term data to accurately assess the conditions of their meadows. Do diversity metrics fluctuate regularly, displaying transient shifts and genetic patchiness? Is diversity low and/or declining and in need of a specific intervention, such as transplantation? Or is it stable, with genotypes well adapted to environmental conditions? Regularly monitoring meadows for changes in baseline conditions over multiple spatial and temporal scales is necessary to detect increases in meadow vulnerability to warming ocean temperatures (Mijangos et al. 2015, O'Leary et al. 2017). As

the effects of climate change continue to amplify, the genetic diversity and phenotypic plasticity of seagrass meadows may provide a means of protection against anthropogenic impacts and promote population maintenance and resilience. It is imperative that these metrics be included in monitoring programs to ensure a sustainable future for seagrasses.

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