Genetic Diversity and Structure of Natural and Restored Seagrass Meadows on the Eastern Shore of Virginia: Causes and Ecological Consequences

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A Dissertation presented to the Graduate Faculty
of the University of Virginia in Candidacy for the Degree of
Doctor of Philosophy

Department of Environmental Sciences

University of Virginia
May 2012

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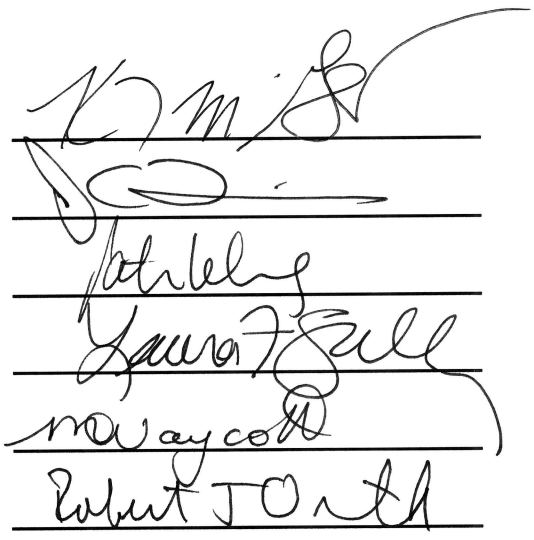

Abstract

Genetic diversity is positively associated with plant fitness, stability, and the provision of ecosystem services. Typically, diversity is lost with disturbance and is not completely recovered by restoration. Therefore, there is concern about the state of seagrass genetic diversity in Virginia. Chesapeake Bay meadows are frequently disturbed, and coastal bay meadows were restored using Chesapeake Bay seeds.

This dissertation assesses the genetic diversity of Virginia seagrass meadows and explores the processes influencing diversity and the ecological implications of that diversity. While I expected that historic and current disturbance in Chesapeake Bay would result in lowered genetic diversity, I found the opposite and instead showed, at three spatial scales, that disturbance shifted the balance towards sexual reproduction (opposed to asexual) and thus enhanced genetic diversity. Also counter to expectations, restored meadows maintained the high genetic diversity of donors, showing no signs of bottlenecks or genetic drift typical of restorations. Restored meadows were, in fact, more diverse than the meadows formed by metapopulation dynamics and recruitment from northern populations. Since the restored populations fit geographically into clustering models, I conclude that restoration did not disrupt regional genetic structure and instead simply accelerated recovery of both areal coverage and genetic diversity. Experimentally, I showed that increased genetic diversity results in higher seagrass density and ecosystem services (productivity, nutrient storage, and habitat) under a range of environmental conditions. Further, a survey showed that the influence of genetic diversity on seagrass

density is comparable to environmental drivers (nutrient concentrations, temperature, and light availability), providing evidence that the positive relationship between genetic diversity and ecosystem functions, determined experimentally by manipulating plants in small plots and controlling for environmental variation, is applicable at larger spatial scales and under real-world conditions. Together these findings suggest that the great success, measured by areal coverage and the value of ecosystem services (worth an order of magnitude more than restoration cost), of the Virginia coastal bay seagrass restoration is due in part to the high genetic diversity of the system. Further, conservation and restoration programs in other regions would benefit from the inclusion of genetic diversity in monitoring and restoration.

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Acknowledgements

Throughout this entire process, I have had the privilege of working with an outstanding set of mentors and colleagues. Jay Zieman introduced me to seagrasses nearly 13 years ago and has been one of my biggest supporters ever since. The guidance and opportunities given to me by Karen McGlathery have been some of the most significant drivers in my success, and her example has taught me a lot about the kind of scientist and advisor I aim to become. The rest of my UVa committee—Pat Wiberg and Laura Galloway—have graciously given well-timed advice along the way.

I was fortunate to work closely with additional committee members outside of UVa, who went above and beyond. JJ Orth inspires me with his never-ending enthusiasm for seagrass ecology. His generously taking me in the field and showing me the ropes as well as his attention to detail have made this dissertation more complete. Michelle Waycott invited me into her home for weeks at a time, patiently explained countless genetic tools and programs, and pushed me to make these studies relevant to both seagrass ecology and to the broader ecology community.

While Ainsley Calladine was not technically on my committee, this work would not have been possible with out the many hours he spent in the lab teaching me techniques, and certainly the figures in this dissertation would been messier without his advice. Dave Smith and Fred Diehl also acted as important mentors. I am a better teacher and a more well-rounded scientist, had more fun during my PhD, and am probably a better person on account of the opportunities and friendship they have shared with me.

The nitty gritty of this project was facilitated by many colleagues; most noteworthy of which is Luke Cole. Luke participated in most of my collection trips and not only helped collect the samples and data but also made outings fun enough that I was willing to go back. Chris Buck often joined us, made sure we didn't get lost, made sure we didn't break too many things, helped us collect data, and laughed with us. I owe a big thank you to Meg Miller, Kelly Hondula, Rachel Baker, Savanna Barry, Gavin Bruno, Tye Burnes, Austin Riopel, Kellen Lauer, Carissa Wilkerson, Kelly Ksiazek, and Dana Gulbransen, who spent time in the field and lab with me working up samples, and to Art Schwarzschild and Donna Fauber, who provided logistical support without which I would not have gotten nearly as much done.

Chapter 1: Introduction

The overall objective of this dissertation is to use genetic tools to characterize the seagrass populations in Virginia, to explore the ecological processes and anthropogenic activities responsible for the observed genetic structure and variability, and to investigate the ecological implications of observed genetic structure and variability.

The importance of genetic structure and diversity in ecology

A high level of genetic diversity in plant populations is associated with increased benefits for plant survival and ecosystem services (Booy et al. 2000). The loss of genetic diversity may cause reduced adaptability to environmental change through loss of fitness (Reed & Frankham 2003), and genetic diversity is often lost through large disturbances, which remove biomass and result in smaller, isolated populations (Frankham et al. 2002).

Conservation and restoration of populations often involves strategies to preserve or enhance genetic diversity. However, introducing new genotypes into a population and changing the population structure may have unintended consequences. Outbreeding depression occurs when locally adapted genotypes interbreed with non-adapted genotypes, resulting in reduced fitness of the progeny. Heterosis occurs when where deleterious alleles are masked or when an increase in heterozygosity results in progeny which are fitter relative to their parents. While heterosis is generally considered positive, it may allow the propagation of deleterious alleles to future generations (Hufford & Mazer, 2003). To avoid these problems during restorations, population structure must be considered. Populations that are nearby and may potentially share genetic material

through natural dispersal are likely to be similar enough that the exchange of genetic material will not result in problems associated with the introduction of foreign genotypes.

The application of genetic tools to seagrass ecology

Seagrasses comprise 72 species of marine flowering plants and are often the dominant macrophyte in shallow coastal bays, lagoons, and estuaries worldwide (Green & Short 2003, McGlathery 2007, Short et al. 2011). Seagrasses form a three dimensional structure, which baffles currents allowing sediment and organic matter to fall out of suspension, clearing the water and sequestering carbon in the sediments. That canopy also acts as a habitat for ecologically and economically important invertebrate, finfish, and marine mammal species, and the primary productivity of these plants acts as the base of a detrital food web (Hemminga & Duarte 2000). These important ecosystem services are being lost at rapid rates as human activity is negatively impacting nearshore waterways limiting light and killing seagrasses (Short & Wyllie-Echeverria 1996, Waycott et al. 2009). Therefore, considerable efforts are being made to slow or reverse the decline as well as to mitigate for the loss of habitat (Greening & Janicki 2006, Orth et al. 2010).

Conservation and restoration strategies are increasingly incorporating genetic diversity (See van Katwijk et al. 2009) since several studies have found positive relationships of diversity and fitness, stability, and the provision of ecosystem services. For example, Williams (2001) showed that both sexual and asexual reproduction rates are positively correlated with genetic diversity, suggesting that diverse assemblages will

survive longer and expand at higher rates. Anthropogenic disturbances are attributed to the loss of seagrass coverage, and genetically diverse assemblages (measured by clonal diversity) have been shown to resist large disturbances (grazing (Hughes & Stachowicz 2004), high temperature events (Reusch et al. 2005), and large algal blooms (Hughes & Stachowicz 2011)) better than assemblages with very low clonal diversity.

Modeling and analysis using genetic tools have shown that many seagrasses species have the potential for long-distance dispersal (Coyer et al. 2004, Olsen et al. 2004, van Dijk et al. 2009, Kendrick et al. 2012), which may protect individual meadows from disturbance by both increasing diversity and providing propagules for recovery. A better understanding of the frequency, extent, influences, and consequences of seagrass metapopulations and dispersal will be essential to effective conservation of these plants (See Kendrick et al. 2012), and because of the large spatial and temporal scales of these processes are best studies using genetic analyses.

The study system: *Zostera marina* meadows in the Chesapeake Bay and the Virginia coastal bays

This study was primarily conducted in eelgrass meadows in the Virginia coastal bays system (part of the Virginia Coast Reserve Long Term Ecological research site) and also included meadows in the nearby Chesapeake Bay. While these sites are close in proximity, they differ with respect to geomorphology, depth, hydrological, environmental conditions, and recent history.

The Virginia coastal bays are small shallow lagoons with no riverine input, and exchange with the ocean is through narrow inlets between barrier islands (Lawson et al. 2007). The watersheds surrounding these bays are characterized by low population density and mixed agricultural and forest land cover, resulting in very low nutrient loading (Cole 2011, Giordano et al. 2011). The shallow nature and low nutrient loading make these bays ideal seagrass habitat; however, from approximately 1933 to 1999, there was no seagrass in this area. A wasting disease impacted *Zostera marina* meadows over the entire Atlantic seaboard in the early 1930s (Cottam 1934, 1935, Rasmussen 1977), and those years coincided with a particularly harsh hurricane season along the Virginia coast. In the Virginia coastal bays, the *Z. marina* populations were decimated, and they did not recover for many years (Orth et al. 2006). A discovery of small patches of seagrass in the southern Virginia coastal bays motivated a large-scale seed addition program initially using Chesapeake Bay meadows as a seed source. This restoration has been extraordinarily successful and seagrass populations in this region are thriving and expanding (Orth et al. 2012).

Chesapeake Bay, in contrast, is one of the world's largest estuaries. It is relatively deep with large riverine input and significant exchange with the ocean. The Chesapeake Bay watershed is very large (164,200 km^2) and human populations in the watershed have increased over the past 100 years causing shifts in the land cover from primarily forested to a diverse mosaic of forests, commercial agriculture, and several large metropolitan areas (Cooper 1995). *Zostera marina* is limited to shorelines in the southern portions of the bay where the water is shallow and salinity is higher than the northern regions. Seagrass coverage has varied over time. Prior to the 1930s, coverage was high, and

subsequently, populations decreased in the 1930s due to the same wasting disease that impacted plants in the Virginia coastal bays. However, populations in Chesapeake Bay recovered naturally from the disease. In more recent years, additional anthropogenic stresses (i.e. reduced water quality and increased temperature) have resulted in continued contractions of seagrass populations (Orth & Moore 1984, Moore & Jarvis 2008).

Study questions

The difference in history of these two areas coupled with the unique success of the restoration in the Virginia coastal bays provides the opportunity to ask a series of questions, which will both increase the theoretical understanding of the impact of genetic diversity and practically contribute to effective conservation and restoration. Six chapters in the dissertation, written as manuscripts to be submitted as co-authored publications, will address the following questions:

Chapter 2: What is the genetic diversity of *Zostera marina* in Chesapeake Bay and in the Virginia coastal bays?

Large disturbances, such as those in Chesapeake Bay that removed large amounts of seagrass biomass (See Orth & Moore 1984, Moore & Jarvis 2010), typically isolate populations and result in a lower genetic diversity (Frankham et al. 2002). Therefore, I hypothesized that the genetic diversity of seagrasses in Chesapeake Bay would be low compared to more stable meadows in other parts of the world. I also hypothesized that meadows in the Virginia coastal bays would have an even greater reduction in genetic diversity than Chesapeake Bay populations because previous studies have shown that

restored seagrass meadows typically have a lower genetic diversity than established meadows (Williams & Davis 1996, Williams 2001).

Chapter 3: How are disturbance history and genetic diversity related in clonal plant systems?

In Chapter 2, I found unexpectedly that both natural and restored *Z. marina* meadows in Chesapeake Bay and in the Virginia coastal bays had high genetic diversity. In Chapter 3, I address how difference in disturbance regimes is related to genetic diversity using these two systems as case studies as well as a regional analysis of sites along the North American Atlantic coast from Nova Scotia to North Carolina. I hypothesized that, because seagrass are clonal plants that also produce a large number of seeds disturbance will increase rather than decrease genetic diversity by opening space where seedlings can survive at greater rates.

Chapter 4: How are plants in the Virginia coastal bay region connected to other regions and how has restoration altered those relationships?

In this chapter, I compare restored meadows with those that have naturally recruited into the region presumably as the result of flowering that broke off and rafted with the currents into the southern Virginia coastal bays (See Harwell & Orth 2002, Källström et al. 2008). Because there are very few, relatively small patches of natural recruitment, I hypothesized that this was a rare event and that the patches were the result of a relatively small seed addition making natural recovery a very slow process. Based on

this, I further hypothesized that those meadows have a lower genetic diversity than the restored meadows we identified as quite diverse in Chapter 2.

Chapter 5: What are the economic implications of this successful seagrass restoration?

In Chapter 4, I found that restoration was faster than natural recovery and effectively created genetically diverse meadows. In this chapter, I used population growth models to estimate the trajectories of both natural recovery and recovery of meadows by seeding and then converted the areal coverage to the economic value that those areas provide in terms of increased nutrient storage and cycling. I hypothesized that natural recruitment would eventually restore this region; however, the acceleration of recovery by seeding would an result in an increase ecosystem services worth more than the amount of money spent on the restoration

Chapter 6: Will incorporating genetic diversity into restoration programs improve the success?

Previous studies have shown that genetically diverse assemblages of seagrass can be more fit (Williams 2001), have increased density, and be more resistant to large disturbances (Hughes & Stachowicz 2004, 2011, Reusch et al. 2005). Restoration efforts are often plagued by disturbance and rely on the high reproduction (increased fitness) for long-term expansion and survival; therefore many restoration guidelines suggest incorporating diversity into restoration plans in an attempt to get restorations that both resist disturbance and recover efficiently (van Katwijk et al. 2009). However, the application of previous results to restoration is difficult since previous studies used

relatively low measures of diversity (unrealistic for the mid-Atlantic region), measures of genetic diversity that could only be applied to clonal communities, and often found results only after nearly catastrophic disturbances (grazing that removed 80% of the biomass (Hughes & Stachowicz 2004), an extreme warming event with a return time over 10000 years (Reusch et al 2005), and an algal bloom that was the largest in 4 years (Hughes & Stachowicz 2011)). We set out to test the robustness of this theory and the applicability towards restoration by using an experiment that mimicked natural restoration, that used realistic levels of diversity, common levels of stress experienced by restoration, and measures of genetic diversity that are broadly applicable to many systems. We hypothesized that genetic diversity would lead to a more successful restoration when measured by the provision of ecosystem services.

Chapter 7: How important is genetic diversity, compared with environmental variability, to the provision of ecosystem services?

In Chapter 6, we found that genetic diversity was important in predicting the density and amount of ecosystem services provided by a seagrass assemblage. Previous research has documented a variety of environmental conditions that also act as good predictors for these variables (i.e. nutrient concentrations (Forqurean & Zieman 1992), salinity (Lirman & Crooper 2003), and light levels (Longstaff et al. 1999)). I hypothesized that while not as widely measured, genetic diversity could improve the understanding of why different seagrass assemblages have such different densities and vary so dramatically.

Chapter 8: Synthesis and Significance

The aim of this dissertation is to interpret the results of these experiments in a way that will both advance ecological theory as well as have practical application towards conservation and restoration science. The final chapter summarizes and synthesize results and places them in the context of our current understanding of population biology, ecological genetics, and coastal ecology.

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Chapter 2: Eelgrass restoration by seed maintains genetic diversity: a case study from a coastal bay system

Abstract

Genetic diversity is positively associated with plant fitness, stability, and the provision of ecosystem services. Preserving genetic diversity is therefore considered an important component of ecosystem restoration as well as a measure of its success. We examined the genetic diversity of restored *Zostera marina* meadows in a coastal bay system along the USA mid-Atlantic coast using microsatellite markers to compare donor and recipient meadows. We show that donor meadows in Chesapeake Bay have high genetic diversity and that this diversity is maintained in meadows restored with seeds in the Virginia coastal bays. No evidence of inbreeding depression was detected (FIS -0.2 to 0) in either donor or recipient meadows, which is surprising because high levels of inbreeding were expected following the population contractions that occurred in Chesapeake Bay populations due to disease and heat stress. Additionally, there was no evidence for selection of genotypes at the restoration sites, suggesting that as long as donor sites are chosen carefully, issues that diminish fitness and survival on account of non-local adaptation can be avoided. A cluster analysis showed that, in addition to the Chesapeake Bay populations that acted as donors, the Virginia coastal bay populations shared a genetic signal with Chincoteague Bay populations, their closest neighbor to the north, suggesting that natural recruitment into the area may be occurring and augmenting restored populations. We hypothesize that the high genetic diversity in seagrasses restored using seeds rather than adult plants confers a greater level of ecosystem resilience to the restored meadows.

Introduction

A high level of genetic diversity in plant populations is associated with increased benefits for plant survival and ecosystem services (Booy et al. 2000). The loss of genetic diversity may cause reduced adaptability to environmental change through loss of fitness (Reed & Frankham 2003). In both marine and terrestrial systems, experimental studies have demonstrated the benefits of genetic diversity to the capacity of populations to resist stressors such as disease, predation, and physical disturbance (Zhu et al. 2000, Hughes & Stachowicz 2004, Reusch et al. 2005, Johnson et al. 2006, Hughes & Stachowicz 2011). In marine systems, lower genetic diversity in the seagrass *Zostera marina* has been shown to reduce survivorship following disturbance (Hughes & Stachowicz 2004, 2011, Reusch et al. 2005). In terrestrial systems, genetically diverse assemblages of primrose plants *Oenothera biennis* were found to serve as a better habitat and support more species of arthropods than less diverse assemblages (Johnson et al. 2006). Also, genetically diverse rice *Oryza sativa* fields have been found to be less susceptible to disease (Zhu et al. 2000). Given the positive benefits associated with higher levels of genetic diversity, it should be considered an essential component of ecosystem restoration.

Strategies to enhance the likelihood of increased genetic diversity through restoration focus on two alternatives. The first is to maximize the use of genetic resources incorporated into captive breeding programs. The second is to use the diversity present in natural populations. Both strategies have been adopted widely, such as when salmon hatcheries that have captive animals sourced from a variety of locations have been used to mitigate population declines in the wild (Waples 1991, 1994). Captive breeding and reintroduction of young into wild populations also have been employed as tactics to

increase the genetic diversity of the endangered Hawaiian thrush and big horn sheep (Kuehler et al. 2000, Ostermann et al. 2001). However, this strategy, where material is sourced from a variety of origins, has also been criticized, because new genotypes are introduced into remnant populations and this potentially results in less fit progeny (Knapp & Dyer 1998).

Many estuarine and coastal areas are experiencing increasing levels of disturbance and/or stress related to human activities, such as eutrophication, low dissolved oxygen, increasing temperatures, and invasive species (Jackson et al. 2001, Lotze et al. 2006, Halpern et al. 2008, Waycott et al. 2009). Knowledge of the value of the plant and animal species that occupy these habitats has resulted in significant efforts to reduce anthropogenic stressors and to emphasize restoration of species and habitats.

Seagrasses (marine angiosperms), of which there are approximately 72 species, are often the dominant macrophytes in estuaries, shallow coastal bays, and lagoons worldwide (Green & Short 2003, Short et al. 2011). Globally, seagrasses are declining (Orth et al. 2006a, Waycott et al. 2009), most often as a result of increasing nutrients and sediments from watersheds being altered by human activities (Waycott et al. 2009). In many degraded systems efforts are being made to mitigate seagrass decline and to improve habitat for seagrass restoration (Greening & Janicki 2006, Orth et al. 2010). There is a growing body of evidence that indicates that genetically diverse assemblages of seagrasses are fitter (Williams 2001) and more resistant to a variety of disturbances (Hughes & Stachowicz 2004, 2011, Reusch et al. 2005). In the seagrass ecosystems dominated by a single species that are typical of northern hemisphere seagrass communities, adopting appropriate restoration strategies to capture adequate levels of

genetic diversity is an important and realistic goal. Monospecific seagrass meadows can act as case studies for evaluating the relative success of implementing different restoration strategies based on maintaining genetic diversity.

Zostera marina (eelgrass) is a seagrass found in temperate and sub-temperate regions of the North Atlantic and North Pacific Oceans and in the Mediterranean Sea (Green & Short 2003). This species of seagrass has been observed to undergo periods of extreme population fluctuations, especially in the North Atlantic (Cottam 1934, 1935). The most notable broad-scale population decline was associated with the spread of *Labyrinthula zosterae*, a fungal parasite, in the 1930s (Rasmussen 1977). While many populations eventually recovered from the impact of this disease (Cottam & Munro 1954), populations in a number of coastal bays in the mid-Atlantic region of the United States did not (Orth et al. 2006b). Most recovery in the Virginia coastal bays is the result of large-scale restoration (Orth et al. 2006b, 2012). While the scale and success of the restoration in the Virginia coastal bays is somewhat unique, mitigation to compensate for seagrass loss through restoration is becoming more globally widespread (Paling et al. 2009, van Katwijk et al. 2009)

One concern surrounding seagrass restoration is the possible loss of genetic diversity when adult plants are used for re-establishing populations (Williams & Davis 1996, Williams 2001). Depending upon the size of the clone, it is entirely possible that adult plants for a small-scale restoration effort could be drawn from a single clone with low genetic diversity. The use of seeds harvested from multiple parents, rather than adult plants, could offset this genetic bottleneck. The successful re-establishment of *Zostera marina* into unvegetated coastal bays in the mid-Atlantic region of the United States

using seeds from a number of source beds (Orth et al. 2012) offered a unique opportunity to test the hypothesis that genetic diversity is not eroded when seeds are used in restoration. Here we present results from our analysis of genetic diversity from both natural *Z. marina* beds in Chesapeake Bay, several of which have served as source beds for restoration, and the restored beds in the Virginia coastal bays.

Materials and Methods

A total of nine *Zostera marina* meadows were sampled in three distinct regions. These included both natural beds in Chesapeake Bay (mouth of the York River, YR; Mobjack Bay, MB; Hungar's Creek, HC; and Fisherman Island, FI), one bay to the immediate north of the restoration sites (Chincoteague Bay, CB), and restored beds in three Virginia coastal bays (South Bay, SB; Spider Crab Bay, SC; and 2 sites in Hog Island Bay, HR6, HR7) (Fig. 1). These Virginia coastal bays are part of the Virginia Coast Reserve Long Term Ecological research site. Several of the Chesapeake Bay sites were sources of seeds (YR, MB, and HC) used in the coastal bay restoration (Table 1). Restored beds sampled in South Bay were seeded from a variety of western Chesapeake Bay sources, including MB and YR. Restored beds sampled in Spider Crab Bay were seeded from SB seeds in 2008. Restored beds sampled in Hog Island Bay were seeded either from Hungar's Creek in 2006 (labeled HR6) or from South Bay (SB) in 2007 (labeled HR7).

Methods for collection, storage, and disbursing of seeds can be found in Marion & Orth (2010). Because we were interested in whether genetic diversity would be maintained in restored beds developed with seeds, we compared donor sites and restored beds for

genetic diversity, resulting in 7 comparisons of donor meadows and recipients. Natural populations at Fisherman Island (FI) and Chincoteague Bay (CB) were also sampled as they represent populations immediately south and north of the restored sites.

At each sampling site, whole seagrass shoots were haphazardly collected by hand from areas approximately 5 m apart, to avoid collecting shoots from the same clones. Leaf tissue was dried and stored at room temperature using silica gel desiccant (AGM, mixture of white and indicating beads) until DNA extraction. All plant samples were collected during the summer months (June to August) of 2008 and 2009.

Total genomic DNA was extracted using DNeasy plant extraction kits (Qiagen) following manufacturer's instructions. A total of 8 microsatellite loci previously used for this species (Reusch et al. 1999) were amplified using fluorescently tagged primers (CT17H, CT3, CT35, GA2, CT19, CT20, GA3, and GA6). Amplification of PCR products followed the procedures recommended by Reusch et al. (1999). PCR products were analyzed by capillary electrophoresis on a MegaBACE 1000 (GE Biosciences) with ET 400-Rox (GE Biosciences) internal size standard in each sample, as per manufacturer's instructions. Fragment lengths for each allele, at each locus, were determined using Fragment Profiler V1.2 (GE Biosciences).

Standard measures of genetic diversity were calculated for each population sampled. Allelic richness (AR), standardized to the smallest population size by rarefaction, was computed using FSTAT 2.9.3.2 (Goudet 2001). Total number of alleles per population (NA), the average number of alleles per locus (A), the average number of low-frequency alleles (A<25%) at each locus, the mean observed (Ho) and mean expected heterozygosity (He), and Wright's inbreeding coefficient (F) were calculated

using GenAlEx 6.3 (Peakall & Smouse 2006). Differences in genetic diversity between donor and recipient populations were analyzed using both a paired t-test and a chi-squared goodness-of-fit test, where paired donor meadows were treated as expected values.

The population structure or relatedness of geographically separated meadows was compared using the standard measure of population differentiation, Fst, calculated in GenAlEx 6.3 (Peakall & Smouse 2006). Within-population inbreeding was estimating using Fis, calculated in GenAlEx 6.3 (Peakall & Smouse 2006). A test of population assignment using Bayesian modeling of all samples was conducted using the software STRUCTURE for assigned numbers of populations of K = 1 to 10 and with 10 replicates with a random start for each value of K (Pritchard et al. 2000). The number of distinct population clusters was determined using the delta K method (Evanno et al. 2005). The relationship between the cluster to which a sample was assigned and geographic origin were analyzed with a Kruskal-Wallis 1-way analysis of variance. Pairwise differences were analyzed with individual Mann-Whitney U-tests, with Bonferroni-corrected alpha values.

Results

Moderate to high levels of allelic diversity were detected across the 9 *Zostera marina* meadows sampled from the Chesapeake Bay, Virginia coastal bays, and Chincoteague Bay. All loci conformed to Hardy-Weinberg equilibrium in at least some of the populations. All populations sampled showed relatively high allelic richness (mean AR = 5.3), with York River having the highest value at 5.7 and Fisherman Island having

the lowest value at 4.4. Across all sites both the observed (mean Ho = 0.72) and expected (mean He = 0.67) heterozygosities were high, which is typical of *Z. marina*. Although in many population samples Ho was greater than He, which resulted in slightly negative inbreeding coefficients (F = –0.2 to 0), the F-values were not significantly different (Table 1).

Restored meadows did not show a significant reduction in allelic richness, mean number of rare alleles, or expected heterozygosity relative to their donor meadows (Fig. 2). A paired t-test between donor and recipient meadows resulted in p-values of 0.39 or greater, and a chi-squared goodness-of-fit using donor values as expected values resulted in p-values equal to or greater than 0.98. The inbreeding coefficient within populations (Fis) approached zero, and there was no significant deviation between donor and recipient pairs (Fig. 2; $t = 0.62$, $df = 3$, $p = 0.58$ and $\chi^2 = 1.08$, $df = 3$ $p = 0.77$).

All Chesapeake Bay sites and restored Virginia coastal bay sites were closely related when analyzed with permuted Fst values (<0.1). Higher pairwise Fst values were observed in comparisons with Fisherman Island and Chincoteague Bay. This was expected as these sites acted as external non-donor recipient reference sites for the present study. Fisherman Island had pairwise Fst values >0.1 with all other meadows. Chincoteague Bay showed a similar deviation from Chesapeake Bay and South Bay sites; however, it had lower pairwise Fst values when compared with Hog Island (2006 and 2007) and Spider Crab Bay, the more northerly restored Virginia coastal bay sites (Table 2).

The relative distinctiveness of sampled meadows was assessed by assigning individuals based on genetically homogenous groups, rather than on sampled locations,

using a Bayesian cluster approach with the software STRUCTURE (Pritchard et al. 2000) and by implementing the ad hoc statistic (ΔK) (Evanno et al. 2005). The highest values for the ΔK statistic identify 4 groups, or genetic populations, that were present among the 9 sampled locations (Fig. 3). Samples from different geographic locations were assigned to each of these 4 different clusters with high probability (Kruskal-Wallis: $\chi^2 = 72$, df = 8, $p < 0.0001$). Significant differences in pairwise comparisons, made with individual Mann-Whitney U-tests using a Bonferroni corrected alpha of 0.001, were observed for many of the comparisons, particularly between reference sites and the donor-recipient locations (Table 3). The southern-most site near the mouth of the Chesapeake Bay, Fisherman Island (FI), was assigned to Cluster 4 and was significantly distinct from each of the other locations. All Chesapeake Bay locations (HC, YR, MB) and the restored meadow at South Bay (SB) were assigned to both Clusters 1 and 2; however, they were not significantly different from one another. The Hog Island Bay beds restored in 2006 (HR6) were not different from the donor meadow of Hungar's Creek (HC). HR6 differed from the York River site and the South Bay site. The Hog Island Bay beds restored in 2007 (HR7) were not different from the South Bay donor meadow, but like HR6 differed from the York site. The Spider Crab Bay site (SC) was similar to the South Bay donor site, as well as the restorations in Hog Island Bay. The northern natural Virginia coastal bay site, Chincoteague Bay, differed from Fisherman Island, all Chesapeake Bay sites, and the older restoration sites of South Bay and HR6. Spider Crab Bay and HR7 were not statistically different from Chincoteague Bay (Table 3).

The proportional assignment of individual samples to each of the 4 modeled genetic clusters supports the observation that the diversity in the restored meadows was

equivalent to that in the donor meadows (Fig. 4). Fisherman Island was distinctive, with few individuals from other sites having a high likelihood of sharing this group; these were assigned to Cluster 4 (Fig. 4). Chesapeake Bay samples (HC, MB, YR), as well as the restored meadows in the Virginia coastal bays, were similar and assigned across Clusters 1 and 2. Neither Cluster 1 nor 2 was specific to 1 location (Fig. 4). Chincoteague Bay samples were distinct and assigned to Cluster 3, along with numerous samples in the Virginia coastal bays, especially Hog Island Bay, which is closest in proximity (Fig. 4). The same patterns persisted when data were grouped as averages of plants collected from 1 location (Fig. 4).

Discussion

Our results demonstrate that the restoration of *Zostera marina* with seeds in Virginia coastal bays has maintained overall population genetic structure and diversity compared to the donor populations. This finding is in contrast to the significant reduction in genetic diversity observed in a restored *Z. marina* meadow in Southern California, USA, where adult plants were used in the restoration effort and were collected from a very small area (200 to 12,000 m^2) (Williams & Davis 1996, Williams 2001). A genetically diverse donor population is required to achieve a genetically diverse restored population, and this was the case with Chesapeake Bay and the Virginia coastal bays. Despite population fluctuations in Chesapeake Bay's *Z. marina* populations in the last 80 years, since the 1930s decline (Orth & Moore 1983, 1984, Orth et al. 2010), current populations exhibit relatively high genetic diversity (Tables 1 & 4).

In the restoration efforts evaluated here, measures of genetic diversity and levels of inbreeding did not differ between the paired donor meadows and recipient meadows (Fig. 1). There is no evidence currently that genotypes are being selected for in the restoration sites. Donor and recipient pairs appear as highly connected, undifferentiated population pairs through high gene flow and low Fst values (Nm ranges between 4.95 and 8.61, while Fst ranges between 0.005 and 0.05), and this supports the conclusion that the donor and recipient populations are genetically comparable. We propose that the success in maintaining genetic diversity in restored populations of the Virginia coastal bays is due to a combination of high levels of genetic diversity present in the donor meadows, collection of seeds from a broad area that does not result in oversampling of closely related individuals, and the introduction of adequate numbers of seeds into donor sites in a manner reflecting relatively 'natural' recruitment processes. While this is an improvement over previous analyses of restorations using adult plants (Williams & Davis 1996, Williams 2001), those studies incorporated plants that were collected from a small area and restorations were relatively small in numbers of transplant units. If adult plants were collected from a large area within a genetically diverse region, reductions in genetic diversity could be improved; however, logistically, it is easier to collect and transplant large numbers of seeds than to transplant large numbers of adult plants. This is underscored by the small scale (<0.5 ha) of most adult transplant restoration efforts (Paling et al. 2009).

Using seeds from local or regional provenances that are likely locally adapted to appropriate environmental conditions would enhance restoration success. In addition to immediate restoration outcomes, the presence of high levels of genetic diversity in

restored populations suggests that the populations will be less likely to show signs of genetic erosion.

Overall, our estimates of genetic diversity are high, but are consistent with the range of values observed in previous studies (Table 4). Olsen et al. (2004) found an insignificant trend of increased diversity with decreased latitude along the western Atlantic coast. Our study adds additional data from closer to the geographic margin of the species, and further supports the observation of a trend of increased diversity with decreased latitude. Compared to the western Atlantic and eastern Pacific populations studied (Reusch et al. 2000, Olsen et al. 2004, Talbot et al. 2004, Coyer et al. 2007, Ort et al. 2010, Wyllie-Echeverria et al. 2010), the Chesapeake and Virginia coastal bay populations described here are more diverse. The only meadows found to have higher values of heterozygosity and numbers of alleles per locus were in Mikawa Bay, Japan (Yoshida et al. 2009; Table 4, present study). The high levels of diversity found in Virginia were unexpected due to the population history of *Zostera marina* in the region. Over the last century, the *Z. marina* meadows in Virginia have experienced many disturbances including disease, reduced water quality and clarity, bioturbation by rays, and high temperature stress (Orth 1975, 1976, Orth & Moore 1984, Moore & Jarvis 2008). The large-scale decline of *Z. marina* populations in the 1930s, which was attributed to disease (Orth & Moore 1984), would be expected to have created a population bottleneck, with subsequent high levels of inbreeding and reduced genetic diversity in remnant populations in the Chesapeake Bay and Chincoteague Bay. While a recently published study found that *Z. marina* populations from both New Jersey and one site in the Chesapeake Bay showed significant signs of inbreeding (Fis > 0.6; Campanella

et al. 2009) (Table 4), our data from Chesapeake and Chincoteague *Z. marina* meadows do not, despite finding similar levels of allelic diversity.

The mechanism by which natural seagrass meadows in Virginia maintain such a high diversity may be quite similar to the mechanism by which restoration by seed maintains high genetic diversity: large numbers of seeds added to open space. The disturbances in Chesapeake Bay (i.e. Orth 1975, 1976, Orth & Moore 1984, Moore & Jarvis 2008) remove seagrass, which reduces competition and thus seedling survival. Phillips et al. (1983) showed that *Zostera marina* flowering increased due to environmental stress and disturbance, which suggests an increase in the source of seeds in disturbed areas. Modeling of clonal terrestrial plants has shown that frequent disturbance and high seedling recruitment can increase overall genotypic diversity (Watkinson & Powell 1993).

Seeds used in the coastal bay restoration sites were collected from as far as 80 km away, as no local source populations were available from the Virginia coastal bays. We detected no direct evidence of genetic erosion through outbreeding depression. Outbreeding depression occurs when locally adapted genotypes interbreed with non-adapted genotypes, resulting in reduced fitness of the progeny (Hufford & Mazer 2003), and usually occurs when different populations mix. Another potential genetic impact of population mixing is heterosis, often referred to as hybrid vigor, that occurs when deleterious alleles are masked or when an increase in heterozygosity results in progeny which are fitter relative to their parents (Hufford & Mazer 2003). Although heterosis is a positive effect of genetic mixing among the first-generation population hybrids, the next generation may experience reduced fitness as deleterious genetic traits are expressed in

future generations. Since genetic structure was maintained by restoration with seeds, as long as donor sites are chosen carefully, these problems are more likely to be minimized.

The use of donor material for restoration from the closest populations, the coastal bay meadows in Chincoteague Bay (CH) or the very small population at the mouth of Chesapeake Bay (FI), may result in problems not encountered when Chesapeake Bay populations were used as donors. These two natural meadows in the Virginia coastal bay region have little gene flow and relatively high Fst values among them and with Chesapeake Bay to the west (Nm ranges between 1.362 and 1.588, Fst = 0.136) (Table 2 & Table 5). Because they are geographically separated and differ genetically, it is possible they may have acquired distinct adaptations due to selection for fitness to local conditions or randomly through genetic drift. Using seeds from these locations could result in outbreeding depression if environmental conditions differ from those in the restoration sites.

We observed the genetic signature of Chincoteague Bay in the restored Virginia coastal bays, principally Hog Island Bay (HB6). When all samples were analyzed using a Baysian cluster model, four distinct genetic clusters emerged, with Chincoteague Bay being relatively unique, except for a few samples in the more northern coastal bays (Fig. 4). This genetic signature could have been the result of two alternative mechanisms. First, small-scale (4 m^2) test plots in South Bay seeded in 1999 used plants from Chincoteague Bay. These plots spread rapidly, and it is possible that flowering shoots with seeds could have drifted to Hog Island Bay and released seeds. Alternatively, flowering shoots with seeds could have drifted out of Chincoteague Bay south along the Atlantic coast and entered the coastal inlet near Hog Island Bay, releasing seeds as they floated over the

bay. Flowering shoots with mature seeds can disperse long (150 km) distances (Harwell & Orth 2002, Källström et al. 2008), and it was suggested that natural recruits observed in 1997 in South Bay may indeed have developed from Chincoteague populations (Harwell & Orth 2002). Based on these previous studies, the Virginia coastal bays are within the colonization envelope of Chincoteague Bay *Zostera marina* populations. The detection of Virginia coastal bay *Z. marina* populations that share a specific genetic signal with Chincoteague Bay (Fig. 4) suggests recruitment via such long-distance dispersal events is likely occurring, although a more targeted analysis would be needed to confirm the most likely source. Natural recruitment into the area suggests that a slow recovery may have already begun before restoration intervention was initiated.

The present study demonstrates that large-scale *Zostera marina* restoration with seed as the source of propagules maintains comparable levels of genetic diversity in donor populations. The donor meadows used in our study had a high genetic diversity, and the subsequent high diversity in the restored areas likely contributed to the success of the restoration by increasing resistance to ecosystem disturbances (for discussion see Hughes & Stachowicz 2004, 2011, Reusch et al. 2005). The positive effect of high genetic diversity is not limited to marine systems, and the use of seeds in the restoration of clonal terrestrial plants might also be advantageous. It should be noted that the Virginia coastal bays experience good water quality (www1.vcrlter.virginia.edu/home1/?q=data_wq), and this has undoubtedly been important to the restoration success in this area given that eutrophication is the most common cause of seagrass loss (Orth et al. 2006a). Where restoration attempts are made with marginal water quality, stresses and disturbances are likely to reduce plant growth and survival. Previous studies suggest that genetically

diverse assemblages of seagrass will be better at surviving disturbances, such as intense grazing events, temperature stress, and algal blooms (Hughes & Stachowicz 2004, 2011, Reusch et al. 2005). The present study also suggests that source material for *Z. marina* restoration can be collected from a relatively great distance away from the recipient site without a concern for genetic problems such as outbreeding depression.

The maintenance of genetic diversity can be used as one measure of restoration success, since high genetic diversity is associated with increased benefits for plant survival and ecosystem services (Booy et al. 2000). In our system, we demonstrate a method of restoration that maintains genetic diversity, and the results of that restoration are positive in terms of increased seagrass coverage and feedbacks on sediment and water-quality characteristics (Hanson & Reidenbach 2012, McGlathery et al. 2012, Orth et al. 2012).

Tables

Table 1. Summary of multilocus genetic diversity estimates for all 9 populations

Sites refer to locations shown on Fig. 1. N: sample size; NA: total number of alleles per population; AR: allelic richness; A: average number of alleles per locus; A<25%: uncommon alleles; Ho: observed heterozygosity; He: expected heterozygosity; F: Wright's inbreeding coefficient. Calculations are based on 8 microsatellite loci.

Site	Site description	N	NA	AR	A	A<25%	Ho	He	F
Fisherman Island (FI)	Southern natural meadow at the mouth of Chesapeake Bay	46	47	4.4	6.7	0.6	0.7	0.6	–0.2
Hungar's Creek (HC)	Natural Chesapeake Bay meadow	48	71	5.5	10.1	0.6	0.8	0.7	–0.1
Mobjack Bay (MB)	Natural Chesapeake Bay meadow	30	63	5.4	9	0.6	0.6	0.6	0
York River (YR)	Natural Chesapeake Bay meadow	30	66	5.7	9.4	0.7	0.7	0.7	0
South Bay (SB)	VA coastal bay meadow restored in 2002 using seed from MB and YR	95	79	5.6	11.3	0.7	0.7	0.7	0
Hog Island Bay (HR6)	VA coastal bay meadow restored in 2006 using seed from HC	167	94	5.6	13.4	0.7	0.7	0.7	–0.1
Hog Island Bay (HR7)	VA coastal bay meadow restored in 2007 using seed from SB	46	66	5.3	9.4	0.7	0.8	0.7	0
Spider Crab Bay (SC)	VA coastal bay meadow restored in 2008 using seed from SB	48	70	5.3	10	0.7	0.8	0.7	–0.2
Chincoteague Bay (CH)	Northern natural VA coastal bay meadow	48	60	4.6	8.6	0.6	0.7	0.6	–0.1

Table 2. Pairwise Fst estimates for all 9 *Z. marina* populations based on 8 microsatellite loci.

*: values not significantly different from zero

	FI	HC	MB	YR	SB	HR6	HR7	SC	CH
Fisherman Island (FI)	0								
Hungar's Creek (HC)	0.124	0							
Mobjack Bay (MB)	0.187	0.048	0						
York River (YR)	0.159	0.031	0.012	0					
South Bay (SB)	0.143	0.019	0.014	0.005*	0				
Hog Island Bay (HR6)	0.117	0.006	0.056	0.031	0.023	0			
Hog Island Bay (HR7)	0.114	0.03	0.095	0.062	0.044	0.018	0		
Spider Crab Bay (SC)	0.109	0.026	0.082	0.051	0.046	0.014	0.037	0	
Chincoteague Bay (CH)	0.136	0.092	0.148	0.111	0.095	0.064	0.067	0.023	0

Table 3. Staistical Separation of *Zostera marina* populations.

The *Zostera marina* samples from each of 9 geographically separated meadows were assigned to different genetically distinct clusters using STRUCTURE. Mann-Whitney U-tests were used to determine differences in populations, and p-values are reported

	FI	HC	PC	PR	SB	HR6	HR7	SC	CH
Fisherman Island (FI)									
Hungar's Creek (HC)	<0.0001								
Mobjack Bay (MB)	<0.0001	0.4							
York River (YR)	<0.0001	0.004	0.2						
South Bay (SB)	<0.0001	0.2	0.9	0.202					
Hog Island Bay (HR6)	<0.0001	0.06	0.004	<0.0001	<0.0001				
Hog Island Bay (HR7)	<0.0001	0.03	0.002	<0.0001	0.0003	0.05			
Spider Crab Bay (SC)	<0.0001	<0.0001	<0.0001	<0.0001	0.002	0.0004	0.9		
Chincoteague Bay (CH)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.12	0.004	

Table 4. Summary of multilocus microsatellite-based genetic diversity around the world.

AR: allelic richness; A: average number of alleles per locus; He: expected heterozygosity; Fis: inbreeding coefficient

Region	Location	Allelic Richness (AR)	Average Number of Alleles per Locus (A)	Expected Heterozygosity (H_e)	Inbreeding Coefficeint (F_{is})	Citation
Eastern Pacific	San Juan Archipelago Washington, USA	2.3–3.1	2.5–10.4	0.29–0.51	-0.11–0.05	Wyllie - Echeverria et al. 2010
	San Francisco Bay California, USA	2.7–5.4	3.1–6	0.24–0.60	-0.2–0.19	Ort et al. 2010 Talbot et al. 2004
	Eastern Pacific: USA and Canada	4.9–6.4		0.17–0.51	-0.14–0.16	Olsen et al. 2004
	Baja California, Mexico		1.5–4.0	0.13–0.54	-0.04–0.36	Coyer et al. 2008
Western Pacific	Mikawa Bay Japan		10.5–12	0.70–0.85	-0.04–0.03	Yoshida et al. 2009
Eastern Atlantic	Baltic Sea, Germany		5–5.9	0.40–0.44	-0.02–0.09	Reusch et al. 2000
	Baltic Sea, Finland	1.17		0.17–0.44	-0.67–0.01	Olsen et al. 2004
	Margot France	3.33		0.32	0.01	Reusch et al. 2000
	Eastern Atlantic, Europe	1.5–2.7		0.33–0.52	-0.26–0.038	Olsen et al. 2004
Western Atlantic	Nova Scotia, Canada	5.67		0.46	-0.022	Reusch et al. 2000
	Western Atlantic Ocean:USA and Canada	2.9–4.9		0.41–0.56	-0.09–0.19	Olsen et al. 2004
	New Jersey coastal bays, New Jersey USA		6.8–10.5	0.69–0.83	0.55–0.71	Campanella et al. 2009
	Chesapeake Bay Virginia USA	**5.4–5.7**	**9–10.1**	**0.6–0.7**	**-0.1–0**	**This Study**
	Coastal Bays Virginia, USA	**4.6–5.6**	**6.7–9.1**	**0.6-–0.7**	**-0.2–0**	**This Study**

Table 5. Pairwise estimates of gene flow for all 9 *Z. marina* populations based on 8 microsatellite loci.

Values above the diagonal are Nm values calculated based on Fst in GenAlEx 6.3 (Peakall & Smouse 2006), and values below the diagonal are calculated based on rare alleles using GenePop (Raymond & Rousset, http://wbiomed.curtin.edu.au/genepop/index.html)

	FI	HC	MB	YR	SB	HR6	HR7	SC	CH
Fisherman Island (FI)		1.762	1.084	1.325	1.494	1.883	1.948	2.037	1.588
Hungar's Creek (HC)	1.168		5.006	7.763	13.249	42.906	7.988	9.348	2.469
Mobjack Bay (MB)	0.516	5.608		20.989	18.067	4.193	2.387	2.792	1.435
York River (YR)	0.639	4.678	3.731		54.986	7.734	3.764	4.641	1.994
South Bay (SB)	0.682	6.234	5.663	4.945		10.448	5.402	5.243	2.38
Hog Island Bay (HR6)	1.362	8.608	6.058	8.245	9.235		13.327	17.192	3.632
Hog Island Bay (HR7)	1.307	4.366	2.194	2.603	4.974	10.833		6.449	3.485
Spider Crab Bay (SC)	1.28	7.123	4.049	4.089	6.8	13.257	6.953		10.536
Chincoteague Bay (CH)	1.362	3.262	3.311	2.689	3.68	4.233	4.089	4.705	

Figures

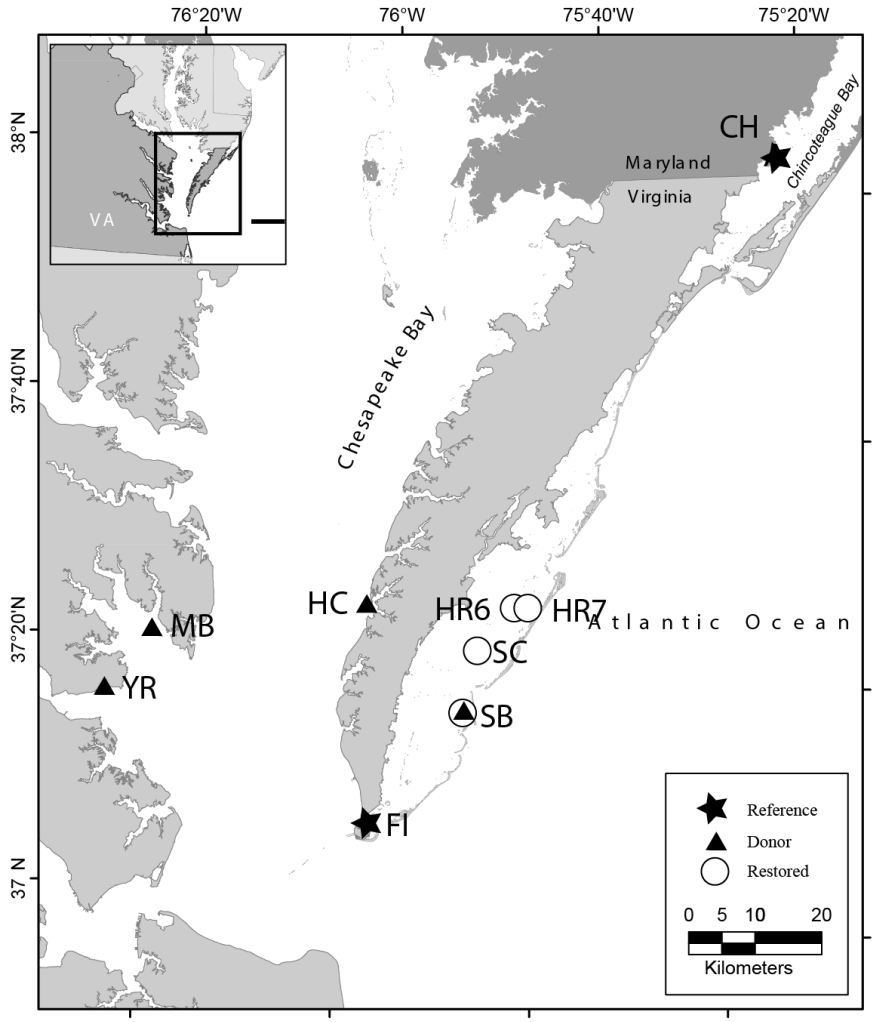

Fig. 1. Site Map.

Chincoteague Bay (CH), Fisherman Island (FI), Hungar's Creek (HC), Mobjack Bay (MB), and York River (YR) are natural *Zostera marina* meadows. Seeds from various Chesapeake Bay sites including MB and YR were used to restore South Bay (SB). HC was used as a donor for HR6 in 2006, and seeds from the restored meadow in SB were used in a restoration in Hog Island Bay in 2007 (HR7) and in a restoration in Spider Crab Bay (SC) in 2008

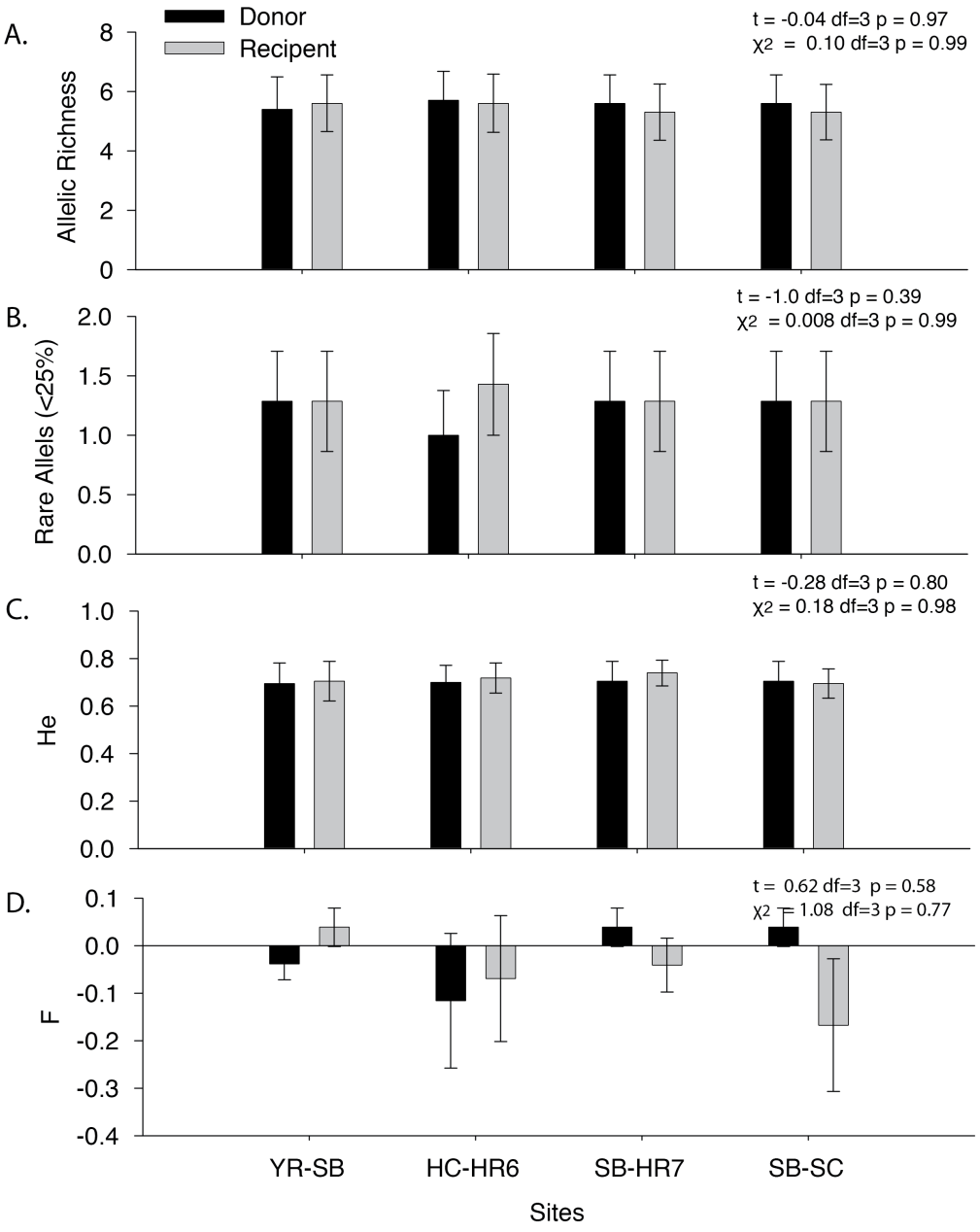

Fig. 2. Comparison of donor and recipient meadows.

Four measures of genetic diversity were used to compare donor (dark) and recipient (light) *Z. marina* meadows (±standard error): (A) allelic richness, (B) frequency of rare alleles (<25%) per population, (C) expected heterozygosity (He), and (D) Wright's inbreeding coefficient (F). Site abbreviations, see Fig. 1

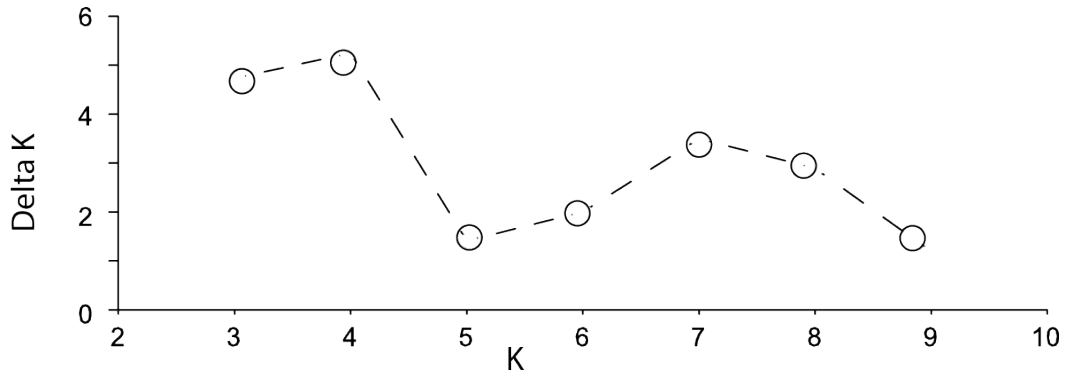

Fig. 3. Evanno Plot of STRUCTURE Results.

The 9 geographically separated *Zostera marina* meadows sampled were grouped into 4 genetically distinct clusters based on the ad hoc statistic ΔK. ΔK was calculated based on 10 runs of the model following Evanno et al. (2005)

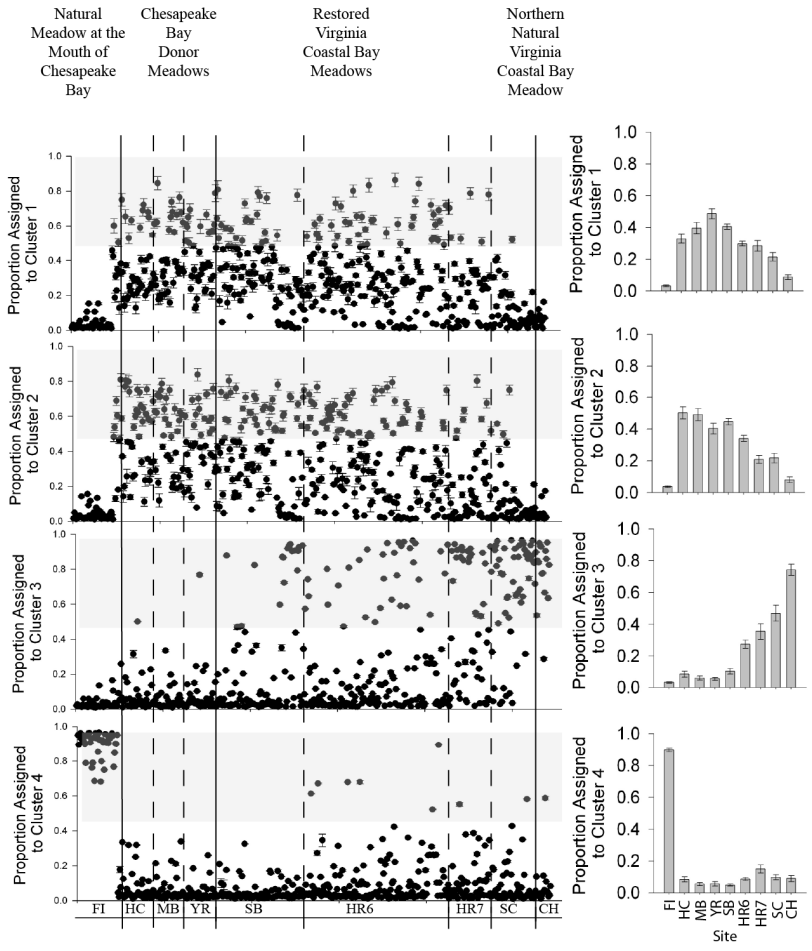

Fig. 4. Cluster assignments of *Zostera marina* populations.

Each sample was assigned to 1 of 4 genetic clusters using the Bayesian cluster model STRUCTURE (Pritchard et al. 2000), and samples tended to cluster regionally. Plots of outcomes for proportional assignments of individual samples to each of the 4 genetic clusters based on 10 independent runs are presented. For each panel, the left plot depicts the mean proportional assignment (±SE) for each individually sampled seagrass shoot; the grey shaded area highlights where assignment to the cluster was >0.5. The right plot depicts the mean proportion assigned to that genetic cluster for all samples collected within that location (±SE). Samples are arranged by geographical location from south to north. Site abbreviations, see Fig. 1

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Chapter 3: Disturbance enhances genetic diversity of eelgrass (*Zostera marina*) meadows

Abstract

Large disturbances to populations are typically characterized by reduction in biomass, decreased size and increased isolation, which reduce genetic diversity and thus overall fitness. This may not hold true for clonal plants such as seagrasses where stresses, defined as conditions that reduce rate of growth, increase the rate of sexual reproduction and decrease growth rates. Increases in stress can lead to shoot mortality and removal of biomass (defined as disturbance), creating gaps where seedlings can establish without competition from clones. By increasing the survival of seedlings, stress and disturbance may shift the balance of reproduction in seagrasses towards sexual as opposed to asexual, effectively increasing genetic diversity. We evaluated the impact of stress and disturbance on genetic diversity of eelgrass (*Zostera marina*) using three case studies. In Hog Island Bay, 4000 m^2 plots planted over a depth gradient show an increase in genetic diversity, evaluated using microsatellites, in deeper waters where the plants often experience light stress and flower in greater proportions. Chesapeake Bay meadows that suffered significant temperature-driven dieback had a positive trend of genetic diversity over time, while meadows that did not die back had a negative trend in diversity over time. Finally, a regional survey demonstrated that plant genetic diversity increased at the southern geographical margin where plants are often stressed and regularly die back in the late fall due to high temperatures. It has been established that genetically diverse eelgrass assemblages are more resistant to disturbances; therefore the response of increased genetic diversity after disturbance could have ecological implications where populations become more resistant to further disturbance.

Introduction

Environmental stresses are unfavorable conditions that cause a variety of plant response: from changes in photosynthesis, to changes in tissue nutrient concentrations, to changes in reproductive strategy, and even to population loss at the most severe stress level (See Collier et al. 2011). Disturbances are events that result in plant mortality and thus can be part of the stress continuum. Large disturbances result in smaller, isolated populations, which often suffer from bottlenecks, characterized by high rates of inbreeding and lowered genetic diversity (Frankham et al. 2002). This loss in genetic diversity often results in decreased population fitness. For example, reductions in lion and cheetah populations because of hunting have resulted in sperm abnormalities and reduced fertility (Wildt et al. 1983, 1987). Prolonged drought in the Sanoran desert resulted in desiccated pools, isolating populations of topminnows, and those isolated populations showed physical deformities, increased susceptibility to parasites, and poorer adaptability to low oxygen conditions (Vrijenhoek et al. 1992, 1994). The impacts of large disturbances on clonal marine plants, however, may not have the same negative effects. In the Baltic Sea, seagrass meadows that experienced frequent, large-scale grazing disturbances did not have a reduced genetic diversity compared to nearby reference sites (Hammerli and Reusch 2003).

The relationship between diversity and disturbance regimes may be more complex in clonal systems (i.e. seagrass) than in non-clonal systems because clonal organisms have the ability to reproduce both sexually and asexually. In addition to adapting to new conditions via sexual reproduction and genetic recombination (Jackson and Hughes 1985, Sackville Hamilton et al. 1987, Honnay and Bossuyt 2005), advantageous genotypes can

proliferate clonally. The balance between these two methods of reproduction can vary with time, environmental conditions, and species-specific characteristics. In the absence of disturbance, clonal reproduction produces physically connected ramets. That integration allows them to share resources and take advantage of stored carbohydrates (See Stuefer 1998), giving clonal recruits an advantage over sexual recruits, which have few reserves and must compete with adult plants. This competitive advantage can be reduced by disturbance, which breaks physical connections between ramets and creates gaps where seedlings are not in direct competition with clones. Further, high stress or disturbance conditions can cause a shift reproductive strategies (Collier et al. 2011). In many species including *Zostera marina*, stresses such as high temperature increase the rates of sexual reproduction (DeCock 1981). A shift to sexual reproduction and the increased success of seedling survival will result in increased genetic diversity because only sexual reproduction can create new genotypes.

Seagrass meadows are ideal systems to study the relationship between disturbance and genetic diversity in a clonal species. Sensitive tools (i.e. microsatellites) are available to describe accurately the genetic variability of many seagrass species (Procaccini and Waycott 1998, Reusch et al. 1999, Van Dijk et al. 2007). Furthermore, because seagrasses are distributed along populated coastlines, they are often impacted by human activities, such as decreases in water quality and water clarity, which can cause seagrass loss (Short and Wyllie-Echeverria 1996, Waycott et al. 2009). Declines in seagrasses result in the loss of important ecosystem services such as habitat provisioning, sediment stabilization, and nutrient retention. Understanding the relationship between disturbance and plant persistence and fitness is important to effective conservation.

Because seagrass species vary physiologically (i.e. in the relative reliance on sexual versus asexual reproduction), there is mixed support in the literature for the positive relationship between disturbance and genetic diversity. In the Wadden Sea and in Chesapeake Bay, *Z. marina* has high flowering rates, high population connectivity, and high genetic diversity in marginal habitats where disturbance is high (Ferber et al. 2008, Reynolds et al. 2012—Chapter 2). However, in some parts of its distributional range (i.e. Portugal), *Z. marina* exhibits a different physiological response to disturbance. It produces few seeds and has low clonal diversity and expected heterozygosity, despite being near the geographical margin where it experiences regular disturbances (Billingham et al. 2003). Likewise, larger-bodied seagrasses, like *Thalassia testudium* and *Posidonia oceanica* that rely heavily on clonal rather than sexual reproduction, show a decrease in genetic diversity where habitats are marginal and disturbances are frequent (Bricker 2009, Diaz-Almela et al. 2007). In these cases the physiological response to severe stress and disturbance is a shift in reproductive strategy towards cloning, so when disturbance removes biomass and opens space but there is a lack of seed to take advantage of the reduced competition from larger clones.

In this study, we evaluate the hypothesis that disturbance enhances genetic diversity, using *Zostera marina* meadows as a model system of a clonal plant with a high rate of sexual reproduction. We consider three different disturbances which act on three different spatial scales: a depth gradient within a meadow where deep areas have reduced seagrass density due to light stress, entire meadows that have experienced large, temperature-driven die-backs, and a regional-scale from the middle of the geographical

distribution to the margin where meadows are temperature stressed and often exhibit yearly temperature-driven die-backs.

Methods

Stress Gradient Within a Meadow

Hog Island Bay is a shallow coastal bay located within the Virginia Coast Reserve Long Term Ecological Research (VCR LTER) site on the eastern shore of Virginia. At this site, 1-acre plots were planted with *Zostera marina* seeds as part of an experimental ecosystem restoration. These plots were planted over a depth gradient from 0.9–1.6 m MSL, and plots at the deep extreme have shown stress from low light conditions, resulting in lower densities (McGlathery et al. 2012).

At each of 9 one acre restored *Z. marina* meadows, we assessed the relative sexual reproductive effort and the overall genetic diversity. In May 2011 (peak flowering season) at each experimental 1 acre plot 10, 0.5 m x 0.5 m quadrats were randomly sampled, and the total number of seagrass shoots and number of flowering shoots were counted. Relative sexual reproductive effort was estimated as the proportional of total shoots that were flowering.

In addition, 24 whole seagrass shoots were collected for genetic analysis, leaving at least 3 m between samples to avoid collecting plants that were part of the same intact clone. Leaf tissue was dried using silica gel desiccant and stored at room temperature until DNA analysis was conducted. Tissue DNA was extracted using DNeasy™ plant

extraction kits (Qiagen) following manufacturer's instructions. Extracted DNA was amplified at 7 polymorphic loci previously described for this species (CT17H, CT3, CT35, GA2, CT19, CT20, GA3) using standard PCR techniques (Reusch et al. 1999). PCR products were analyzed using capillary electrophoresis on a MegaBACE 1000 (GE Biosciences). Average number of alleles per locus (A) and expected heterozygosity (H_e) were calculated for each plot using GenAlEx 6.3 (Peakall and Smouse 2006). Allelic richness (A_R) standardized to the smallest population size by rarefaction, was computed using FSTAT 2.9.3.2 (Goudet 2001).

Relationships between stress and both sexual reproductive effort and overall genetic diversity were explored using a regression with depth (a proxy for stress).

Meadow Scale

The summer of 2010 was a particularly warm summer in Chesapeake Bay, with frequent temperatures above 30°C resulting in large-scale seagrass die-back (Moore et al. 2012). Two meadows in Chesapeake Bay, Allen's Island at the mouth of the York River and Pepper Creek in Mobjack Bay) were sampled at three different times: in the summer of 2009 before the die-back, in the fall of 2010 immediately after the die-back, and in the summer of 2011 during plant recovery. Additionally in 2009 and 2011, plants were collected from South Bay, within the Virginia Coast Reserve, where meadows were not negatively impacted by high temperatures in the summer of 2010 (Moore et al. 2012). At each sampling period, 48 shoots were haphazardly collected, processed, genotyped, and analyzed for alleles per locus, expected heterozygosity, and allelic richness as above.

Differences in diversity over time were analyzed using a regression, and the differences in allelic richness between the initial and final samplings were analyzed with a t-test.

Regional Scale

A literature search resulted in 29 *Z. marina* meadows that were sampled in a similar manner and analyzed at the same microsatellite loci (Olsen et al. 2004, Chapter 4). These sites spanned the western Atlantic Coast from Nova Scotia Canada to North Carolina USA, representing the southern half of the geographical extent of this species along the eastern coast of North America (Green and Short 2003). In the southern portion of this region, temperatures are consistently near the lethal limit for this plant, and some meadows often die back in the summer exhibiting a semi-annual life cycle (Jarvis 2009).

We hypothesized that plants near the southern geographical margin were most stressed by temperature and even experience die-back indicative of disturbance and thus we used latitude as a stress gradient leading to disturbance at the margin. The relationship between genetic diversity and stress was assessed with a regression.

Results

Within Meadow

In Hog Island Bay, where plant density was low at the deepest extent (McGlathery et al. 2012), relative sexual reproduction also increased with depth (R^2=0.4,

p=0.06) (Fig 1). The increase in sexual reproduction resulted in an increase in allelic richness with depth (R^2=0.7, p=0.008) (Fig. 1).

Among Meadows

The two eelgrass meadows in Chesapeake Bay that experienced a temperature-driven die-back showed a strong, but statically insignificant, positive relationship with allelic richness between the sampling periods (Allen's Island: m=1.4, R^2=0.9, p=0.2 and Pepper Creek: m=0.1, R^2=0.3, p=0.2). South Bay, the meadow that did not die back, showed a negative relationship with diversity. There was only a statistically significant difference in starting and ending diversity in the temperature stressed meadow at Allen's Island (t=2.2, p=0.05 vs Pepper Creek: t=0.4, p=0.7 and South Bay: t=0.95, p=0.3).

Regional Scale

Along the U.S. Atlantic coast *Z. marina* allelic richness decreased with latitude (R^2=0.33, p=0.001), with highest diversities near the geographical margin where plants are often temperature stressed and can subsequently exhibit a semi-annual life cycle (Jarvis 2009). The analysis was rerun without the most northerly population to ensure the interpretation of the results, and the relationship was still significant (R^2=0.20, p=0.04).

Discussion

In this region, previous studies have shown that seeds can be essential in the recovery of *Zostera marina* meadows from disturbance (Jarvis and Moore 2010), and in

this study, we demonstrate using three case studies that *Z. marina* can have enhanced diversity following disturbed conditions. This increase in genetic diversity can be important since elevated genetic diversity in this plant is associated with resistance to a variety of disturbances (Hughes and Stachowicz 2004, Reusch et al. 2005, Hughes and Stachowicz 2011, Chapter 6). This increased resistance can be evident with only a small increase in diversity. Reynolds et al. showed that experimental plots survived low-light conditions longer with an increase in diversity of only 1.1 alleles per locus (Chapter 6), which is smaller than the increase in allelic richness that we observed over a within-meadow light-stress gradient (difference in number of alleles per locus was 2.0) (Fig. 1) and in a meadow that was recovering from a heat disturbance (Fig. 2). Because disturbances, such as non-optimum temperatures, grazing events, algal blooms, and low light conditions that induce die-back, often occur repeatedly at specific sites, this increase in diversity and subsequent elevation of resistance to environmental disturbances could have ecological impacts minimizing coverage loss during future events.

The severity and duration of disturbance may be influence the degree to which the genetic diversity of the system changes. Hammerli and Reusch (2003) suggested that swan grazing in the Baltic alleviated dominance of a well-adapted clone. This is analogous to the 'intermediate disturbance hypothesis' where there is an optimum removal of top competitors by disturbance or predation which will enhance species diversity, with dominance by well-adapted species at lower disturbance and survival of only a few species at the highest disturbance level (Connell 1978). All of the stresses and disturbances in our study were relatively severe, and in at least 2 cases (the Hog Island Bay transect and the regional transect) further disturbance would have resulted in a

complete loss of plants and thus diversity. In Hog Island Bay, deeper areas along the depth gradient are void of seagrasses (See McGlathery et al. 2012), and along the U.S. Atlantic Coast, more southerly warmer areas are dominated by a different seagrass species. In both of these cases, the relationship between disturbance and genetic diversity appears linear (Fig. 1 and 3). This may suggest that in this species and in this region of the geographical range, the relationship between disturbance and genetic diversity more resembles a tipping point (Fig. 1 and 3) rather that the common bell shaped response predicted by the intermediate disturbance hypothesis. Likewise, Reusch did not find support for intermediate disturbance hypothesis when he manipulated disturbance in 1 m x 1 m plots (2006).

The positive relationship of disturbance and stress with genetic diversity observed in this study would decrease with an insufficient supply of seed, which can occur from either lack of seed production or from seed loss. *Z. marina*, has a relatively short-lived seed bank (<1 year) (Orth and Moore 1983, Moore et al. 1993), so disturbances that occur prior to that season's flowering may result in a lack of seed. Losses of seed to burial, scouring, or herbivory in disturbed areas would also limit seedling recruitment and successful sexual reproduction, thus limiting the enhancement of genetic diversity. This may explain the relatively small, statically insignificant increase in diversity at the thermally disturbed area at Pepper Creek. There was less overall recovery of *Z. marina* at that site compared to Allen's Island (pers. obs.) and some colonization by *Ruppia maritima*, which might compete with and limit the success of *Z. marina* seedlings. Delayed recovery and enhancement of diversity could still occur with an influx of seeds into the area. Seeds can be naturally imported into the meadow from adjacent undisturbed

areas. Flowering shoots can break off and raft with currents, carrying seeds over long distances (>100 km) into new areas (Harwell and Orth 2002, Kallstrom et al. 2007). This region is characterized by high rates of dispersal and connectivity among populations, with few populations in this region showing any signs of isolation or inbreeding depression (Chapter 4). These site characteristics and the external source of genotypes likely facilitates the observed positive relationship between disturbance and genetic diversity and lends one explanation of the unexpected increase in genetic diversity at the geographical margin of this species (Eckert et al. 2008). The impacts of glacial history on eelgrass populations along the east coast of the US cannot be discounted as a driver for these patters (Olsen et al. 2004).

High flowering rates and population connectivity, however, are not sufficient to observe positive trends in genetic diversity. South Bay, the Virginia coastal bay site, consistently had a high rate of flowering (~25% of total shoots flower, pers. obs. 2007–2009) and is highly connected to other populations in the region (Chapter 4); however, we did not observe a positive trend in genetic diversity over time (Fig. 2). This site, while physically close to the two Chesapeake Bay sites, differs in environmental characteristics. It consistently has lower temperature and clearer water than the Chesapeake Bay sites (Moore et al. 2012). The summertime temperatures in South Bay were consistently above 20–25°C, which is known to trigger flowering (DeCock 1981). However, the slightly warmer water (1°C difference) combined with slightly less light reaching the plants (difference K_d= 0.5 m^{-1}) in Chesapeake Bay not only induced flowering but caused die-back (Moore et al. 2012) that might have increased the rate of seedling success, thus creating a trend of increased diversity at those sites (Fig. 2). Analogously, shallow plots

in Hog Island Bay were more dense, so even though they had a lower percentage of shoots that were sexually reproducing, seed production was still high. However, the density of the plots likely inhibited the success of seedling survival, and thus those plots may rely more on cloning and have a reduced genetic diversity (Fig. 1).

Anthropogenic disturbances are causing loses of seagrass worldwide (Short and Wyllie-Echeverria 1996). Since seagrasses are foundation species that are important in maintaining good water quality and acting as a habitat and nursery for many ecologically and economically important species, this loss has cascading effects (Orth and van Montfrans 1987, Duarte et al. 2008). Previous experimental studies have shown that genetically and genotypically diverse assemblages of these plants better resist a wide range of disturbances, including high temperature and low light, than genetically or genotypically depauperate assemblages (Hughes and Stachowicz 2004, Reusch et al. 2005, Hughes and Stachowicz 2011, Chapter 6). This study demonstrates that *Zostera marina* has an evolutionary response of enhanced diversity to these same disturbances, which can create a positive feedback where disturbance will increase the resistance of the community to further disturbance.

Figures

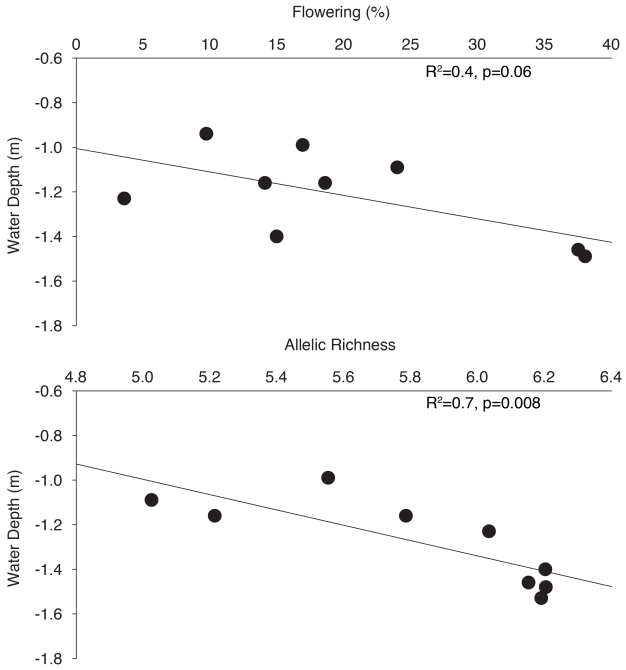

Fig. 1. Within meadow light gradient.

One acre plots were planted in Hog Island Bay, Virginia, USA with 100,000 *Zostera marina* seeds (n=9). The plots spanned a natural depth gradient from 0.9–1.6m. Previous research in this area has shown that light limitation is a stress over this depth gradient, and at the densities will be very low at the deepest depth (McGlathery et al. 2012). (A) Relative sexual reproductive effort was assessed over the stress gradient by counting the proportion of total reproductive shoots in 1/4m^2 quadrats (n=9). (B) Allelic richness was calculated using rarefaction from 24 samples analyzed at 7 microsatellite loci.

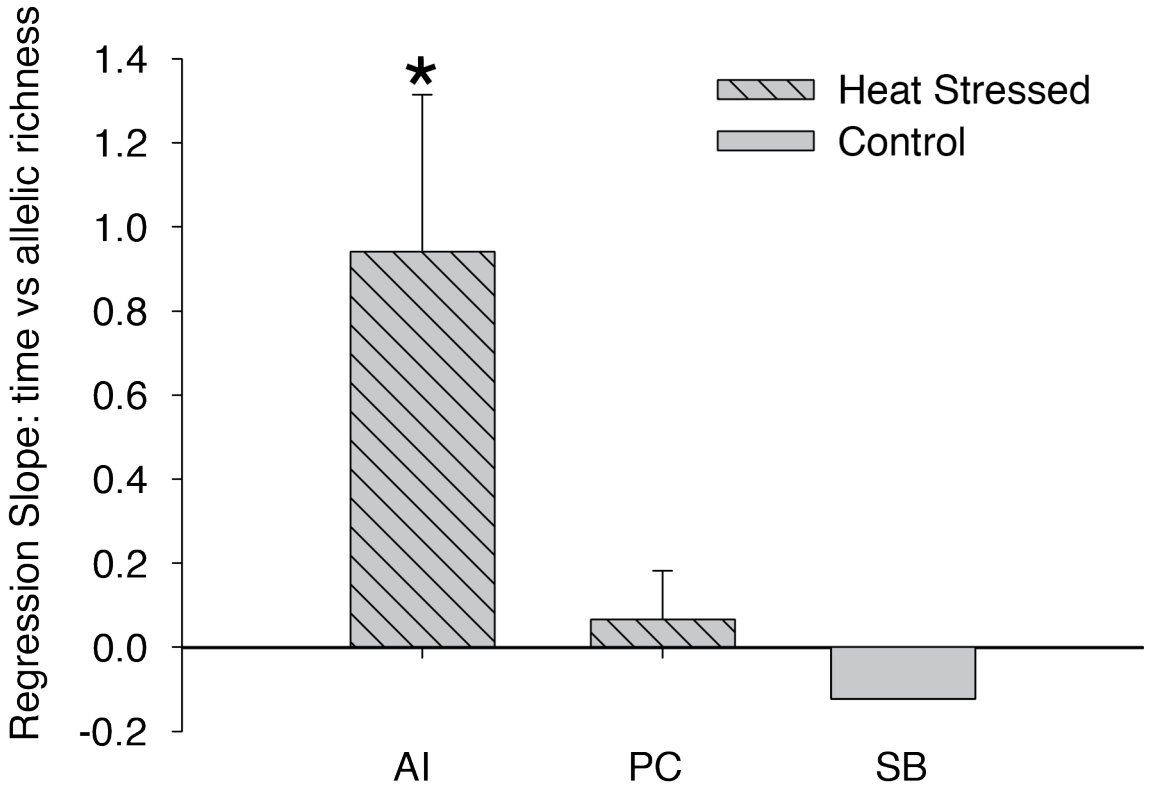

Fig. 2. Temperature induced die-back.

In 2010, two *Zostera marina* meadows in Chesapeake Bay (Allen's Island (AI) and Perrin River (PR)) experienced large-scale die backs due to very warm temperatures. South Bay (SB), in the southern Virginia coastal bay system has a higher oceanic exchange rate, thus lower temperatures and did not experience a die-back. The change in genetic diversity (AR) over time (the slope of the regression) is plotted for each meadow. The rate for Soth Bay (SB) was calculated with only two points and therefore lacks an error bar. A * represents a statistically significant difference in beginning and ending genetic diversity (allelic richness).

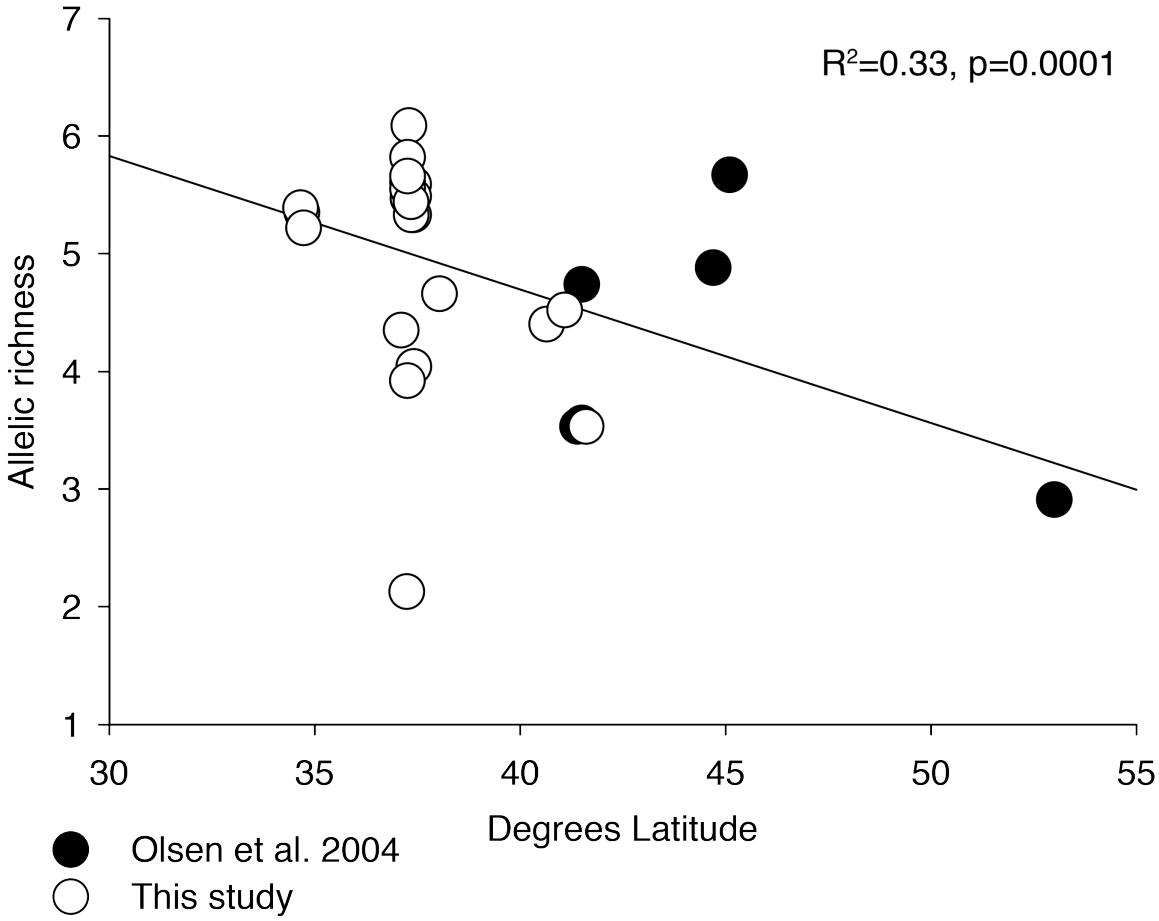

Fig. 3. Latitudinal gradient.

All 29 meadows spanned a region from the southern geographical limit of the species in North Carolina, USA (34°N) to near the center of the geographical distribution in Quebec, Canada (53°N). This represents a temperature gradient from moderate temperatures to a near lethal limit at the southern latitudes where plants regularly die back in the fall. A regression was used to evaluate the relationship between genetic diversity and temperature stress.

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Chapter 4: Restoration efforts recover landscape genetic connectivity in a dispersal-limited ecosystem

Abstract

Eelgrass (*Zostera marina*) populations in the Virginia coastal bays were eradicated by a disease in the 1930s. Only small patches of eelgrass naturally recovered, and most recovery was achieved by restoration using seeds. We surveyed 23 eelgrass populations along the Western Atlantic seaboard to evaluate population genetic structure and connectivity among restored and naturally recruited meadows. We determined that while populations were genetically distinct, there was considerable migration among populations. Over the 1000 km coastline surveyed, F_{ST} values ranged from 0 to 0.5. Migration among populations was estimated using F_{ST}, rare alleles, and Bayesian modeling methods. All methods showed a general north to south migration pattern, suggesting that typical natural recruitment into the Virginia coastal bays was from natural meadows to the north. Clustering analysis indicated that all of the natural meadows in the region paired with a sampled meadow north of the region; however, the likely sources of the recruitment varied, supporting the hypothesis that recruitment was relatively sporadic and likely to occur rarely. A comparison of the naturally recruited populations to restored populations showed that the naturally recruited populations were less diverse, and showed signs of inbreeding. Restored populations fit geographically into clustering models, suggesting that they did not disrupt regional genetic structure when they jump-started recovery. First-order estimates indicate that the diversity achieved by active restoration in 10 years would take between 157–185 years to achieve by natural recruitment.

Introduction

Large-scale disturbances are becoming more frequent in near-shore communities due in large part to human activities (Jackson et al. 2001, Lotze et al. 2006, Halpern et al. 2008, Waycott et al. 2009). Natural recovery of those systems occurs via dispersal and metapopulation dynamics (Kendrick et al. 2012). Metapopulations are groups of populations that are linked by effective dispersal and thus geneflow (Levins 1970, Cain et al. 2000), providing the propagule source for natural recovery. Dispersal is the movement of genes from a source population into a new settlement site (Pineda 2007), and is only effective when the gametes or seeds released reach an appropriate habitat, settle, survive, and reproduce (Kinlan et al. 2003). Barriers to effective dispersal include inadequate source of propagules, physical barriers, lack of dispersal agents, and lack of suitable habitat or settlement space (van der Pijl 1982). Biotic and abiotic dispersal agents are often limited in coastal compared with terrestrial systems, and coastal systems have an abundance of barriers compared with the open ocean (Kendrick et al. 2012**).**

Restoration is an artificial dispersal agent and augments population connectivity by taking propagules from one population and seeding a new area, either in an effort to speed up natural recovery or to circumvent barriers to dispersal. Care must be taken so that restoration efforts do not introduce problems, which are unlikely to be associated with natural metapopulation dynamics. Many restoration guidelines call for taking propagules from nearby sources where populations have similar genetic structure and variability (Broadhurst et al. 2008). For example, seed transfer zones have been developed for many terrestrial species including Douglas Fir trees (*Pseudotsuga menziesii* F.) (Campbell 1991), Fourwing saltbush (*Atriplex canescens*) (Sanderson et al.

2004), and native grass (*Festuca roemeri*) (Wilson et al. 2008). The goal of seed zones is to limit the negative impacts of the introduction of novel genotypes such as genetic swamping or heterosis (Hufford and Mazer 2003). In simple terms, collection should happen from an area that is potentially naturally connected via metapopulation dynamics. Restoration, at its best, should simply speed up the natural process of recovery. However, nearby seed sources are often lacking or impractical to harvest, and furthermore, the data needed to delineate seed zones is also not always available. When sources for restoration material are collected from outside of natural dispersal ranges, novel genotypes may not be locally adapted, or may interbreed with locally adapted genotypes and result in less fit progeny (i.e. outbreeding depression) (Hufford and Mazer 2003). These deleterious impacts may be propagated outside of the restoration area through effective dispersal and metapopulation dynamics.

In this study, we examine a system that has been highly disturbed and is recovering both naturally and through human-mediated restoration. Anecdotal evidence suggests that the Virginia coastal bays were once carpeted with seagrass (*Zostera marina* L); however, a widespread fungal parasite (*Labyrinthula zosterae*) decimated seagrass populations all over the Atlantic Ocean in the 1930s (Rasmussen 1977). While many populations slowly recovered from the impact of this disease, including those populations in Chesapeake Bay (Cottam & Munro 1954, Orth & Moore 1984), seagrass populations in the coastal bays of Virginia remained locally extinct for nearly 60 years (Orth et al. 2006). Small patches of natural recovery found in the mid 1990s, as well as long term water quality (www1.vcrlter.virginia.edu/home1/?q=data_wq) and light modeling analyses (Lawson et al. 2007) suggested that this area could support seagrass. As a result,

large-scale restoration using seed collected primarily from adjacent Chesapeake Bay was initiated to speed the recovery of these coastal bays. In this study, we use population genetic analysis (using microsatellite markers) to compare natural recovery with successful restoration of seagrass in the Virginia coastal bays and natural populations to north, south and west. Our aims were to first, estimate the existing genetic structure and natural population connectivity across the region to determine connectivity and potential sources for the natural recruitment. Additionally, we evaluated the degree to which restoration changed the natural population genetic structure of the region through the introduction of genetic diversity from an adjacent system. Second, we contrast the genetic diversity of naturally recruited meadows and meadows restored with seed, and we make a first-order comparison of recovery efficiency, estimated as time required to re-establish genetic diversity.

Methods

Sample Collection

Eelgrass tissues samples were collected from 23 meadows from a geographical range from Woods Hole, MA, USA to Beaufort, NC, USA. This represents the southern 1/3 of the geographical extent of this species along the eastern coast of North America (Green & Short 2003). Sampling intensity was highest around Virginia, USA in the Chesapeake Bay and the coastal bays on the ocean side of the Delmarva Peninsula (Fig. 1). The Virginia coastal bays have a history of total seagrass loss occurring around 1933

as the result of disease and storm impacts. Recovery is recent (<15 years), and the young meadows now in that region are either a result of large-scale restoration (See Orth 2006) or relatively recent natural recruitment. See Table 1 for a summary of sampling sites.

At each of the 23 sampling locations, entire plants were collected haphazardly from areas at least 3 m apart to avoid collecting the same clones. The number of replicates from each site varied and is reported in Table 1. Leaf tissue was dried using silica gel desiccant and stored at room temperature until DNA analysis was conducted. Plants from the 2 New York sites (PEC and WGSB) are a subset of the samples previously described (Brisbin 2010, Peterson et al. in review).

Methods follow those described in Reynolds et al (2012—Chapter 2): DNA was extracted from leaf tissue using DNeasy™ plant extraction kits (Qiagen) following manufacturer's instructions. Extracted DNA was amplified at 7 polymorphic loci previously described for this species (CT17H, CT3, CT35, GA2, CT19, CT20, GA3) using standard PCR techniques (Reusch et al. 1999). PCR products were analyzed using capillary electrophoresis on a MegaBace 1000 (GE Biosciences).

Data analysis

For each population sampled, a suite of general genetic diversity measurements were calculated. Allelic richness (A_R), standardized to the smallest population size by rarefaction, was computed using FSTAT 2.9.3.2 (Goudet 2001). The average number of alleles per locus (A), mean expected hetrozygosity (H_e), and Wright's inbreeding coefficient (F) were calculated using GenAlEx 6.3 (Peakall & Smouse 2006). Evidence

of recent bottlenecks was analyzed using a Wilcoxon test of the two-phase model (TMP) in the software package BOTTLENECK v. 1.2.02 (Cornuet and Luikart 1996).

Objective 1: Estimation of regional genetic structure and connectivity

The similarity of geographically separated meadows was analyzed using 3 measures of population differentiation. F_{ST} (estimated as Q, Weir and Cockerham 1984), and F'_{ST} (Hendrick 2005) were calculated using the software Genodive v 2.0 (Merimans and Van Tierden 2004). D_{EST} (Jost 2008) was calculated using SMOGD v. 1.2.5 (Crawford 2010). F'_{ST} and D_{EST} are have normalized variance and therefore are better suited to comparisons amoung species. The clustering of populations by F_{ST} was visualized using a neighbor-joining tree as implemented in the program Mega 5 (Tamura et al. 2011).

All samples were assigned into genetic clusters (K: 1–10) regardless of geographic origin using a Baysian modeling approach conducted in STRUCTURE (Pritchard et al. 2000). All model runs had a random start value, a burn-in period of 50000, and 100000 reps and were repeated with an n of 10. The number of distinct population clusters was determined using the delta K method of Evanno et al. (2005). The proportion of assignments to each cluster was used to create a population distance matrix by calculating average variance among populations and dividing by the variance among populations for each cluster. The grouping of clusters was visualized using a neighbor-joining tree as implemented in the program Mega 5 (Tamura et al. 2011). The geographical distribution of clusters was analyzed using a linear regression analysis of

each cluster against latitude, and the effect of restoration on that distribution was analyzed by regressions with and without those populations.

The relationship between geographical distance and similarity of populations was considered using an isolation by distance analysis. For this analysis, restored populations were omitted, since the dominant movement of genes was a result of human manipulation through restoration as opposed to natural processes. Geographical and genetic distance matrixes were calculated in GenoDive v 2.0 (Merimans and Van Tierden 2004). Geographical distances that involved meadows in Chesapeake Bay were manually corrected to include traveling distance around the Delmarva Peninsula as opposed to straight distances, which would require travel over land. Regression of the 2 matrices was performed in SAS.

Migration among populations was analyzed using three techniques. Pair-wise estimates of geneflow (N_m) were calculated based on rare alleles using GenePop (Raymond and Rousset, http://wbiomed.curtin.edu.au/genepop/index.html) and from F_{ST} calculated in GenAlEx 6.3 (Peakall & Smouse 2006). The direction and rate of recent migration (N_m) was estimated using a multi-locus genotype based Baysian approach with the software BayesAss (Wilson & Rannala 2003).

Objective 2: Differences in natural recruitment and restored populations

In the Virginia coastal bays, there were 5 restored populations (HR6, HR7, SC, SBN, and SB) and 3 small populations (HN, SSB, and FI) that have naturally recruited into the area. Mean allelic richness, expected heterozygosities, and Wright's inbreeding coefficient (F) were compared using a t-test.

To compare the rate of natural recovery via metapopulations and large-scale anthropogenic restoration, we made a series of first-order calculations to estimate the time necessary for natural recovery to produce populations with the same genetic diversity as those produced by large-scale restoration. The calculation was made for an 80-year period, and we first made the conservative assumption that natural recovery of allelic richness was a linear process. This assumption is conservative in that allelic richness will increase as migration events into the area increased, allelic richness would in fact increase logarithmically. However, as we only have a static measure of migration rate over a long period of time, estimated from our measures of genetic diversity, a linear model can be applied as a starting point to estimate the minimum rate of population expansion. To model population expansion, we plotted total migration rate against each allelic richness. For each of the natural and restored populations, we summed the migration rates (Nm) from all of the other populations. We used the Nm calculated based on F_{ST}, as this measure gives an integrated estimate of migration event. Other measures of gene flow were not appropriate as there were few rare alleles to use in the rare allele methods of estimating migration and because the Nm calculated using the program BayesAss only estimates very recent migration events. We conducted a regression analysis between total migration (summed Nm values) and allelic richness for the natural and restored populations independently. We determined the migration rate at the point where allelic richness for the two groups was the same and hypothesized that was the inflection point of the logarithmic curve. We used the linear approach of an increase in migration rate for natural populations to estimate a time in which that would occur.

Results

Previous studies have described the Chesapeake Bay and Virginia coastal bays as one of the most diverse regions in the species' range (Reynolds et al. 2012 —Chapter 2). The survey data reported in the present study also show that most populations are quite diverse, with mean allelic richness of 5.0 and a mean expected heterozygosity of 0.6. However, there was one population in this survey that deviated from that trend. The naturally recruited Southern South Bay in the coastal bay region had a low allelic richness of 2.13 and an expected heterozygosity of only 0.3 (Table 1).

No population showed significant evidence of inbreeding depression or a population bottleneck. The allele frequency distribution was a normal L-shaped distribution for each of the sampled populations, and the probability values for a 2-tail test for heterozygostity excess or deficiency ranged between 0.06 and 1, suggesting no signs of severe populations bottlenecks.

Objective 1: Estimation of regional genetic structure and connectivity

Populations in this study showed some genetic structure. While some geographically distinct populations did not vary genetically, many did. We used three measures of genetic differentiation within populations that varied in magnitude ($0>F_{ST}>0.5$; $0>F'_{ST}>0.8$; $0>D_{EST}>0.6$); however, pattern of differences between sites did not vary between the different measures. The highest value ($F_{ST}=0.49$, $F'_{ST}=0.78$, $D_{EST}=0.6$) was between the two anomalously low diversity sites: Allen's Island in Chesapeake Bay and Southern South Bay in the Virginia coastal bays. The sites that were most similar were the sites in Western Chesapeake Bay (with the exception of Allen's

Island) and the South Bay (SB) restoration site, which was restored using seed from Chesapeake Bay (Table S1, Supporting Information). Populations clustered geographically using the neighbor-joining technique with populations north of the Virginia coastal bays clustering together, Chesapeake Bay populations clustering together, and North Carolina populations clustering together. The restored populations in the Virginia coastal bays clustered between the populations in Chesapeake Bay and the regions to the north, while two of the three naturally recruited Virginia coastal bay populations were relatively unique (Fig. S1, Supporting Information). A regression analysis showed that 3 of the 5 clusters had a significant relationship with latitude, with cluster 1 being dominant in the south and clusters 4 and 5 being dominant in the south. Cluster 3 best described the unique, low diversity, naturally recruited population of SSB in the Virginia coastal bays, and Cluster 2 was most abundant in Chesapeake Bay (Fig. S2, Supporting Information). Adding the restored Virginia coastal bay sites to these regression did not change any of the patters or the statistical significance (Fig. S2, Supporting Information).

The Bayesian cluster analysis in STRUCTURE (Pritchard et al. 2000) followed by a delta K analysis (Evanno et al. 2005) found 5 distinct genetic clusters among the 23 geographically separated populations sampled. The distinct genetic clusters were not distributed evenly over the geographic range. Generally, populations in Chesapeake Bay were dominated by 2 different clusters and all looked quite similar. One Chesapeake Bay population at the mouth of the York River [Allen's Island (AI)] lacked a signal from only one of those genetic clusters. The restored populations in the Virginia coastal bay populations were assigned to genetic clusters that looked quite similar to Chesapeake Bay

populations, from which seeds were originally taken to restore these populations. The meadows that naturally recruited into the Virginia coastal bays, however, were different from one another. Two of those populations, one in Hog Island Bay (HN) and Fisherman Island near the mouth of the Chesapeake (FI) were similar to populations to the north: Chincoteague Bay (CB) and Woods Hole (WH). The third naturally-recruited population, South-south Bay (SSB), grouped in its own distinct cluster. The populations in North Carolina, at the geographical margin of the species, shared the same 2 genetic clusters as those populations in Chesapeake Bay; however, the dominance between the genetic clusters was reversed. Those two signals were also found in populations to the north of the restoration area (Fig. 1). When visualized with a neighbor-joining tree, populations once again clustered geographically by region: the region to the north of Virginia, Chesapeake Bay, and North Carolina, while the restored populations grouped between the northern region and Chesapeake Bay and the naturally recruited Virginia coastal bay meadows mostly paired with a meadow in the northern region (Fig. S1, Supporting Information).

Isolation by distance showed a rather weak positive relationship between geographic distance and genetic distance (R^2=0.2, $p<0.0001$). There was a single population that obviously deviated from this pattern. South-South Bay (SSB) is the naturally recruited population that had an anomalously low diversity, and its pair-wise genetic distance was always high despite any change is geographic distance. It was therefore left out of the isolation by distance calculations.

Estimates of migration (Nm) calculated using rare alleles varied from 0.2 to 17.3, Southern South Bay (SSB) having the lowest connectivity to Allen's Island (AI) in

Chesapeake Bay, and the two restoration treatments in Hog Island Bay (HR6 and HR7) having the highest rate of intermigration. This method of estimating migration was the only method that found a significant amount of migration with the Southern South Bay site (Spider Crab Bay (SC)=2.3 and Chincoteague Bay (CH)=1.7) (Supp. Table 2, Fig. 2). When estimates of migration were based on F_{ST} values, rates ranged from 0.3 to 124.8, with the lowest rates again being between Southern South Bay (SSB) and Allen's Island (AI), and the highest rates being between two sets of paired sites in western Chesapeake Bay Sandy Point (SP) with Four Point Marsh (FP) and Sandy Point (SP) with Brown's Bay (BB) (Fig. 2, Table S2, Supporting Information). Unlike estimates of migration based on F_{ST} and rare alleles, migration rates calculated using Bayesian statistics in the program BayesAss showed only recent (within the last few generations) migrations and also estimated directionality. Those rates ranged from 0.02–32.2, with the lowest migration rates being from one of the furthest south site of Morgan's Island (MI) in North Carolina to the furthest north site in Woods Hole (WH), with the reverse migration being similarly small (0.8). The highest rates were from the 2007 restoration plots in Hog Island Bay (HR6) into the 2006 restoration plots in Hog Island Bay (HR7), with the reverse migration being of similar magnitude (31.0) (Fig. 2, Table S3, Supporting Information).

Objective 2: Differences in natural recruitment and restored populations

As a whole, populations that recruited naturally into this region had a lower allelic richness (3.5 ± 0.7 SE vs 5.5 ± 0.6 SE; t=79.07, df=5, p=0.001) and a lower expected heterozygosity (0.5 ± 0.07 SE vs 0.7 ± 0.005 SE; t=95.49, df=5 p=0.008) than populations that recruited into the area by restoration. All inbreeding coefficients were

close to zero, and there was no difference in inbreeding coefficients between naturally recruited and restored populations (-0.07 ± 0.07 SE vs -0.08 ± 0.03 SE; t=0.1, df=5, p=0.9). South-South Bay (SSB), the anomalous low diversity site, did not significantly skew the results. Results were similar when that population was omitted from the analysis (Fig. 3).

The linear model of increase in allelic richness 125 years, reach the same level of diversity as populations restored by seeding. The assumption that the increase in migration rates is linear with time is likely false. However, more complex logarithmic approximations of the time needed for natural recovery to reach the genetic diversity achieved by large-scale restoration were very similar (185 years) (Fig. 4).

Discussion

Metapopulation dynamics are important in the natural recovery of ecosystems that have experienced catastrophic loss. In this study, we found evidence that naturally recruiting seagrass in the Virginia coastal bay system has resulted from dispersal from meadows to the north. This natural recovery has occurred slowly. Nearly 80 years after meadows were lost due to disease, only three small *Zostera marina* populations have recruited. Our genetic analyses find that those patches show signs of genetic drift, most likely due to a small founding population. Through intervention via seed-based restoration, areal coverage (See Orth et al. 2012), and genetic diversity have increased. Not only has this restoration been successful in quickly restoring large, diverse seagrass populations to the coastal bays, it has not changed the overall regional population genetic structure. The clustering of restored meadows between meadows to the north and south of

the region suggests that restored genetic structure of the region likely reflects of the regional structure prior to the 1930s disease, and the good fit of restored populations in the regressions of structure over latitudinal gradients support this hypothesis.

Z. marina has evolved mechanisms which allow for long distance dispersal that enable ecosystem connectivity and establish regional metapopulations (Kendrick et al. 2012). Long-distance dispersal in *Z. marina* can occur when flowering shoots break free and raft with the currents. Seeds can remain viable within rafting shoots for up to 3 weeks, meaning that seeds can be transported long distances (100–150 km), potentially into habitats that are suitable for germination and survival (Harwell & Orth 2002, Källström et al. 2008). The primary, near shore currents along the mid-Atlantic coast run from north to south (Leatherman et al. 1982). Therefore one would expect that most movement of reproductive shoots would be from north to south, unless seeds were transported by other vectors such as an animals, storm currents, or boat propellers that do not necessarily depend on the prevailing wind or currents. Genetic evidence from natural meadows supports north-south movement of seeds into the Virginia coastal bays, with the naturally recruited meadows pairing with an established meadow to the north. Southern South Bay (SSB) was somewhat anomalous in that its genetic structure is relatively unique; however, one estimate of migration (the rare allele method) showed connection with Chincoteague Bay (CB), consistent with the southerly migration (Fig. 2). Since Southern South Bay does not look like any of the populations sampled (Fig. 1), this recruitment event is either old and has undergone genetic drift so that it no longer resembles the seed source, or the source of the seeds was from outside of our sampling area. Because this population has a reduced genetic diversity compared to all other

populations, it may also be derived from a small recruitment event that now shows effects of founder events. There is no evidence of a population bottleneck, so if this is the case, this is not a recent recruitment event and bottleneck effects have disappeared with time.

Most sites in this survey, spanning over 1000 km of coastline, exhibited similar genetic structure and high rates of connectivity among meadows, so that we would not expect there to be significant numbers of rare alleles. The pairs of sites with significant migration rates uniquely identified by analysis using rare alleles are those that are relatively far apart [i.e. Chincoteague Bay (CB) and Pepper Creek in Chesapeake Bay (PC)], or where the site's low diversity suggest an older, isolated migrations (i.e. Chincoteague Bay (CB) and Southern South Bay (SSB)). Estimates based on overall similarity of populations (F_{ST}) show much stronger relationships, where we expect high migration rates among populations that are very close. For example, Brown's Bay (BB) and Pepper Creek (PC) are geographically close both being Western Chesapeake Bay and show significant migration (Nm=83.08), and Brown's Bay (BB) in Chesapeake Bay was used a seed source for the South Bay (SB) restoration resulting in a very high apparent migration rate of Nm=62.25. Because migration rates are high, it is logical that few rare alleles would exist, and thus the rare-allele method would underestimate migration rates. The migration rates based on Bayesian modeling (BayesAss) have the benefit of estimating directionality; however, these only reflect what has happened in the last few generations. While there are some differences in these estimates, all of the different methods indicate metapopulation dynamics and indicate a general northern to southern migration.

Natural recruitment by dispersal and metapopulation dynamics is a slow process due to natural barriers in the Virginia coastal bay system. Dispersal barriers would most likely limit the founding seed population and thus naturally recruiting populations should exhibit a founder effect and genetic drift (Kendrick et al. 2012). On average, only 10% of total shoots flower (Olesen 1999), and of those flowering shoots, only a portion will break off and raft with the current at an appropriate time in their life cycle so that they have fertilized fruits that will develop into viable seeds. The tidal channels connecting the Virginia coastal bays to the open ocean are relatively narrow, limiting the connection to the open ocean. Previous research has shown that around 5–10% of seeds in this area germinate and develop into viable seedlings (Orth et al. 2006). Even if seedlings establish and create a small patch of seagrass, they may not survive and reproduce in order to create sizable meadows (Olsen & Sand-Jenson 1994). The three natural meadows in this region showed a decreased genetic diversity (Fig. 3) and were dominated by a single genetic cluster (Fig. 1), suggesting that these meadows have been subject to genetic drift, concurrent with a small seed addition and in contrast with the large addition of seeds during restoration. Based on migration rates, we estimate that natural recovery would take 125–185 years to achieve the same level of allelic diversity as natural populations. While the southern Virginia coastal bays have good water quality and can support seagrass expansion (Lawson et al. 2007, www1.vcrlter.virginia.edu/home1/?q=data_wq), coastal bays to the north are experiencing large declines in seagrass primarily due to water quality (Short et al. 2006) and thus may have insufficient effect population size and number of propagules limiting long term capacity for natural recruitment.

In this region, it appears that long distance dispersal and natural recruitment are very slow, sporadic processes. Restoration may in many ways be more successful than natural recruitment. The Virginia coastal bay habitat already being modified and ecosystem services are being enhanced by the return of seagrasses to the Virginia coastal bays (McGlathery et al. 2012). The ecological, economic, and cultural benefits of this restoration will be orders of magnitude more than that of a naturally restored region because they will be available for a longer period of time (See Fonseca et al. 2000). Full recovery by natural processes would not be accomplished for many years. The success of this restoration is in part due to careful source selection, technique that maintains the diversity of this well-chosen donors (Reynolds et al. 2012—Chapter 2), and excellent habitat conditions. While the movement of seeds over long distances by active restoration may have negative impacts on the larger ecosystem by adding in foreign genotypes resulting in outbreeding depression (Hufford & Mazer, 2003), the overall distribution of genetic structure and evidence of migration in the region surrounding this restoration suggest that there is little concern for these problems. Indeed, Chesapeake Bay (to the south of the restoration site) shares a genetic signal with populations to the north of the restoration site (the New York sites of Western Great South Bay (WBSG) and Peconic Estuary (PEC)), and Nm values suggest that there has been historical migration between the two areas (Figs 1, 2). Because the restored meadows group in between meadows to the north and south of the region, it is likely that the restored genetic structure is similar to that which existed prior to the 1930s die-back. A system can fully recover via natural mechanisms; however, recovery will be slow and may be hindered by meadow declines and impacts to nearby systems. Large-scale restoration is a faster process, and when the

connectivity of the larger system is taken into account and care is taken not to disrupt natural genetic structure, that faster recovery will result in the provision of more ecosystem services.

Tables

Table 1**.** Summary of multilocus genetic diversity estimates for all 23 *Zostera marina* populations based on seven microsatellite loci. Sites refer to locations shown on Fig. 1. N=sample size, A_R=allelic richness, Na= average number of alleles per locus, H_e=expected heterozygosity, F=Wright's inbreeding coefficient.

	Site	Code	Location				N	A_R	Na	H_e	F
Virginia Coastal Bays	Woods Hole	WH	41.6065	N	70.6485	W	48	3.53	5.86	0.53	-0.19
	Western Great South Bay	WGSB	40.6489	N	73.2116	W	24	4.4	7.29	0.56	-0.02
	Peconic Estuary	PEC	41.0816	N	72.2843	W	24	4.52	6.71	0.61	0.12
	Chincoteague Bay	CH	38.0398	N	75.346	W	48	4.66	8.57	0.63	-0.06
	Hog Island Bay Restoration (2006) *	HR6	37.4104	N	75.7296	W	167	5.59	13.29	0.72	-0.07
	Hog Island Bay Restoration (2007) *	HR7	37.4095	N	75.724	W	94	5.33	10.57	0.73	-0.11
	Hog Island Bay Natural Recovery	HN	37.4154	N	75.7225	W	43	4.04	6.86	0.59	0.01
	Spider Crab Bay *	SC	37.3524	N	75.8047	W	48	5.33	10	0.7	-0.17
	Northern South Bay *	SBN	37.2725	N	75.8054	W	24	5.47	8.71	0.7	-0.08
	South Bay *	SB	37.2663	N	75.812	W	95	5.61	11.14	0.71	0.04
	Southern South Bay	SBS	37.2393	N	75.8528	W	46	2.13	3.57	0.38	0
	Fisherman's Island	FI	37.1079	N	75.9683	W	46	4.35	6.71	0.61	-0.22
	Hungar's Creek	HC	37.4079	N	75.987	W	48	5.49	10.14	0.7	-0.12
Chesapeake Bay	Cape Charles	CC	37.2665	N	76.0236	W	48	5.55	9.86	0.68	-0.11
	Pepper Creek	PC	37.3421	N	76.3158	W	30	5.44	9	0.63	0.03
	Four Point Marsh	FP	37.3408	N	76.4063	W	24	5.44	8.14	0.64	0.03
	Brown's Bay	BB	37.2946	N	76.3824	W	16	6.09	8.57	0.65	-0.08
	Sandy Point	SP	37.2627	N	76.3984	W	23	5.82	8.71	0.67	-0.03
	Perrin River	PR	37.2636	N	76.4178	W	30	5.66	9.43	0.69	-0.04
	Allen's Island	AI	37.2572	N	76.4228	W	23	3.92	5.57	0.58	-0.25
	Morgan's Island	MI	34.6903	N	76.62	W	21	5.35	7.71	0.7	0
	Middle Marsh	MM	34.6578	N	76.5259	W	13	5.39	6.71	0.69	-0.06
	Phillips Island	PI	34.7316	N	76.6856	W	24	5.22	7.14	0.72	-0.01

Figures

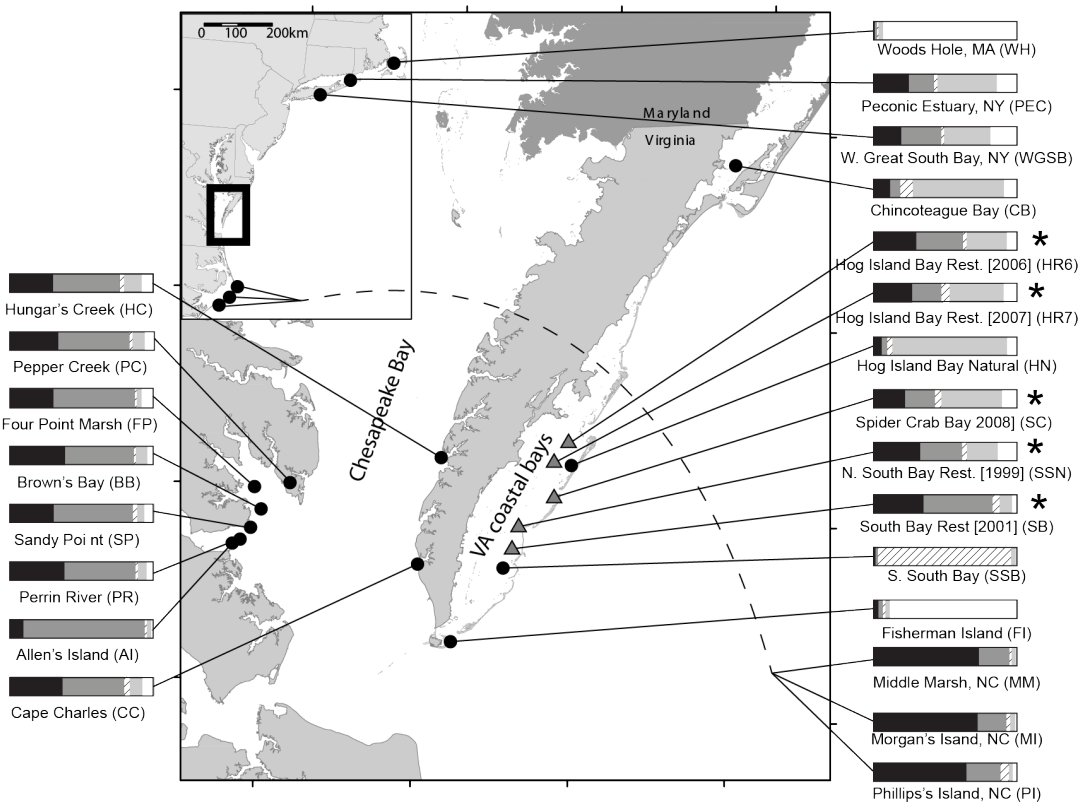

Fig. 1**.** Comparison of genetic structure among sites.

Site map of 23 geographically separate *Zostera marina* sampling locations on the east coast of North America. Proportional plots showing the assignment of individuals within each sampled population to the five Bayesian modeled genetic clusters (implemented in STRUCTURE; Pritchard et al. 2000) which were identified using the *ad hoc* statistic ΔK, (*sensu* Evanno et al. 2005). Sites that were in restored meadows, as opposed to naturally recruited meadows, are highlighted by an * beside the location name.

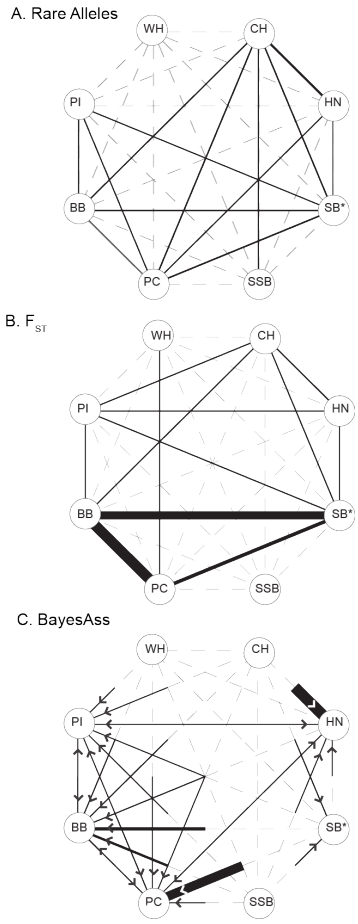

Fig. 2**.** Migration Rates.

Each circle represents a sampled population, and the populations are arranged approximately north to south going clock-wise. All pair-wise populations are connected with a dashed line. Solid lines represent a significant Nm value. The size of the line is proportional to the Nm value. (A) Nm values were calculated based on rare alleles using GenePop (Raymond and Rousset, 1995) and range from 0.2 to 17.3. (B) Nm values were calculated based on $F_{ST,}$ calculated in GenAlEx 6.3 (Peakall & Smouse 2006) and range from 0.3 to 124.8. (C) Nm values and direction were estimated using a multi-locus genotype based Baysian approach with the software BayesAss (Wilson & Rannala 2003), and values range from 0.02 to 32.2. Site codes are described in Table 1.

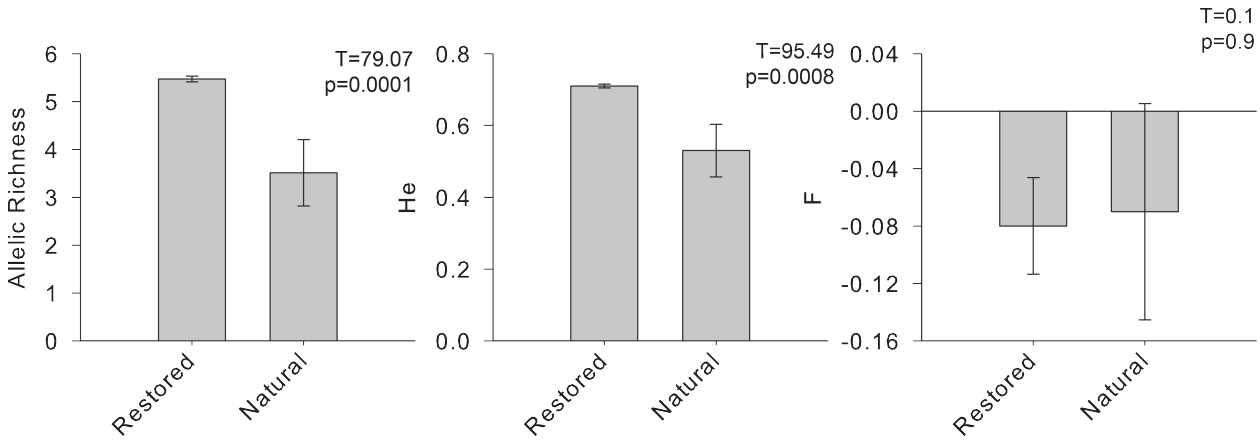

Fig. 3. Comparison of young naturally recruited and restored meadows using four measures of genetic diversity (± standard error).

(A) Allelic richness; (B) expected Heterozygosity (H_e), and(C) Wright's inbreeding coefficient (F). Statistic results are derived from analysis using student's T-test.

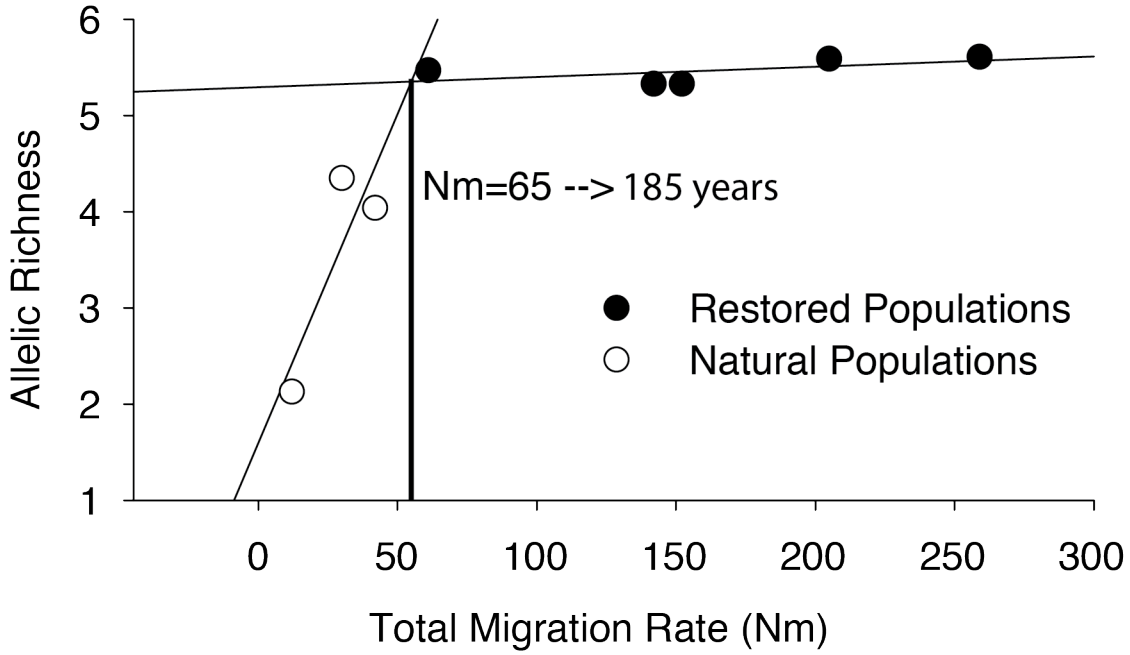

Fig. 4. Comparison of recovery efficiency, estimated as time required to re-establish genetic diversity.

For both restored (filled circles) and naturally recruited (open circles), cumulative migration rate (based on F_{ST}) were regressed against genetic diversity. We considered the point where the two regression lines intersected the inflection point on the logarithmic curve representing the relationship between migration and increases in diversity and population size. The intercept for the natural populations was considered the migration rate that natural recovery would need to achieve to create populations equal to restored populations. We used a linear approach (migration over time) to estimate a year in which that would occur.

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Appendix (Supplementary Material)

Table S1. Summary of genetic relatedness for all 23 *Zostera marina* populations based on seven microsatellite loci.

F_{ST} (estimated as Q, Weir and Cockerham (1984) Evolution, 38, 1358–1370), and F'_{ST} (Hendrick (2005) Evolution, 59, 1633–1638) were calculated using the software Genodive v 2.0 (Merimans and Van Tierden (2004) Molecular Ecology Notes, 4, 792-794). D_{EST} (Jost (2008) Molecular Ecology, 17, 4015–4026) was calculated using SMOGD v. 1.2.5 (Crawford (2010) Molecular Ecology Resources, 10, 556–557)

	WH	WGSB	PEC	CH	HR6	HR7	HN	SC	SBN	SB	SBS	FI	HC	CC	PC	FP	BB	SP	PR	AA	MI	MM	PI
	FST																						
	F'ST																						
WH	DEST																						
	0.217																						
	0.474																						
WGSB	0.13																						
	0.169	0.023																					
	0.389	0.058																					
PEC	0.09	0.01																					
	0.149	0.109	0.087																				
	0.357	0.278	0.237																				
CH	0.17	0.07	0.06																				
	0.142	0.053	0.028	0.056																			
	0.373	0.13	0.063	0.16																			
HR6	0.19	0.06	0.03	0.06																			
	0.142	0.063	0.04	0.031	0.01																		
	0.365	0.155	0.097	0.081	0.021																		
HR7	0.22	0.09	0.06	0.04	0.01																		
	0.173	0.101	0.113	0.081	0.073	0.061																	
	0.384	0.232	0.279	0.205	0.198	0.16																	
HN	0.23	0.09	0.15	0.10	0.11	0.10																	
	0.128	0.054	0.037	0.023	0.007	0.007	0.055																
	0.33	0.148	0.107	0.067	0.01	0.01	0.152																
SC	0.16	0.06	0.05	0.01	0.00	0.00	0.07																
	0.173	0.117	0.101	0.084	0.057	0.045	0.09	0.055															
	0.434	0.319	0.298	0.252	0.172	0.128	0.242	0.183															
SBN	0.24	0.12	0.09	0.07	0.06	0.05	0.12	0.02															
	0.193	0.103	0.063	0.096	0.025	0.036	0.141	0.048	0.064														
	0.505	0.279	0.178	0.285	0.075	0.109	0.398	0.151	0.202														
SB	0.29	0.17	0.08	0.19	0.04	0.08	0.25	0.10	0.11														
	0.412	0.358	0.351	0.239	0.268	0.239	0.35	0.266	0.301	0.287													
	0.745	0.64	0.662	0.476	0.619	0.525	0.661	0.569	0.607	0.644													
SBS	0.44	0.29	0.32	0.23	0.39	0.33	0.39	0.30	0.32	0.43													
	0.106	0.214	0.178	0.139	0.119	0.122	0.146	0.115	0.11	0.152	0.357												
	0.246	0.525	0.465	0.372	0.348	0.354	0.36	0.334	0.317	0.445	0.7												
FI	0.12	0.24	0.16	0.17	0.12	0.19	0.24	0.11	0.19	0.17	0.42												
	0.176	0.067	0.037	0.091	0.005	0.031	0.1	0.023	0.072	0.023	0.324	0.137											
	0.435	0.163	0.088	0.26	0	0.081	0.26	0.062	0.216	0.061	0.677	0.383											
HC	0.19	0.08	0.04	0.13	0.01	0.07	0.15	0.04	0.10	0.02	0.43	0.11											
	0.199	0.08	0.052	0.113	0.018	0.047	0.113	0.043	0.065	0.025	0.321	0.138	0.005										
	0.504	0.216	0.148	0.333	0.048	0.141	0.309	0.137	0.209	0.073	0.68	0.393	0.003										
CC	0.23	0.10	0.07	0.16	0.03	0.11	0.20	0.05	0.10	0.03	0.39	0.06	0.00										
	0.234	0.117	0.069	0.148	0.052	0.068	0.178	0.084	0.094	0.011	0.378	0.192	0.044	0.038									
	0.551	0.293	0.185	0.407	0.141	0.19	0.452	0.253	0.282	0.022	0.738	0.511	0.113	0.109									
PC	0.25	0.17	0.09	0.23	0.08	0.13	0.30	0.13	0.12	0.01	0.48	0.14	0.03	0.01									
	0.24	0.127	0.079	0.142	0.041	0.067	0.183	0.074	0.09	0.012	0.395	0.177	0.033	0.033	0.009								
	0.539	0.278	0.175	0.374	0.093	0.16	0.434	0.204	0.226	0.01	0.726	0.447	0.05	0.071	-0.009								
FP	0.28	0.12	0.09	0.22	0.07	0.14	0.29	0.16	0.13	0.02	0.52	0.14	0.02	0.02	0.01								
	0.213	0.128	0.076	0.117	0.039	0.057	0.156	0.064	0.076	0.004	0.389	0.152	0.031	0.026	0.003	0.005							
	0.492	0.306	0.19	0.323	0.084	0.133	0.385	0.186	0.211	-0.014	0.721	0.4	0.047	0.063	-0.009	-0.074							
BB	0.24	0.09	0.04	0.17	0.03	0.15	0.27	0.08	0.10	0.00	0.51	0.11	0.01	0.00	0.00	0.00							
	0.214	0.124	0.067	0.124	0.034	0.057	0.165	0.062	0.075	0.004	0.367	0.163	0.025	0.024	0.005	-0.002	0.002						
	0.51	0.304	0.169	0.35	0.08	0.149	0.423	0.187	0.214	-0.007	0.709	0.442	0.041	0.059	-0.005	-0.077	-0.055						
SP	0.24	0.16	0.06	0.18	0.04	0.12	0.25	0.11	0.12	0.01	0.48	0.11	0.02	0.01	0.00	0.00	0.00						
	0.209	0.103	0.059	0.111	0.026	0.035	0.146	0.053	0.074	0.002	0.336	0.164	0.029	0.028	0.012	0.011	0.007	0.006					
	0.534	0.286	0.177	0.336	0.069	0.099	0.407	0.176	0.249	-0.003	0.703	0.476	0.078	0.09	0.035	0.001	0.005	0					
PR	0.29	0.13	0.08	0.19	0.03	0.08	0.30	0.08	0.11	0.01	0.48	0.15	0.03	0.05	0.01	0.02	0.00	0.00					
	0.317	0.224	0.198	0.253	0.152	0.169	0.274	0.184	0.151	0.128	0.467	0.271	0.152	0.143	0.134	0.133	0.142	0.119	0.125				
	0.661	0.447	0.424	0.633	0.358	0.386	0.605	0.492	0.329	0.315	0.787	0.647	0.332	0.355	0.275	0.162	0.196	0.176	0.293				
AA	0.44	0.33	0.28	0.51	0.30	0.36	0.47	0.35	0.29	0.21	0.60	0.46	0.27	0.25	0.18	0.22	0.20	0.16	0.19				
	0.27	0.14	0.116	0.163	0.089	0.092	0.145	0.105	0.103	0.115	0.335	0.21	0.094	0.093	0.148	0.136	0.136	0.13	0.113	0.215			
	0.691	0.393	0.355	0.501	0.287	0.298	0.405	0.36	0.357	0.385	0.683	0.617	0.299	0.307	0.456	0.392	0.427	0.417	0.396	0.561			
MM	0.50	0.24	0.24	0.33	0.23	0.22	0.23	0.22	0.24	0.31	0.45	0.37	0.25	0.20	0.29	0.33	0.29	0.35	0.31	0.50			
	0.266	0.123	0.116	0.153	0.078	0.084	0.134	0.091	0.104	0.112	0.334	0.208	0.085	0.087	0.156	0.14	0.136	0.132	0.106	0.226	0.003		
	0.659	0.33	0.341	0.462	0.231	0.247	0.356	0.304	0.348	0.363	0.638	0.597	0.245	0.28	0.468	0.356	0.391	0.389	0.362	0.524	0		
MI	0.47	0.21	0.24	0.34	0.21	0.22	0.24	0.20	0.25	0.25	0.42	0.43	0.23	0.23	0.31	0.30	0.25	0.33	0.21	0.50	0.01		
	0.201	0.133	0.112	0.123	0.076	0.075	0.116	0.078	0.07	0.107	0.282	0.166	0.082	0.083	0.15	0.144	0.126	0.123	0.113	0.204	0.027	0.022	
	0.495	0.342	0.314	0.362	0.23	0.219	0.298	0.249	0.203	0.341	0.544	0.47	0.227	0.256	0.439	0.351	0.33	0.338	0.373	0.447	0.057	0	
PI	0.33	0.22	0.22	0.26	0.20	0.17	0.19	0.16	0.17	0.23	0.34	0.40	0.22	0.21	0.29	0.33	0.29	0.27	0.28	0.44	0.05	0.03	

Table S2. Summary of migration ratess among all 23 *Zostera marina* populations based on seven microsatellite loci.

Nm values based rare alleles were calculated using GenePop (Raymond and Rousset, http://wbiomed.curtin.edu.au/genepop/index.html), and Nm values based on FST were calculated in GenAlEx 6.3 (Peakall & Smouse (2006) Molecular Ecolology Notes, 6, 288−295).

	WH	WGSB	PEC	CH	HR6	HR7	HN	SC	SBN	SB	SBS	FI	HC	CC	PC	FP	BB	SP	PR	AA	MM	MI
WH	Rare FST																					
WGSB	0.80 0.90																					
PEC	0.87 1.23	3.28 10.62																				
CH	1.43 1.43	2.02 2.04	4.56 2.62																			
HR6	1.72 1.51	3.09 4.47	4.34 8.68	4.36 4.21																		
HR7	1.48 1.51	2.73 3.72	3.77 6.00	5.54 7.81	17.27 24.75																	
HN	1.10 1.20	1.72 2.23	1.27 1.96	9.39 2.84	4.76 3.17	5.23 3.85																
SC	1.97 1.70	3.35 4.38	4.37 6.51	5.07 10.62	13.26 35.46	7.93 35.46	5.43 4.30															
SBN	0.88 1.20	3.02 1.89	3.63 2.23	5.13 2.73	6.03 4.14	4.37 5.31	2.41 2.53	5.15 4.30														
SB	1.11 1.05	2.97 2.18	3.02 3.72	3.95 2.35	9.13 9.75	8.59 6.69	2.85 1.52	7.04 4.96	5.04 3.66													
SBS	0.29 0.36	0.77 0.45	0.40 0.46	1.64 0.80	1.54 0.68	1.62 0.80	0.76 0.46	2.29 0.69	0.97 0.58	1.39 0.62												
FI	0.68 2.11	0.48 0.92	0.38 1.15	1.49 1.55	1.36 1.85	0.85 1.80	1.35 1.46	1.28 1.92	0.62 2.02	0.71 1.39	0.34 0.45											
HC	1.88 1.17	2.99 3.48	3.36 6.51	3.47 2.50	8.61 49.75	6.28 7.81	3.50 2.25	7.12 10.62	4.63 3.22	6.34 10.62	1.39 0.52	1.17 1.57										
CC	1.17 1.01	3.19 2.88	2.63 4.56	3.85 1.96	9.44 13.64	5.22 5.07	2.60 1.96	5.33 5.56	4.04 3.60	7.93 9.75	1.30 0.53	0.67 1.56	8.79 49.75									
PC	0.99 0.82	4.12 1.89	3.17 3.37	3.54 1.44	6.06 4.56	3.89 3.43	1.59 1.15	4.05 2.73	2.98 2.41	5.80 22.48	0.95 0.41	0.52 1.05	5.61 5.43	9.65 6.33								
FP	0.62 0.79	2.64 1.72	2.57 2.91	2.28 1.51	4.68 5.85	3.25 3.48	1.17 1.12	2.93 3.13	4.10 2.53	5.27 20.58	0.70 0.38	0.42 1.16	5.47 7.33	3.23 7.33	3.38 27.53							
BB	0.63 0.92	2.66 1.70	2.52 3.04	2.70 1.89	6.30 6.16	4.17 4.14	1.46 1.35	3.24 3.66	4.19 3.04	5.08 62.25	0.74 0.39	0.68 1.39	5.54 7.81	4.42 9.37	3.93 83.08	3.91 49.75						
SP	0.75 0.92	2.03 1.77	2.60 3.48	2.29 1.77	6.79 7.10	3.17 4.14	0.93 1.27	3.74 3.78	2.75 3.08	6.48 62.25	0.83 0.43	0.46 1.28	4.17 9.75	3.89 10.17	3.42 49.75	3.02 124.75	3.06 124.75					
PR	1.17 0.95	3.51 2.18	2.25 3.99	2.94 2.00	8.25 9.37	4.72 6.89	1.71 1.46	4.09 4.47	3.46 3.13	5.10 124.75	0.71 0.49	0.64 1.27	4.68 8.37	4.46 8.68	3.73 20.58	4.36 22.48	3.27 35.46	4.83 41.42				
AA	0.34 0.54	1.13 0.87	0.55 1.01	0.85 0.74	0.89 1.39	0.97 1.23	0.34 0.66	0.70 1.11	1.61 1.41	1.21 1.70	0.18 0.29	0.28 0.67	0.93 1.39	0.73 1.50	1.11 1.62	1.34 1.63	0.98 1.51	1.40 1.85	1.04 1.75			
MI	0.45 0.68	1.24 1.54	1.55 1.91	1.37 1.28	2.01 2.56	1.97 2.47	0.71 1.47	1.52 2.13	2.55 2.18	2.57 1.92	1.01 0.50	0.38 0.94	1.55 2.41	1.86 2.44	1.12 1.44	1.30 1.59	1.50 1.59	1.08 1.67	1.44 1.96	0.64 0.91		
MM	0.46 0.69	1.42 1.78	1.08 1.91	1.75 1.38	1.55 2.96	1.58 2.73	1.18 1.62	1.64 2.50	2.21 2.15	1.31 1.98	0.77 0.50	0.31 0.95	1.10 2.69	1.21 2.62	1.08 1.35	0.95 1.54	1.21 1.59	0.91 1.64	1.30 2.11	0.42 0.86	3.07 83.08	
PI	0.69 0.99	1.14 1.63	1.19 1.98	1.30 1.78	1.92 3.04	2.04 3.08	0.78 1.91	1.63 2.96	2.12 3.32	1.77 2.09	0.74 0.64	0.48 1.26	1.65 2.80	1.42 2.76	1.61 1.42	1.30 1.49	1.48 1.73	1.38 1.78	1.48 1.96	0.75 0.98	3.11 9.01	1.68 11.11

Table S3. Summary of directional migration rates among all 23 *Zostera marina* populations based on seven microsatellite loci. Nm values estimated using a multi-locus genotype based Baysian approach with the software BayesAss (Wilson & Rannala (2003) Genetics, 163, 1177–1191).

From /	WH	WGSB	PEC	CH	HR6	HR7	HN	SC	SBN	SB	SBS	FI	HC	CC	PC	FP	BB	SP	PR	AA	MM	MI	PI
WH		0.327	0.231	0.068	0.046	0.076	0.169	0.252	0.384	0.052	0.066	0.035	0.285	0.140	0.283	0.402	0.629	0.407	0.208	0.342	0.317	0.083	0.343
WGSB	0.037		0.147	0.039	0.078	0.092	0.166	0.194	0.426	0.064	0.042	0.026	0.206	0.143	0.266	0.381	0.578	0.435	0.232	0.352	0.384	0.100	0.353
PEC	0.027	22.498		0.059	0.056	0.131	0.203	0.314	0.511	0.096	0.064	0.036	0.169	0.152	3.482	0.412	0.543	0.469	0.544	0.580	0.357	0.099	0.402
CH	0.025	3.040	1.831		0.039	0.073	28.909	16.183	9.339	0.397	0.052	0.036	0.779	0.232	0.256	0.433	0.538	0.471	0.260	0.460	0.425	0.128	0.447
HR6	0.029	0.308	0.222	0.040		30.982	0.203	0.218	0.441	0.250	0.052	0.025	0.150	0.177	0.222	0.403	0.525	0.439	0.267	0.674	0.351	0.106	0.279
HR7	0.030	0.301	0.127	0.046	32.117		0.172	0.222	0.378	0.084	0.070	0.022	0.185	0.139	0.230	0.343	0.476	0.386	0.318	0.392	0.407	0.094	0.291
HN	0.027	0.311	0.130	0.046	0.054	0.104		0.187	1.147	0.053	0.052	0.026	0.130	0.137	0.191	0.422	0.528	0.431	0.276	0.375	0.382	0.107	0.337
SC	0.027	0.326	0.158	0.038	0.032	0.077	0.176		0.389	0.041	0.058	0.023	0.166	0.136	0.250	0.365	0.565	0.361	0.202	0.442	0.419	0.086	0.267
SBN	0.026	0.330	0.127	0.037	0.037	0.092	0.163	0.204		0.059	0.047	0.027	0.121	0.158	0.239	0.382	0.539	0.375	0.249	0.416	0.384	0.082	0.277
SB	0.033	0.414	0.676	0.093	0.048	0.128	0.199	1.808	0.963		0.067	0.034	1.790	0.767	23.832	7.743	6.925	9.589	26.474	2.041	0.525	0.118	0.286
SBS	0.029	0.277	0.152	0.039	0.060	0.154	0.205	0.246	0.500	0.491		0.026	0.172	0.187	0.217	0.312	0.521	0.343	0.249	0.393	0.367	0.107	0.411
FI	0.032	0.330	0.269	0.042	0.036	0.054	0.154	0.255	0.433	0.056	0.054		0.248	0.134	0.232	0.415	0.528	0.383	0.259	0.310	0.321	0.099	0.301
HC	0.028	0.301	0.155	0.041	0.073	0.101	0.176	0.180	0.407	0.107	0.053	0.026		0.152	0.231	0.348	0.544	0.419	0.285	0.385	0.335	0.131	0.285
CC	0.029	0.350	0.153	0.042	0.056	0.093	0.159	0.197	0.396	0.080	0.048	0.026	0.183		0.238	0.375	0.506	0.357	0.252	0.504	0.394	0.107	0.352
PC	0.028	0.293	0.126	0.040	0.043	0.100	0.169	0.214	0.376	0.075	0.052	0.030	0.157	0.130		0.389	0.541	0.423	0.205	0.449	0.332	0.105	0.300
FP	0.026	0.389	0.139	0.040	0.050	0.087	0.183	0.239	0.391	0.088	0.060	0.031	0.138	0.139	0.240		0.538	0.544	0.199	0.301	0.381	0.103	0.291
BB	0.036	0.296	0.126	0.044	0.028	0.079	0.140	0.192	0.361	0.065	0.044	0.034	0.181	0.152	0.259	0.433		0.436	0.204	0.400	0.339	0.103	0.328
SP	0.029	0.306	0.142	0.037	0.027	0.086	0.168	0.222	0.373	0.067	0.060	0.030	0.195	0.171	0.236	0.408	0.622		0.201	0.396	0.391	0.113	0.299
PR	0.033	0.378	0.416	0.056	0.062	0.109	0.196	10.314	12.996	0.279	0.100	0.025	26.645	28.648	0.396	16.677	13.547	14.020		21.444	0.378	0.142	0.357
AA	0.029	0.314	0.172	0.038	0.052	0.080	0.147	0.186	0.614	0.117	0.050	0.034	0.183	0.134	0.281	0.345	0.560	0.401	0.225		0.370	0.118	0.291
MM	0.030	0.307	0.147	0.045	0.071	0.085	0.180	0.252	0.395	0.094	0.055	0.037	0.144	0.156	0.234	0.367	0.515	0.418	0.239	0.343		0.098	0.265
MI	0.023	0.321	0.162	0.043	0.047	0.121	0.183	0.285	0.518	0.117	0.063	0.028	0.172	0.323	0.200	0.363	0.682	0.407	0.246	0.360	24.022		25.254
PI	0.024	0.329	0.131	0.035	0.063	0.061	0.189	0.249	0.314	0.075	0.051	0.029	0.189	0.164	0.242	0.374	0.493	0.418	0.265	0.318	0.381	0.076	

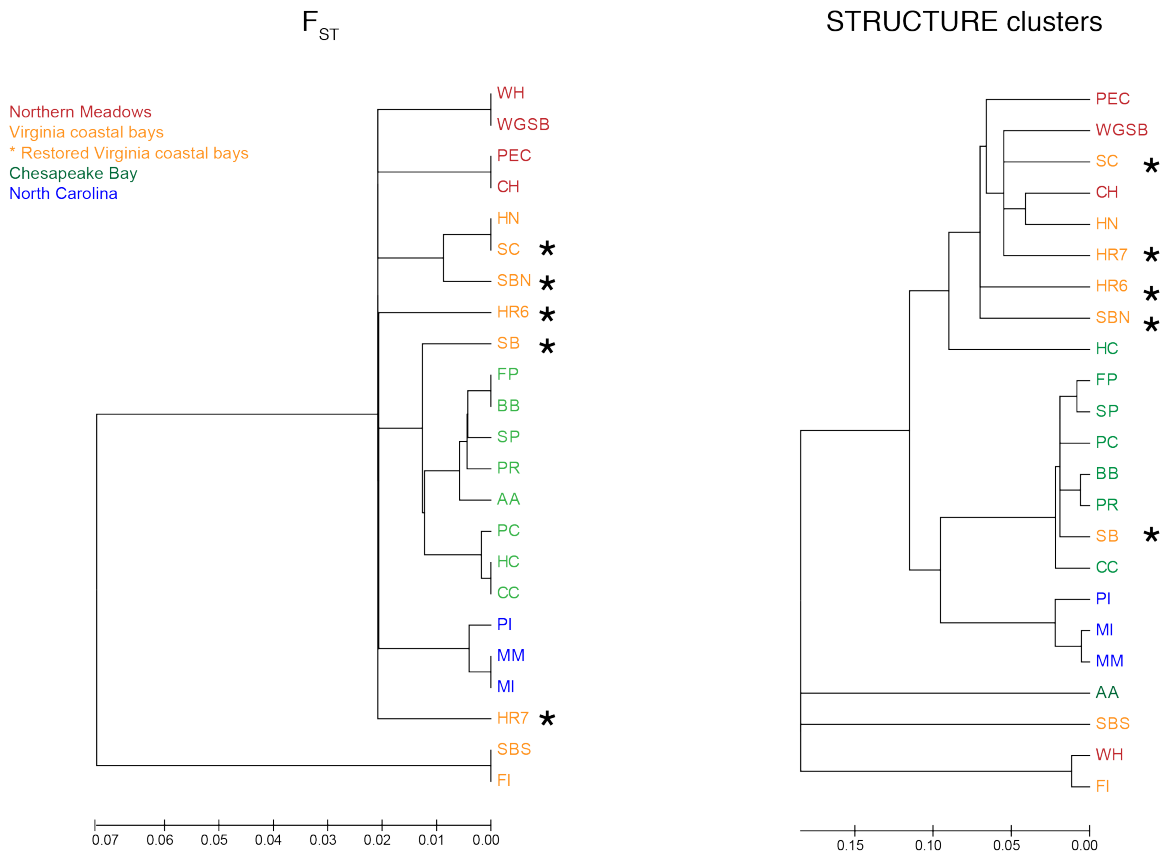

Fig. S1. Clustering of all 23 *Zostera marina* populations based on seven microsatellite loci.

Clusters were visualized using a neighbor-joining tree as implemented in the program Mega 5 (Tamura et al. (2011) Molecular Biology and Evolution, 28, 2731–2739). The left tree used an FST distance matrix and the right tree used a distance matrix created from STRUCTURE results (Pritchard et al. (2000) Genetics, 155, 945). The proportion of assignments to each cluster was used to create a population distance matrix by calculating average variance among populations and dividing by the variance among populations for each cluster. Samples are shaded by geographical region.

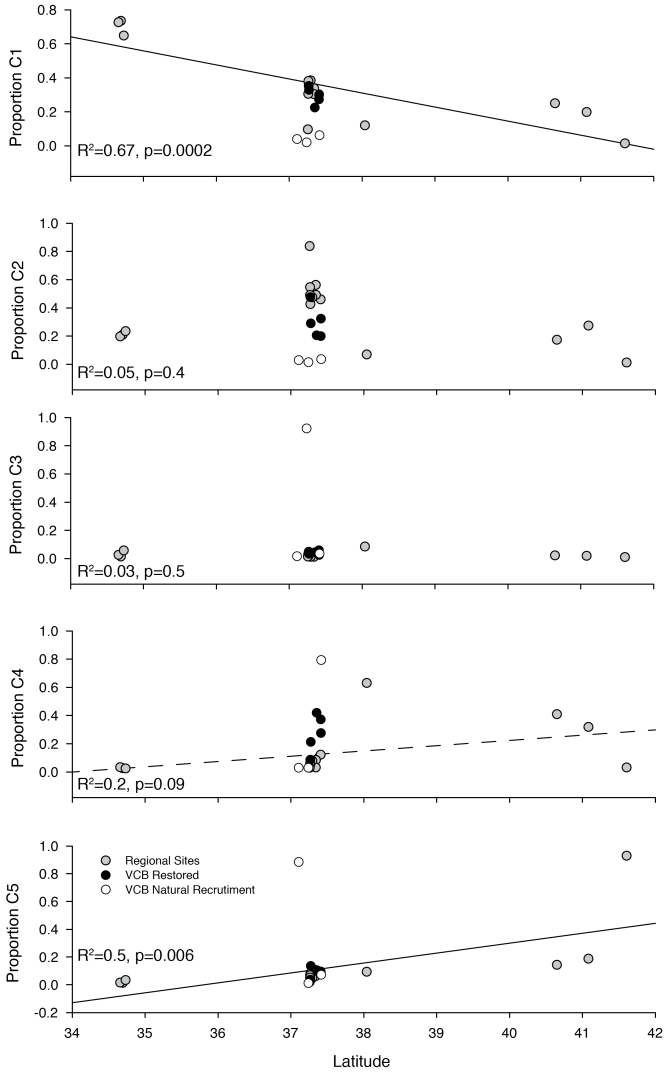

Fig. S2. The distribution of genetic clusters over the geographical gradient. Samples from each of 23 geographical locations were assigned to 1 of 5 genetic clusters. For each population, the physical latitude was regressed against the genetic cluster assignment. The significant relationships suggest that genetic structure is distributed according to location, and the good fit of the restored sites suggests that restoration did not disrupt the overall regional genetic structure.

Chapter 5: The economic value of ecosystem services returned through restoration

Abstract

The goal of ecosystem restoration is to augment the recovery of a community and the associated ecosystem services. We model areal seagrass recovery and estimate the value of returned ecosystem services in a system where the seagrasses were lost due to disease. We estimate that the current value of ecosystem services is more than eight times the cost of restoration. Further, we estimate natural recovery would take more than 100 years to reach the coverage achieved by restoration in just 10 years.

Monograph

Humans directly and indirectly gain goods and services from natural ecosystems, and while these services are not paid for or traded on the open market, their value is fundamental and significant at local and global scales (Costanza et al. 1997). Ecosystem perturbations result in the loss of these goods and services, subsequently impacting human welfare and economic stability. Ecological restoration aims to re-establish communities, which function and provide services similar to unimpacted ones. Ecosystem restoration is costly but effective at increasing biodiversity and ecosystem services, though often falling short of re-creating pristine ecosystems (Benayas et al 2009). We demonstrate that for a large-scale marine plant restoration the value of increasing ecosystem services is substantial, recovering the cost in a short time.

Seagrasses have been restored to the Virginia coastal bays following the 1933 loss due to disease, which subsequently caused the loss of associated species including the commercially important bay scallop. Discovery of small patches of natural eelgrass (*Zostera marina*) recovery in the late 1990s motivated the investment of time and grant funds (around to $2 million) into restoration. By 2010, 80 ha of lagoon bottom were seeded and had expanded into nearly 2000 ha of eelgrass coverage. The resulting increase in ecosystem services includes increases in faunal abundance, carbon sequestration, and nitrogen cycling (McGlathery et al. 2012, Orth et al. 2012).

Costanza estimated that the nitrogen removal achieved by seagrass and algal meadows is worth approximately $19,002 USD ha^{-1} $year^{-1}$ (1997), and conservatively 50% can be attributed to seagrass alone (Waycott et al. 2009). Measured nitrogen removal in this system is consistent with published estimates (Cole 2011), allowing the

application of global values, which results in a current nutrient cycling value of over $16 million USD year^{-1}. This yearly value is 8 times the total amount invested in the restoration over the last decade, recouping costs by 2003 (Fig. 1).

We used measured annual seagrass cover to generate three population growth models, which predict areal increase for two categories: restored and naturally recruited meadows. We discounted the linear model because it failed to show significant natural meadow expansion. The results estimate that natural recovery to the 2010 areal coverage would have taken around 110 years (i.e. 2043). This estimate is likely fast as small size and random site selection consistent with natural recruitment could easily result in meadow isolation, slowing the areal expansion of natural patches (i.e. Fisherman Island). Using the 110-year conservative estimate, the seeding conducted between 2000 and 2010 accelerated the recovery by 30 years. Assuming that the areal coverage from restoration remains constant over the next 30 years, the added value of having additional seagrass would be nearly $400 million USD (Fig. 1). This value is conservative as coverage is expected to continue to expand over the next 30 years, further increasing the economic value (Fig. 1).

Fisheries are expected to increase via seagrass restoration. Global values for animals harvested from seagrass meadows is between $8–2511 USD ha^{-1} year^{-1} (Barbier et al 2011). Using an average value for seagrass associated fisheries ($235 USD ha^{-1} year^{-1}), we estimate the current value of this ecosystem service to be 0.4 million USD year^{-1}. We do not yet have direct evidence that the fisheries, produced by these young meadows, are within the range of literature values, so application of global values is speculative.

There is substantial economic value in the ecosystem services provided through the restoration of the Virginia coastal bays. We acknowledge that these are first order estimates and therefore contain significant uncertainty but expect that the actual value is higher as presented values. Nutrient cycling estimates use 1991 prices (Costanza et al. 1997), and the estimates of fisheries market value are from 1993–2006 (Barbier et al. 2011). Current values of both of these services are likely to be higher in 2012. In addition the value of the system would be even higher if other services were to be included. For example, surveys have shown that carbon is being sequestered in the sediments of these restored seagrass meadows as increased organic matter (McGlathery et al. 2012, Orth et al. 2012). Further, seagrasses trap sediment and reduce flow, protecting shorelines from erosion (Barbier et al. 2011). These are valuable services, for which there are few estimates. Overall, we present conservative economic values aiming to present a minimum estimate of the ecosystem services provided by these restored ecosystems.

Methods

The areal extent of seagrass across the Virginia coastal bays has been monitored annually via aerial imagery (http://web.vims.edu/bio/sav). We plotted coverage annually using areal photographs of all meadows in the Virginia coastal bays identifying separately those not originating from restoration activities (seeding) [i.e. natural recovery—Hog Island Bay (prior to 2007), the small meadow south of Ship Shoal inlet (prior to connection with South Bay to the north), and Fisherman Island.]. We assume natural recruitment occurred via rafting shoots from meadows outside the region, which

is supported by genetic data (Reynolds et al. 2012—Chapter 2, Chapter 4). The time-course data generated were used to model meadow growth.

We modeled the population growth of both the planted areas and the natural areas in this region using 3 methods: linear growth, exponential growth, and logistic growth. The linear growth model fit yearly coverage data to a line (y=mx+b). The exponential growth model followed the equation $x_t = x_0(1 + r)^t$ (where x=population area, r=growth rate, t=time). The logistic growth model was based on the growth rate calculated in the exponential model, but predicted areal coverage was capped by applying the equation (dN/dt)=rN*(1-N/Max) (where N=population size, t=time, and Max=maximum population size). We defined maximum population size as areas with a water depth between 0.6–1.6 m; the modeled and observed range for this species in this region (Carr et al. 2012, McGlathery et al. 2012). This would overestimate available habitat since sediment characteristics (e.g., organic matter, grain size) can also limit growth (Koch 2001); therefore, to be conservatice, we only use 50% of the suitable habitat based on water depth .

Economic value was calculated using two well documented ecosystem services: (a) nutrient cycling in seagrass and algal meadows (Costanza et al. 1997) modified for only seagrass (Waycott et al. 2009) i.e. \$9502 ha^{-1} $year^{-1}$ and (b) the economic value for fisheries using reported assessments of fisheries yield (the mean was used except where a range was given, then the minimum value was used) (McArther et al. 2006, Samonte-Tan et al. 2007, Watson et al. 1993). Literature values range from \$8–2511 USD ha^{-1} $year^{-1}$, as meadows varied regionally and market value of species differed. We used a calculated mean of \$237 USD ha^{-1} $year^{-1}$.

To evaluate the economic benefit, we calculated the year in which value of ecosystem services provided by the restoration were sufficient to repay the cost of restoration and monitoring efforts (around $2 million). We made this estimate by summing the value of nutrient cycling and fisheries each year. Additionally, we evaluate the economic benefit as a result of accelerated recovery and calculate the elevated value of ecosystem services generated. To do this, we compared the area of restored seagrass meadows in 2010 to the area of natural populations using the three different model projections of natural population growth. We determined a date (year) when we estimate the natural populations would have achieved the same coverage, integrated the yearly difference in area between the restored and natural meadows, and then converted that area to economic value. Again, this is a conservative estimate as planted areas will continue to expand and deliver valuable ecosystem services.

Figures

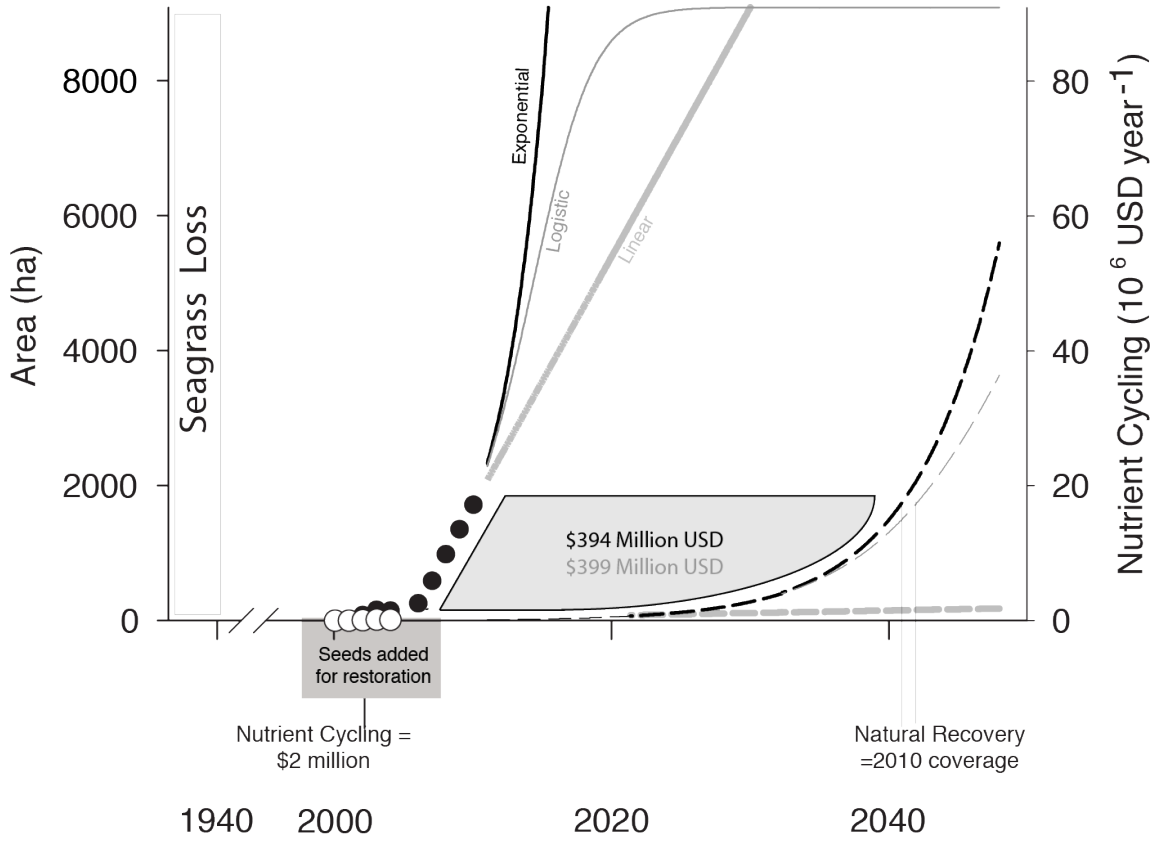

Fig. 1. Economic value of restoration.

Measured and modeled seagrass coverage and values for ecosystem services in the Virginia coastal bays. Filled dots (data) and solid lines (models) represent restored meadow area and empty dots (data) and dashed lines (models) represent naturally recruited areas.

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Chapter 6: Genetic diversity enhances restoration success by augmenting ecosystem services

Abstract

Disturbance and destruction of near-shore marine ecosystems as a result human activities is becoming more frequent, and restoration is often used to mitigate losses. A common metric to evaluate the success of restoration is the return of ecosystem services. Previous research has shown that biodiversity, including genetic diversity, is positively associated with the provision of ecosystem services. We conducted a field based restoration experiment using techniques and sites similar to actual large scale restoration projects which demonstrated that a small increase in genetic diversity enhanced ecosystem services (invertebrate habitat, increased primary productivity, and nutrient retention). In our experiment, plots with elevated genetic diversity had plants that survived longer, increased in density more quickly, and provided more ecosystem services (invertebrate habitat, increased primary productivity, and nutrient retention). We used the number of alleles per locus as a measure of genetic diversity, which, unlike clonal diversity, used in earlier research, can be applied to any organism. Additionally, unlike previous studies where positive impacts of diversity occurred only after a large disturbance, this study assessed the importance of diversity in response to potential environmental stresses (high temperature, low light) along a water depth gradient. We found a positive impact of diversity along the entire depth gradient. These results suggest that ecosystem restorations will significantly benefit from obtaining sources of restoration materials (transplants or seeds) with high genetic diversity and from restoration techniques that can maintain that genetic diversity.

Introduction

Ecological restoration is the process of augmenting the recovery of a degraded, damaged, or destroyed ecosystem (SER 2004). A typical restoration goal is to create a stable functional ecosystem, which provides ecosystem services similar to less impacted reference systems. Ecosystem resistance and resilience (stability), and the provision of ecosystem services, such as primary and secondary production are often positively correlated with measures of biodiversity (Kinzing et al 2001, Loreau et al. 2002, Hooper et al. 2005). Given the positive benefits of biodiversity, it is often incorporated into and used as a measure of restoration success (Ruiz-Jaem & Mitchell 2005). While biodiversity is often measured as species diversity, biodiversity is a hierarchical concept that can be measured at the scale of ecological guilds down to species, and even to variability within species (Reusch & Hughes 2006). In communities dominated by a single foundation species, such as temperate seagrass meadows, kelp forests, or cattail marshes, genetic diversity may be the most appropriate measure of biodiversity. The term genetic diversity is often broadly used to describe a number of measures, all of which may be important (Porcaccini et al. 2007). The number of unique individuals within populations is more appropriately called genotypic diversity or clonal diversity). Heterozygosity (measured within an individual) and allelic diversity (measured at the population level) are true measures of diversity.

The positive impacts of genetic diversity have been documented in a variety of systems. For example, planting genetically diverse varieties of crops tends to produce greater yields as well resistance to herbivory and disease (Cantelo & Sanford 1984, Wolf 1995). Genetic bottlenecks and inbreeding in small or endangered populations often

result in decreased levels of heterozygosity and reduced fitness levels (Reed & Frankham 2003, Leimu et al. 2006). Natural and manipulated marine plant assemblages have shown that clonal diversity is positively associated with density and some measures of ecosystem function (habitat and nutrient cycling) after large-scale disturbances (Hughes & Stachowicz 2004, Reusch et al. 2005, Hughes & Stachowicz 2009, Hughes & Stachowicz 2011).

Zostera marina (eelgrass) meadows are ideal model systems for studying the relationship between genetic diversity and ecosystem functioning. Eelgrass is a broadly distributed species in the Northern hemisphere with coverage on both the East and West coasts of both the Atlantic and Pacific Oceans (Green & Short 2003). The natural range of genetic diversity measured in this plant is high (both within and between meadows), probably due both to its adaptability to a wide range of environmental conditions and its ability to reproduce both sexually and asexually (Reusch 2001, Olsen et al. 2004, Hughes & Stachowicz 2009). It is also a well-studied species; previous work has shown that genetic diversity was positively correlated with plant density and with the density and diversity of organisms that use the seagrass as a habitat (Hughes & Stachowicz 2004, Reusch et al. 2004).

Seagrass genetic variability, as for all clonal plants, can be measured as either genotypic or genetic diversity. Genotypic diversity is a measure of the number of unique individuals, sometimes referred to as clonal diversity. Positive effects of genotypic diversity can occur when one or a few individuals are particularly adapted to local conditions. Theoretically where genotypic diversity is high, it is more likely that one or more individuals will be well-adapted. While genotypic or clonal diversity measures the

number of unique genetically-defined individuals or clones per area, it does not capture the degree of genetic variation among individuals. Genetic diversity describes the makeup of the unique genotypes present and can be measured in a variety of ways. The most often used measure of genetic diversity is heterozygosity (combinations of alleles within individuals) and is a measure independent of the number of alleles per population. Another measure of genetic diversity is allelic richness (among all individuals in populations) which captures the total diversity present in populations but is independent of the combinations of alleles. A community with a high genetic diversity and an abundance of genotypes (and thus phenotypes) is likely to have individuals that are occupying various niches. Complementarity is another mechanism by which genetic diversity increase population fitness and occurs when individuals have a variety of phenotypes, which allows the population access to different pools of resources limiting competition. Increasing the pool of genetic diversity among clonal genotypes also improves evolutionary potential and adaptive capacity under changing environmental conditions. Further, findings related to genetic diversity can be applied broadly to many systems that include both clonal and non-clonal species.

As genetic diversity is more difficult to manipulate than clonal diversity in natural systems, most studies that have contributed to our understanding of the relationship between genetics and ecosystem function have primarily used clonal diversity as their measure of genetic diversity. Manipulative experiments have shown that as a result of disturbance, clonal diversity—measured as number of unique genotypes—improved habitat quality (Reusch et al. 2005, Hughes & Stachowicz 2009) and plant resistance to further disturbance (Hughes & Stachowicz 2004, Reusch et al. 2005, Hughes &

Stachowicz 2011). There is some evidence that genetic diversity measured as heterozygosity is positively correlated with eelgrass fitness (Williams 2001, Hammerli & Reusch 2003). However, when analyzed in the same system, it is not clear that genotypic and genetic diversity have the same influence on either plant fitness (Arnaud-Haond et al 2010) or habitat quality (Hughes & Stachowicz 2009).

Previous results demonstrating that clonal diversity enhances ecosystem resistance to disturbance have been used to direct seagrass restoration (van Ktwijk et al. 2009); however, their applicability to large-scale restoration is debatable. Typical stresses that seagrass restoration efforts face include unfavorable light conditions due to sediment resuspension (Ramage & Schiel 1999) and bioturbation (Davis et al. 1998, Hauxwell et al. 2004). However, the documented positive effect of clonal diversity has been shown only during or after very large, albeit natural, disturbance events: for example, a grazing event that removed up to 75% of the biomass (Hughes & Stachowicz 2004), a warming event that has a return time of 10,000 years (Reusch et al. 2005), and the largest macroalgal bloom recorded at a site in a 4 year period (Hughes & Stachowicz 2011). These large disturbances do occur in nature but are not typical and may be beyond what the stresses that typical restored ecosystems would experience, and the role of genetic diversity in providing resistance to more common stressors is not clear.

We conducted a realistic field based restoration experiment, using techniques, sources, and restoration sites currently being employed by ongoing large-scale restoration, that demonstrate that a small increase in genetic diversity to a system with high baseline diversity can improve restoration success when measured by the provision of ecosystem services (habitat, productivity, and stability). This increase is evident even

when specific stresses and disturbances are not. Because our experimental system has high levels of heterozygosity with little variability, we use allelic diversity as our measure of genetic diversity. The outcomes of this study will be broadly applicable to understanding ecosystems and their restoration and unusual in that few studies have demonstrated the effects of allelic diversity on ecosystem functioning experimentally in the field.

Materials and Methods

Experimental Set-up

In May of 2007, flowering shoots were collected from 3 sites: (1) Mobjack Bay (UTM N 4141439 W 435119); and (2) the York River in Chesapeake Bay (UTM N 4125059 W 374285), and (3) South Bay (UTM N 4124724 W 428005), which is part of the Virginia coastal bay system. Seeds from the flowers were then used in a restoration experiment at Hog Island Bay (UTM N 4140648 W 435429), also part of the Virginia coastal bay system. Hog Island Bay and South Bay are part of the Virginia Coast Reserve Long Term Ecological Research (LTER) site. All necessary permits were obtained for the described field studies. The restoration site is part an area set aside for seagrass research and restoration by the Virginia Marine Resources Commission and access and collection were permitted through collaboration with the Virginia Institute of Marine Sciences.

In Hog Island Bay, 36 2 m x 2 m plots were seeded at a density of 100 seeds m^{-2} using an approach that has been successfully applied to restoration in this region (Orth et al. 2006, Marion & Orth 2010). Seeds were distributed underwater and gently covered

with sediment by hand. The plots were distributed in 8 blocks of 4 plots each along a depth gradient of about 0.8 m (range -0.78–1.5 MSL). To establish variation in genetic diversity in the experiment, in each block one plot was planted with seeds from each of the 3 source populations, and the fourth plot was planted with seeds from all of the source populations combined. The experiment was monitored over 3 growing seasons.

Twice during the experiment, differences in light and temperature conditions along the depth gradient were analyzed. Temperature was monitored for one month using HOBO temperature loggers that read every 15 min. Light profiles were taken at the center of each block using a LiCor spherical 4Pi sensor (n=3 for each block).

The genetic diversity of the plants in each plot was measured once during the experiment, 20 months after seeding. Six mature shoots from each plot were collected. At the time of collection, this was more than 10% of total shoots. DNA was extracted from each shoot using Qiagen DNeasy Plant extraction kits and, amplified at 8 microsatellite loci (loci: CT17H, CT3, CT35, GA2, CT19, CT20, GA3, and GA6) using standard PCR techniques (Reusch et al. 1999), and fragment were analyzed by capillary electrophoresis on a MegaBACE 1000 (GE Biosciences) with an internal ET ROX 400 size standard (GE Biosciences).

Shoot density, invertebrate density, seagrass productivity, and leaf nitrogen content were measured in each of the plots for 3 growing seasons (2008–2010). Monthly density (May–August) was counted in 4 haphazardly placed ¼ m^2 quadrats, and the maximum height of 5 haphazardly chosen shoots were measured. Once during the growing season, invertebrate density and productivity were estimated. Three shoots from each plot were carefully extracted and preserved in isopropyl alcohol until invertebrates

could be sieved, counted, and identified in the laboratory. Plant productivity was estimated by marking all shoots in a 0.01 m^2 quadrat (Zieman & Wetzel 1980). Three independent samples of young tissue were taken, dried, ground and analyzed on a Carlo Erba Elemental Analyzer.

Data Analyses

We assessed differences in genetic diversity among the different seed sources. For every plot an average of all alleles at each of the 8 loci was calculated using GenAlEx 6.3 (Peakall & Smouse 2005). Differences in diversity between treatments were analyzed using ANOVA followed by Tukey pairwise comparisons. Data were log transformed to meet the assumption of homogeneity of variance. The overall difference in genetic makeup between groups was analyzed using F_{ST} (estimated as Q, Weir & Cockerham 1984) in the software package Genodive v 2.0 (Merimans & can Tienderen 2004).

Since the numbers of alleles per locus were statistically higher for the South Bay and all combined treatments than for the Mobjack Bay or York River donors, we refer to these combined treatments as relatively high (South Bay donor and all donors) and relatively low (York River and Mobjack Bay both in Chesapeake Bay). To determine overall differences in density and the provision of ecosystem services, summer data were pooled by block and by the diversity categories. Differences in pooled data were analyzed with a t-test. In addition, the direct relationship of measured parameters to genetic diversity was explored with a regression.

Differences in light and temperature conditions with depth were analyzed using a standard regression. The potential impact of differences in temperature and light to the plants was considered by comparing the mean density of all plots at each depth.

Data were also blocked into 3 groups by dividing the depth gradient into 3 equal parts. Environmental stresses varied with among those depth groups: higher temperatures in shallow depths which often rose above 30°C (<0.9 m), lower light in deeper water that led to eventual mortality as predicted from models (Carr et al. 2012) (>1.3 m), and less stressful conditions with respect to light and temperature at moderate depths (0.9–1.3 m). We analyzed the impacts of genetic diversity on seagrass density at each of these depth intervals. For each block, a difference between high-diversity treatments and low-diversity treatments was calculated, and a chi-square goodness of fit test with an expected value of 0 (no effect of genetic diversity) was conducted. Differences between the effect of genetic diversity on density under separate stress regimes (shallow temperature stressed and mid-depth unstressed) was analyzed using a paired t-test, pairing the difference between high- and low-diversity treatments at each sampling date.

For those plots that did not survive the light stress, the length of time that each of those deeper plots survived was plotted against depth. A blocked ANOVA (block: diversity treatment, factors: water depth and block) was used to determine if the plants in the high-diversity treatment were more resistant to the light stress of deep water and thus survived for a longer amount of time.

Results

Plants in plots that were seeded from different sources differed in the average number of alleles per locus ($F=7.16$, $p=0.002$). Other experiments in this region have shown that regional genetic diversity of seagrass is high (Reynolds et al. 2012), and the plants in this experiment also had a high diversity relative to other studies. However, the

experimental plots seeded both from South Bay seeds and all of the seeds combined had a greater number of alleles per locus (4.4 ± 0.3 s.e.) than those plots seeded from the Chesapeake Bay sites of Mobjack Bay and the York River (3.5 ± 0.3 s.e.) (Fig. 1).

Measured genetic diversity was positively correlated with density and areal productivity during peak growing season (June) during the 3 years monitored (Fig. 2). The number of invertebrates was also positively correlated with genetic diversity during the summer (Fig. 2); however, in 2010 invertebrate density was measured during the fall and there was no relationship (R^2=0.1, p=0.5).

We will refer to the South Bay and combined plots as 'high diversity' and the Mobjack Bay and York River plots as 'low diversity' even though both measures are high compared to other geographical regions (Reynolds et al. 2012) (Fig. 1). Although there was a difference in overall diversity, the two groups of populations were quite similar in overall genetic makeup, with a pairwise F_{ST} value of 0.01. During the months of high growth (June and July), plants in the high-diversity treatment were more dense (F=2.72, p=0.007) (Fig. 3a). Maximum height was marginally higher in high-diversity plots than in low-diversity plots (F=2.68, p=0.1) (Fig. 3b). Shoot-specific productivity did not differ between treatments (F=0.4, p=0.5), but because of increased density, overall areal productivity was higher in high-diversity plots (F=6.52, p=0.01) (Fig. 3c). Nitrogen content of the leaves did not vary with diversity treatment (F=1.05, p=0.5); however, because high diversity plots were more productive, they had higher nitrogen standing stock (F=6.25, p=0.01). Likewise, there was no difference in the number of invertebrates per shoot (F=1.15, p=0.6), but again since the high-diversity plots were more dense, there were more invertebrates per area in more diverse plots (F=2.22, =0.02) (Fig. 3d).

Environmental characteristics varied over the depth gradient. Light decreased with depth ($m=0.02$, $R^2=0.5$, $p=0.03$) (Fig. 4a), and while minimum daily temperature did not vary with depth ($m=0.8$, $R^2=0.12$, $p=0.3$), maximum daily temperature was greater, often above 30°C, ($m=0.1$, $R^2=0.8$, $p=0.0002$) in shallower water (Fig. 4b). Plant density and survival varied along this range in environmental conditions. Shallow plots were less dense than plots at the moderate depth, and the density of plants at the moderate depth expanded throughout the three years (Fig. 4c). In both the shallow (temperature stressed) and mid-depth (relatively unstressed) blocks, high diversity plots were more dense than low diversity plots (Shallow: $\chi^2=11{,}000$ $p<0.0001$; Mid-depth: $\chi^2=12{,}000$ $p<0.0001$) (Fig 4d). The plants in the deeper water died during the second growing season. During the first growing season while all plots still had live plants (2008), the high-diversity plots at these deeper sites were consistently more dense than the low-diversity plots over all sampling months ($\chi^2=8.9$, $p=0.03$). That positive relationship decreased during the second year as plants died and eventually the density of all of the deep plots became 0 ($\chi^2=9.7$, $p=0.2$) (Fig 4d). Because the plots were monitored monthly, an approximate time of survival (in months) could be determined. Plots with a higher genetic diversity lived about one month longer ($F=2.8$, $p=0.1$), suggesting that genetically diverse assemblages are more resistant to chronic light stress.

High-diversity treatments were more dense across the environmental gradient; however, the magnitude of difference between high and low diversity treatment varies temporally. There was a consistent pattern where the difference between the high- and low-diversity plots was greatest in June when the plots were shallow and heat stressed, while the difference between the high- and low-diversity plots was greatest in July when

plots were at a moderate depth and apparently unstressed (Fig. 4d). Despite temporal variability, the overall density difference between high- and low-diversity plots was consistent among shallow, heat-stressed plots and mid-depth, unstressed plots (Fig. 5).

Discussion

Genetic diversity, measured as allelic diversity, was positively associated with seagrass density, which cascaded upward into positive impacts on invertebrate density, nitrogen retention, and areal productivity. This is in accordance with previous studies, which demonstrate a positive effect of clonal diversity on ecological parameters (Hughes & Stachowicz 2004, Reusch et al. 2005, Hughes & Stachowicz 2011); however, these results are unusual in that the enhancement of ecosystem services occurred without obvious signs of ecological stress or disturbance. The enhancement of ecosystem services also occurred despite a relatively small increase in genetic diversity in region that has been documented as one of the most diverse in the world (Reynolds et al. 2012). These results suggest that the success of seagrass restoration will increase when efforts are made to use transplants with a high genetic diversity and with techniques that maintain that diversity (i.e. seeding; Reynolds et al. 2012).

Previous work has shown that genotypic diversity, measured as clonal richness, (Hughes & Stachowicz 2004, Reusch et al. 2005, Hughes & Stachowicz 2011) and genetic diversity, measured as heterozygosity (Williams 2001, Hammerli & Reusch 2003), are positively associated with plant fitness and ecosystem stability. This study, conducted as a field experiment, demonstrates a similar relationship with allelic diversity. This is significant since previous measures of diversity (clonal richness and

heterozygosity) are not appropriate for many systems. Because our experimental restoration plots were initially planted with seed, all recruits are genetically distinct and as a result clonal diversity would be 1.0 at the start of the experiment for all sites. Because clonal was constant, and we did not detect any clonal dominance across the experiment, it was an unsuitable measure to describe variability. In addition, heterozygosity was very high and largely invariant (Reynolds et al. 2012), making this unsuitable for describing variability. Allelic diversity, however, is a robust measure of diversity and was able to be applied in this system and would also be applicable in most other systems. It should be noted that it is clear from the literature that genotypic diversity is potentially important for *Zostera marina* and that over time, we are not discounting the possibility that genotypic diversity may become a more important measure in this system once the effect of initial establishment is overcome.

Allelic diversity was associated with increases in both seagrass density and ecosystem services: habitat, productivity, and nitrogen retention. The mechanism for this enhancement of ecosystem services is the increase in density as there was not a shoot-specific increase in ecosystem services. In this study as well another in the same system, density was an appropriate measure of restoration success and a suitable variable for understanding the impact of genetic diversity on seagrass ecosystems (McGlathery et al. 2012).

This study found a positive impact of allelic diversity on density under different environmental stress regimes: chronic light stress that killed the plants, temperature stress that decreased density, and low stress levels with no apparent effect on the plant. While the importance of diversity in terrestrial systems has been shown in the absence of stress

(Crutsinger et al. 2007), this is one of the first studies to show the positive effect of genetic diversity under low stress conditions in seagrass systems. The overall relationship between density and diversity does not differ in the warm shallow stressed water and the low stress moderate depths (Fig. 5); however, in temperature-stressed plots, there is an earlier separation of high diversity and low diversity plots both initially and during each season sampled (Fig. 4d). This suggests that diversity is important regardless of stress, but disturbance or stress can cause a shift in response. We hypothesize that when stressed plants require more resources, causing competition and earlier importance of niche complementarity than in systems where plants are not stressed and not severely resource limited. Plants in the deep plots, which experienced the lethal low light stress, acted similarly to all other plants during the first year (Fig. 4d). During the second year, plants started to die and patterns were more difficult to detect, but more genetically diverse plots lived longer, showing some ecosystem resistance to the stress that lead to their death. This increased stability is similar to past results using clonal diversity as opposed to genetic diversity. Manipulative experiments have shown that experimental plots with a larger number of individual clones (range 1-8) have better resisted large disturbances due to geese grazing (Hughes & Stachowicz 2004), extreme temperature events (Reusch et al. 2005), and large macroalgal blooms (Hughes & Stachowicz 2011). Our findings are important and unique in that this was a very common chronic stress as opposed to very large disturbance. One of the most common causes of seagrass decline is decreased water quality, which promotes planktonic and epiphytic algal growth reducing light levels and shading seagrasses (Short & Wyllie-Echeverria 1996). While plants in our experiment were not resilient and did die, if the reduction in light was shorter term such as occurs

with sediment suspension during a storm event or a short-term nutrient pulse, the plants that survived longer may have outlived the disturbance and continued to survive.

Both complementarity and dominance of a few genotypes have been described as mechanisms for genotypic diversity enhancement of disturbance resistance in other studies (Hughe & Stachowicz 2011). For strong dominance to occur, a small number of genotypes would need to be more abundant. We found no clones in our analysis, and this is typical of this region, where flowering rates, seed production, and clonal diversity are high (Reynolds et al. 2012). Further, overall low pairwise F_{ST} values suggests that there may not be many unique alleles in these more diverse populations. Instead there must be more combinations of similar alleles, which is more likely to lead to complementarity as opposed to dominance.

One of the strengths of our experiment is that it replicates realistic conditions for restoration. Seeds were collected from sites that are regularly used for restoration projects and planted in the same manner and in close proximity to a site that is being used for large-scale restoration (Reynolds et al. 2012, McGlathery et al. 2012). Our results suggest restorations that achieve high levels of genetic diversity will be more successful and will create more resilient seagrass ecosystems: where plants survive longer, reproduce more rapidly, more quickly increase in density, and provide more ecosystem services.

Figures

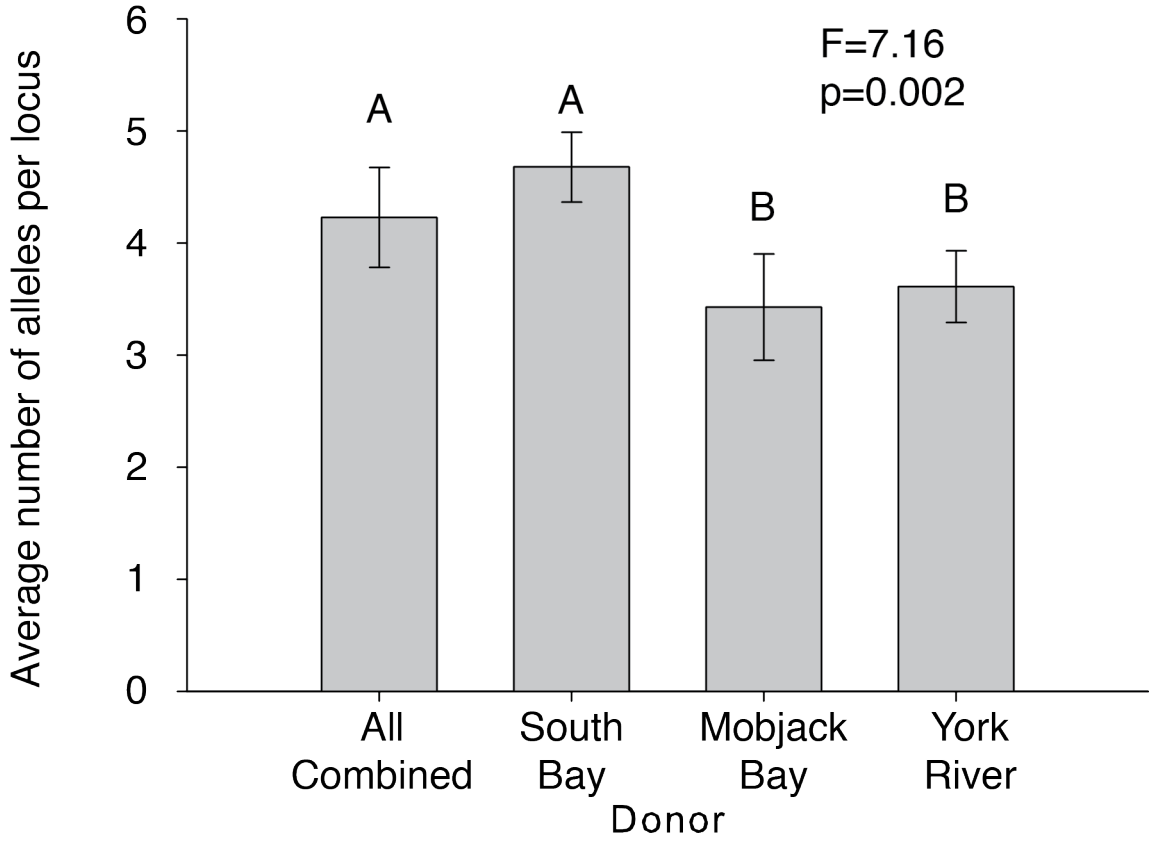

Fig. 1. Plot genetic diversity.

Zostera marina seeds were collected from Mobjack Bay and the York River in Chesapeake bay and from South Bay, part of the Virginia coastal bay system. Seeds were planted in Hog Island Bay, also part of the Virginia coastal bay system in plots as either individual sources or as plots with all seed sources combined. Plots planted with seeds from either Mobjack Bay or the York River had a lower overall genetic diversity (measured by alleles per locus) than plots planted from either South Bay or from all three seed sources combined.

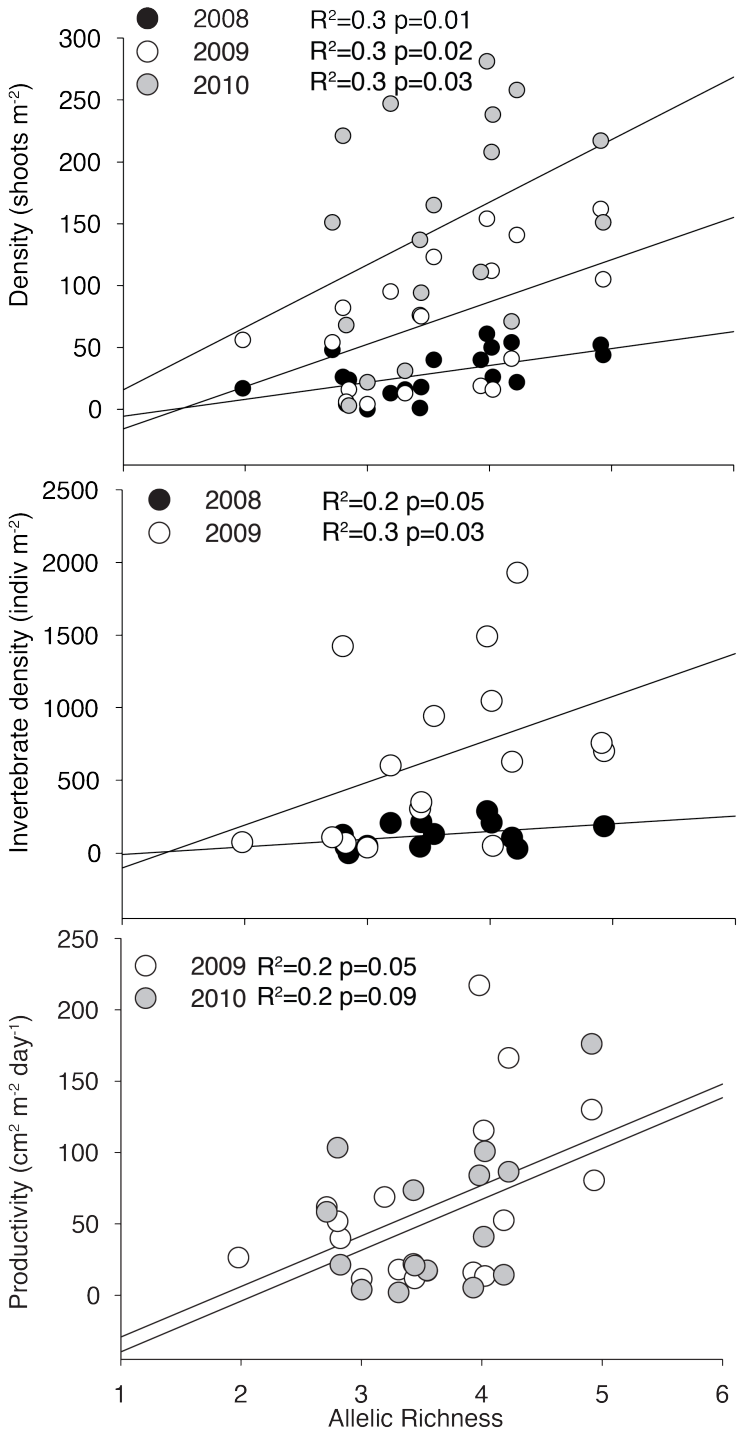

Fig. 2. Linear relationship between diversity and ecosystem services.

Plant density (A), invertebrate density (B), and areal productivity (C) during the peak growing season (June) was regressed against plot genetic diversity.

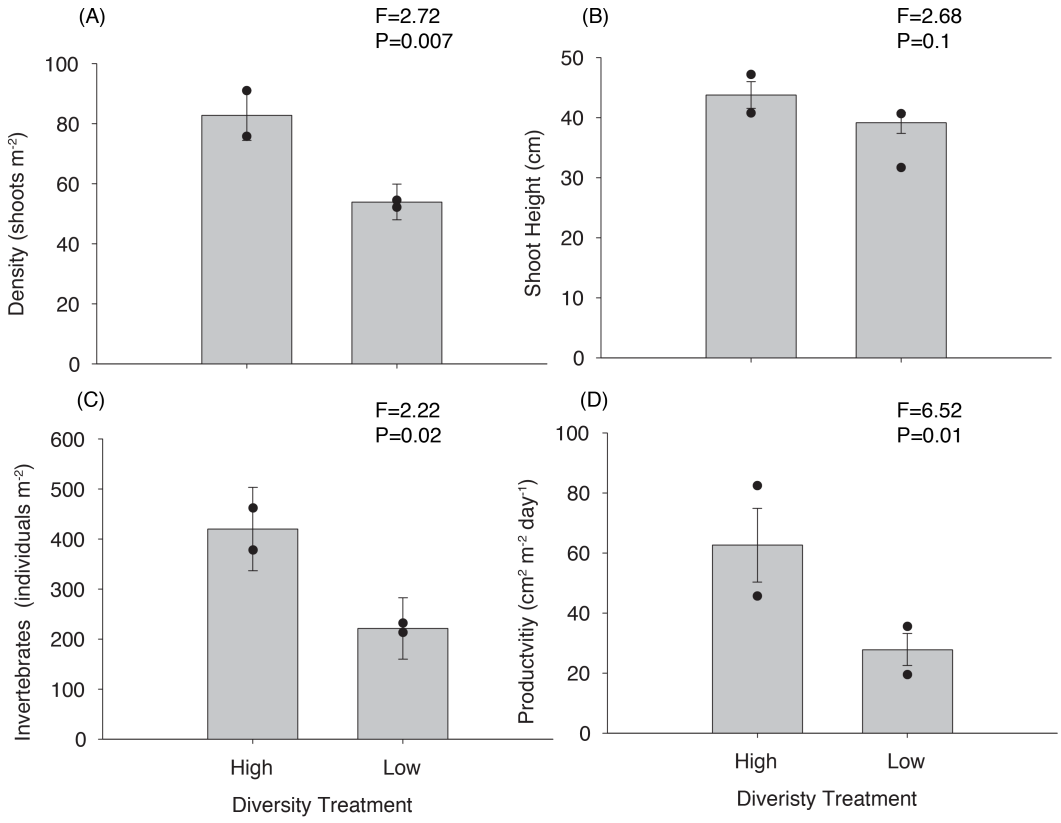

Fig. 3. Relationship of Genetic to Ecosystem Services.

Experimental *Zostera marina* plots were planted in Hog Island Bay in two levels of genetic diversity: relatively high (4.4 alleles per locus ± 0.3 s.e.) and relatively low (3.5 alleles per locus ± 0.3 s.e.). During the peak summer growth (June and July), plant characteristics [density (A) and shoot height (B)] and measured ecosystem services [habitat function estimated as invertebrate density (C) and areal productivity (D)] were measured, and differences between high diversity and low diversity plots were analyzed with a T-test. Error bars represent standard error. Dots represent the mean of plots from individual seed sources.

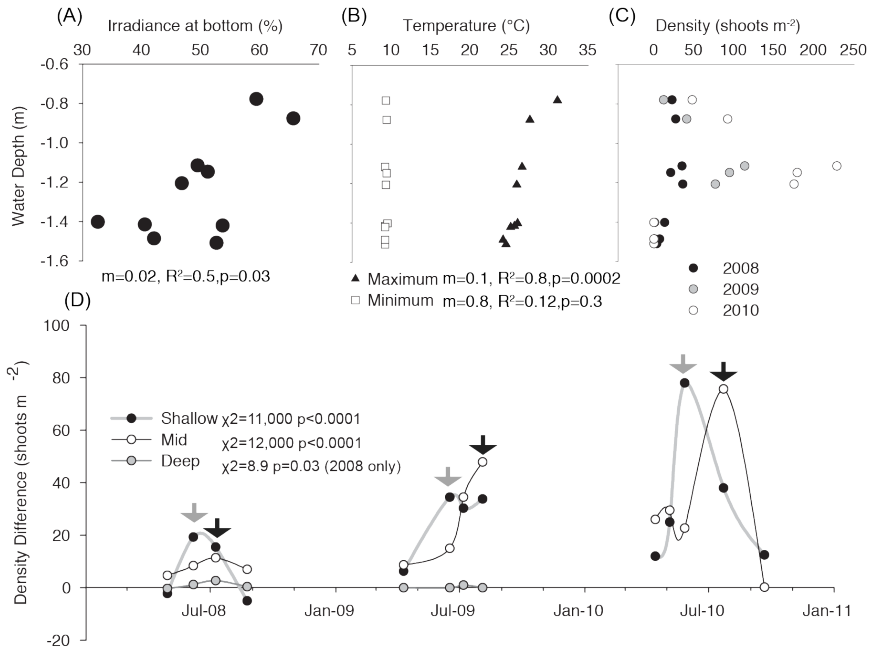

Fig. 4. Depth differences.

Experimental *Zostera marina* plots were planted in Hog Island Bay over a depth gradient of 0.8 m (range -0.78–1.5 MSL). Environmental conditions [light (A) and temperature (B)] varied with depth and resulted in differences in plant density (C). Plots at a depth less than 1m and plots with a depth greater than 1.4m had lower densities, while mid-depth plots had high densities that increased each year. Plots, replicated at each depth, were assigned to one of two levels of genetic diversity: relatively high (4.4 alleles per locus ± 0.3 s.e.) and relatively low (3.5 alleles per locus ± 0.3 s.e.). (D) Differences in density between the high diversity and low diversity plots were analyzed with a chi-square test (expected value of 0). Plants at the deepest depths died during the second growing season; therefore, differences were analyzed for the first year, while all plots had live plants, independently. Arrows indicate the timing of maximum density difference between high and low diversity plots.

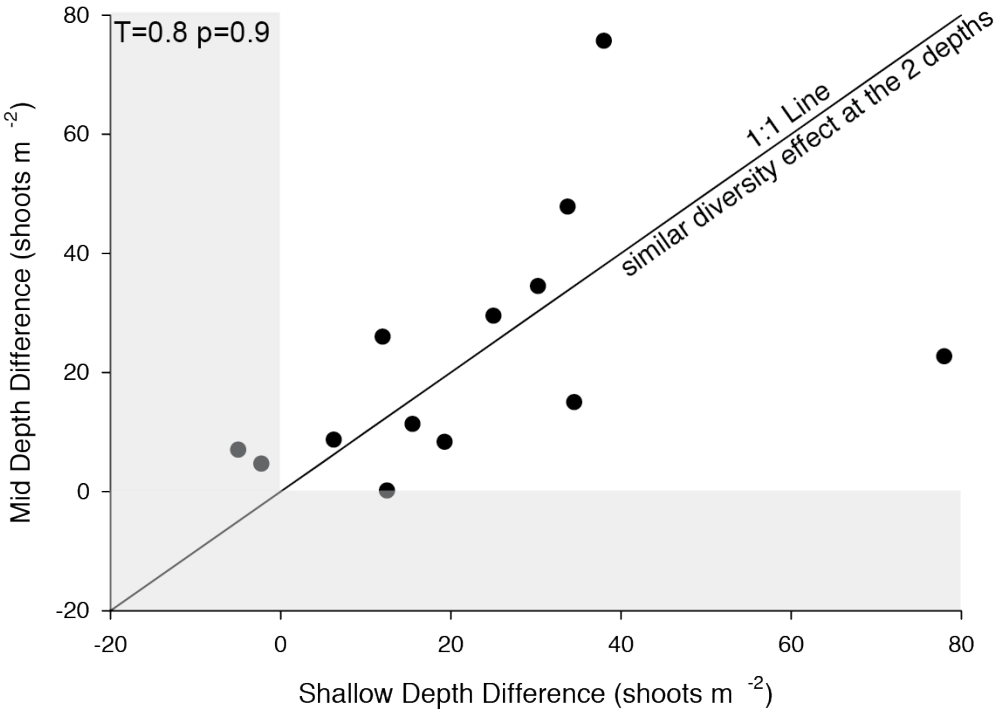

Fig. 5. Impact of genetic diversity under stressed and not stressed conditions. Experimental *Zostera marina* plots were planted in Hog Island Bay in two levels of genetic diversity: relatively high (4.4 alleles per locus ± 0.3 s.e.) and relatively low (3.5 alleles per locus ± 0.3 s.e.). Plots were replicated at depth. Plots at a depth less than 1m were apparently heat stressed had reduced densities, while plots at depths between 1 and 1.4 m had high densities that increased over time. For each sampling date, a difference in density between high diversity and low diversity plots was calculated at each depth. A paired t-test (paired at sampling date) was used to determine if there was a greater effect of diversity on density at the different stress levels. The solid line is a 1:1 line representing no differences in the effect of diversity between the two depths. The grey areas represent samples where the effect of genetic diversity on plant density was not significant.

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Chapter 7: Genetic diversity validated as an important driver of seagrass density and ecosystem services

Abstract

Seagrasses are foundation species that provide a number of ecosystem services. Plant density can act as an easily measured proxy for many ecosystem services including habitat, primary productivity, sediment stabilization, and nutrient cycling. Environmental parameters such as light levels and nutrient availability are well documented as influencing shoot density in seagrass meadows. However, the relative importance of genetic diversity in determining seagrass density compared to environmental variables remains largely unexplored. In this study, we measured seagrass density, genetic diversity, water depth, sediment organic matter, and sediment nitrogen in replicate 4000 m^2 plots located in the oligotrophic Virginia coastal bays. A stepwise linear regression model indicated that water depth, genetic diversity, and sediment nitrogen all were significant predictors of seagrass density. Further, a regional survey of meadows that varied significantly in environmental parameters (i.e. temperature, nutrient loading) showed a strong positive relationship between seagrass genetic diversity and density. The importance of this relationship is therefore comparable to such parameters as light availability, nutrient concentrations, and temperature variation. Monitoring, conservation, and restoration plans, which aim to maximize seagrass density and the provision of ecosystem services, should include genetic diversity alongside more traditional environmental parameters.

Introduction

Seagrasses are foundation species and the meadows that they create provide a wealth of ecosystem services including: high rates of primary productivity; habitat for ecologically and economically important invertebrate, finfish, mammal, and reptile species; nutrient and sediment filtration; and sediment stabilization (Hemminga and Duarte 2000, McGlathery et al. 2012). Individual meadows vary in the amount of ecosystem services that they provide. For example, as meadows age, they remove more nitrogen from the system by means of denitrification (Cole 2011). Seagrass meadows located in tropical regions act as a nursery habitat for more species and more individuals than meadows located in temperate regions (Williams & Heck 2001). Seagrass species with relatively large amounts of below-ground biomass (e.g. *Thalassia testudium*) stabilize sediments more effectively than smaller species with less below-ground biomass (e.g. *Halodule wrightii*) (Fonseca & Fisher 1986). Even meadows of the same species in the same region can vary in the amount of primary production and the number of invertebrates that they harbor, with higher genetic diversity assemblages providing enhanced levels of ecosystem services (Hughes & Stachowicz 2004, Reusch et al. 2005, Chapter 6).

Understanding the factors that influence the provision of ecosystem services by individual meadows will aid in developing effective conservation and restoration strategies. Seagrasses are being lost at rapid rates worldwide primarily due to anthropogenic disturbances (Short & Wyllie-Echeverria 1996, Waycott et al. 2009), and this results in the direct loss of ecosystem services. Many of these ecosystem services have significant economic value. For example, it is estimated that the nutrient cycling and

removal service is worth an average of $19,000 USD ha^{-1} yr^{-1} (Costanza et al 1997), and the fisheries associated with seagrass range in value from $8–2511 ha^{-1} $year^{-1}$ (Watson et al. 1993, McArthur & Boland 2006, Samonte-Tan et al. 2007).

While it is difficult to measure the total amount of ecosystem services that a meadow provides, density is a good proxy for several of those services, at least for *Zostera marina* meadows (McGlathery et al. 2012, Chapter 6). Previous studies have shown that environmental factors and plant physiological responses influence seagrass density. In Florida Bay, nutrient additions from bird excrement resulted in increases in seagrass density (Forqurean & Zieman 1992). Shading reduced the density of the seagrass *Halophila ovalis* in Moreton Bay, Queensland, Australia (Longstaff et al. 1999). Individual seagrass species have optimum salinities, and changes in salinity influence plant density (Lirman & Cropper 2003). Nutrient loading, light levels, and salinity are parameters that are routinely monitored by organizations that track the health of seagrass meadows (Fourqurean 2003, Short et al. 2006, Johnson et al. 2011).

Genetic diversity is rarely measured by monitoring programs; however, several recent experimental studies have shown that genetic diversity is also positively correlated with *Z. marina* density under a variety of conditions (Hughes & Stachowicz 2004, Reusch et al. 2005, Hughes & Stachowicz 2009, Chapter 6). Each of these studies manipulated diversity in relatively small plots (1–4 m^2) that were close in proximity (separated by 2–5 m) and therefore were exposed to similar environmental conditions. The goal of the present study was to determine the importance of genetic diversity relative to environmental parameters (i.e. water depth and light limitation, sediment nitrogen content and organic matter) under a range of realistic environmental conditions.

We used well-studied replicate 4000 m^2 plots to rank predictors of seagrass density and the provision of ecosystem services, and then we explored the relationship between genetic diversity and seagrass density along a 100 km stretch of coastline where environmental parameters such as temperature and nutrient loading vary.

Methods

Within-Meadow Analysis

Hog Island Bay is a shallow lagoon within the Virginia Coast Reserve (VCR) coastal bay system and is part of the VCR Long Term Ecological Research site. In this lagoon, large (0.4 ha) replicate plots have been planted with *Zostera marina* seeds (See McGlathery et al. 2012). We used 10 of those plots for this survey. All plots were planted in either 2007 or 2008 and varied in depth from 0.9 to 1.6 m. During the summer of 2010, density was estimated in each plot by counting the shoots in 10 replicate 0.25 m x 0.25 m quadrats. Organic matter was estimated as mass loss on ignition of dry sediments from 5 replicate 60 mL syringe cores taken to a depth of 5 cm. A separate set of cores was dried, ground, and analyzed for nitrogen content using a Carlo Erba Elemental Analyzer with a 1020°C combustion tube, 650°C reduction tube, and helium as a carrier gas. Finally, 24 *Z. marina* shoots were analyzed for genetic diversity. Plants (separated by at least 5 m) were collected and preserved with silica gel desiccant until DNA could be extracted, following the protocol described in Reynolds et al. (2012—Chapter 2), using DNeasy™ plant extraction kits (Qiagen) following manufacturer's instructions. Extracted DNA was amplified at 7 polymorphic loci previously described for this species (CT17H, CT3,

CT35, GA2, CT19, CT20, GA3) using standard PCR techniques (Reusch et al. 1999). PCR products were analyzed using capillary electrophoresis on a MegaBace 1000 (GE Biosciences), and Allelic richness (A_R) standardized to the smallest population size by rarefaction, was computed using FSTAT 2.9.3.2 (Goudet 2001).

Previous research has shown that the provision of ecosystem services (modification of sediments, habitat, and productivity) is closely related to density of shoots in this region (McGlathery et al. 2012, Chapter 6). A stepwise linear regression analysis was used to determine which parameters (water depth, genetic diversity, sediment organic matter, and nitrogen content) are the best predictors of seagrass density as a proxy for the provision of ecosystem services. Seagrasses in the system become light limited at a depth near 1.6 m at mean sea level (MSL) and two stable states—seagrass or bare-sediments—can exist based on environmental forcing (Carr et al. 2012a, McGlathery et al. 2012).

Regional Analysis

Seven *Z. marina* meadows spanning the 120 km latitudinal gradient from the northern Virginia coastal bays (Chincoteague Bay) to the mouth of Chesapeake Bay (Fisherman Island), including meadows on both the western (Mobjack Bay and the York River) and eastern shores (Hunger's Creek, Cape Charles) of Chesapeake Bay, as well as meadows in the Virginia coastal bays (South Bay) were sampled. These meadows vary significantly in that the southern Virginia coastal bays are restoration sites (South Bay), the Chesapeake Bay sites can be temperature stressed (Hunger's Creek, Mobjack Bay,

and the York River) (Moore & Jarvis 2008, Moore et al. 2012), and the Chincoteague Bay sites are often stressed by excess nutrients (Short et al. 2006).

At each of these meadows, 24 *Z. marina* shoots were collected and analyzed for allelic richness as described above. Additionally, density of *Z. marina* was estimated by counting the number of shoots in 10 replicate 0.25 m x 0.25 m quadrats. The relationship between allelic richness and density in this region was analyzed using standard linear regression.

Results

Within-Meadow Analysis

In Hog Island Bay, water depths ranged from 0.9 m to 1.6 m, allelic richness ranged from 5.0 to 6.2, sediment organic ranged from 1 to 2.5%, and sediment nitrogen ranged from 0.001% to 0.1%. Seagrass density ranged from 4 to 342 shoots m^{-2} and co-varied with other measured parameters. Seagrass density was negatively correlated with depth (R^2=0.4, p=0.04), but was not significantly correlated with the other environmental factors (AR: R^2=0.05, p=0.5; sediment organic matter: R^2=0.04, p=0.5; sediment nitrogen content: R^2=0.1, p=0.3). However, a step-wise linear regression model selected depth, allelic richness, and sediment nitrogen as significant drivers of seagrass density (Depth: R^2=0.4; Depth+AR: R^2=.5; Depth+AR+N: R^2=0.7) (Fig. 1).

When only the shallower plots above the 1.5 m depth (MSL) where seagrass density declines and exists in a bistable state with bare bottom (Carr et al. 2010, 2012a,

McGlathery et al. 2012), density was positively correlated with allelic richness (R^2=0.7, p=0.05) and sediment organic matter (R^2=0.6, p=0.06), negatively correlated with water depth (R^2=0.6, p=0.1), and not correlated with sediment total nitrogen content (R^2=0.1, p=0.5). A step-wise linear regression model selected both allelic richness and organic matter as significant drivers for seagrass density (AR: R^2=0.6, AR+OM: R^2=0.9). (Fig. 1).

Regional Analysis

Across all sites, density had a strong, but marginally significant, positive relationship with meadow allelic richness (R^2=0.5, p=0.09) (Fig. 2).

Discussion

Density in this region can be used a proxy for the provision of ecosystem services (McGlathery et al. 2012). In the Virginia coastal bays, shoot density has been shown to be a significant driver of the number of invertebrates living within the canopy, the overall areal productivity, the rate of nitrogen fixation, and the modification of the sediment including increases in sediment organic matter and fining of sediments (Cole & McGlathery 2012, McGlathery et al. 2012, Hansen and Reidenbach 2012, Chapter 6). In this study, we show that while many environmental factors can influence the density of seagrass meadows, genetic diversity is among the most important predictors of density on local and regional spatial scales.

Although genetic diversity was an important determinant of seagrass density, water depth was sometimes more important. Previous studies in the Virginia coastal bays

have shown that density dramatically declines at a depths below 1.5 m (McGlathery et al. 2012), and modeling results identify 1.6 m as the tipping point between the alternative stable seagrass states and a bistable region where either seagrass or bare sediment can exist based on environmental conditions (Carr et al. 2012a,b). Including genetic diversity in the linear regression model significantly improved our explanation of the variance in seagrass density (Fig. 1). When the deepest plots, where seagrass coverage is likely to be unstable were excluded, genetic diversity was the most important predictor of seagrass density, suggesting that it is only superseded in this system when the depth-related stressor (inferred as light limitation in deeper waters) was tipped over some threshold.

Other parameters considered that could influence seagrass density varied little over the depth gradient (i.e. sediment nitrogen), and there were other parameters not considered in this study (i.e. water column nutrients, water temperature). However, these parameters were implicitly included in the regional survey, which showed a strong, albeit marginally significant, correlation between genetic diversity and seagrass density (Fig. 2). The regional survey included sites that are known to regularly experience near lethal temperatures (i.e. Chesapeake Bay; Moore & Jarvis 2008, Moore et al. 2012) and sites that do not (i.e. Virginia coastal bays (Moore et al. 2012)), sites that have low nutrient loading (i.e. Virginia coastal bays (Cole 2011)), and sites that have high water column nutrients (i.e. Chincoteague Bay (Short et al. 2006)).

This is the first study to show that genetic diversity has an influence on plant density (and thus ecosystem services) over large spatial scales (4000 m^2 —entire meadows) and where environmental conditions vary. Small-scale (0.25–4 m^2)

experimental studies have manipulated clonal and genetic diversity to show that there is a positive correlation with genetic diversity and plant density, habitat function, and nutrient cycling (Hughes & Stachowicz 2004, 2009, Reusch et al. 2005, Chapter 6). Our study suggests that the importance of this relationship is comparable to such key parameters as nutrient concentrations, light availability, and temperature variation.

Understanding the factors that influence the provision of ecosystem services is essential to develop effective management strategies for the conservation of seagrass habitats. Seagrasses are declining worldwide at rapid rates primarily as a result of human impacts on water quality (Short & Wyllie-Echeverria 1996, Waycott et al. 2009), and with those declines come the loss of important ecosystem services. Habitat for commercially important species (Anderson 1989) and nutrient cycling (Costanza et al. 2007) are among the many economically valuable ecosystem services provided by these plants, and motivate efforts to both reduce impacts on seagrass habitats and restore degraded meadows. Worldwide, seagrass monitoring programs track a similar suite parameters in order to evaluate the overall meadow trajectories: plant cover and density, species diversity, tissue nutrient content, water quality, salinity, sediment texture, sediment organic matter, and sediment nutrients (Fourqurean 2003, Short et al. 2006, Johnson et al. 2011). Previous studies have shown that genetically diversity seagrasses are more resilient to environmental stresses (Hughes & Stachowicz 2004, Reusch et al. 2005), and our study demonstrates that genetic diversity is a good predictor of seagrass density and the amount of ecosystem services provided by the meadow. Together, these findings suggest that the preservation of genetic diversity should also be incorporated into conservation, restoration, and monitoring plans.

Figures

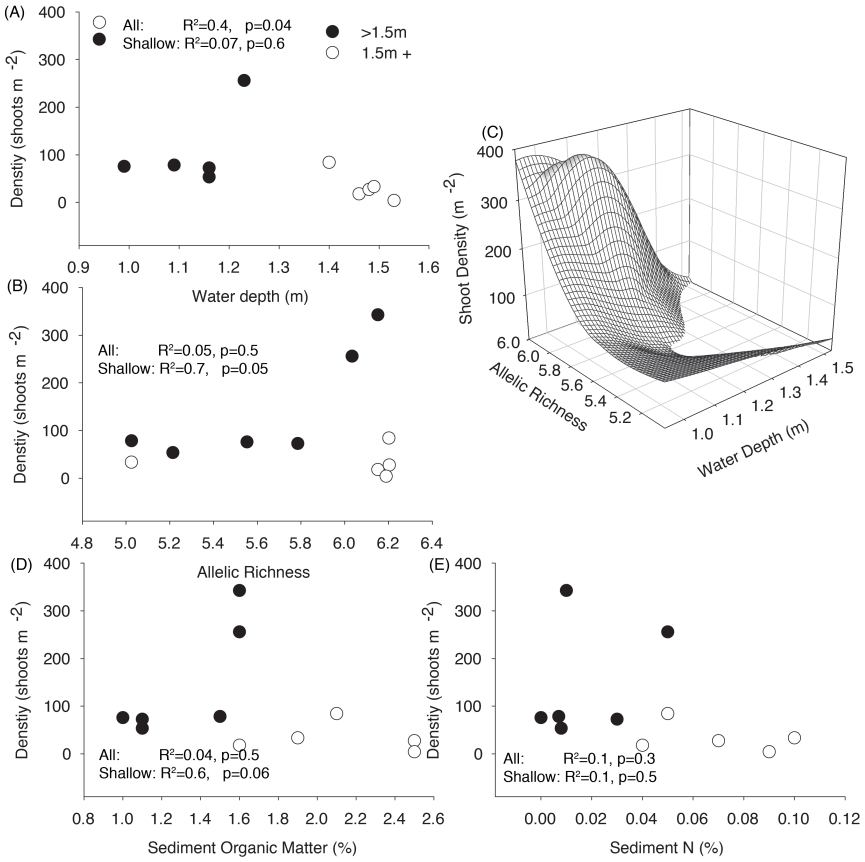

Fig. 1. Predictors of seagrass density in Hog Island Bay.

Within Hog Island Bay lagoon, plant density in replicate 4000 m^2 *Zostera marina* restoration plots was compared water depth (A), to plant genetic diversity [estimated using microsatellite techniques (B)], sediment organic matter (D), and sediment nitrogen content (E). The dark circles represent shallow plots, while the white circles represent plots that are deeper. Previous studies in this lagoon have shown that deeper plots are less dense and less stable. Significant regressions are illustrated with a solid line and marginally significant relationships are illustrated with a dotted line. The * marks which data are used in the regressions. Panel C is a 3D representation of the correlation between density and the two most influential predictors: water depth and genetic diversity.

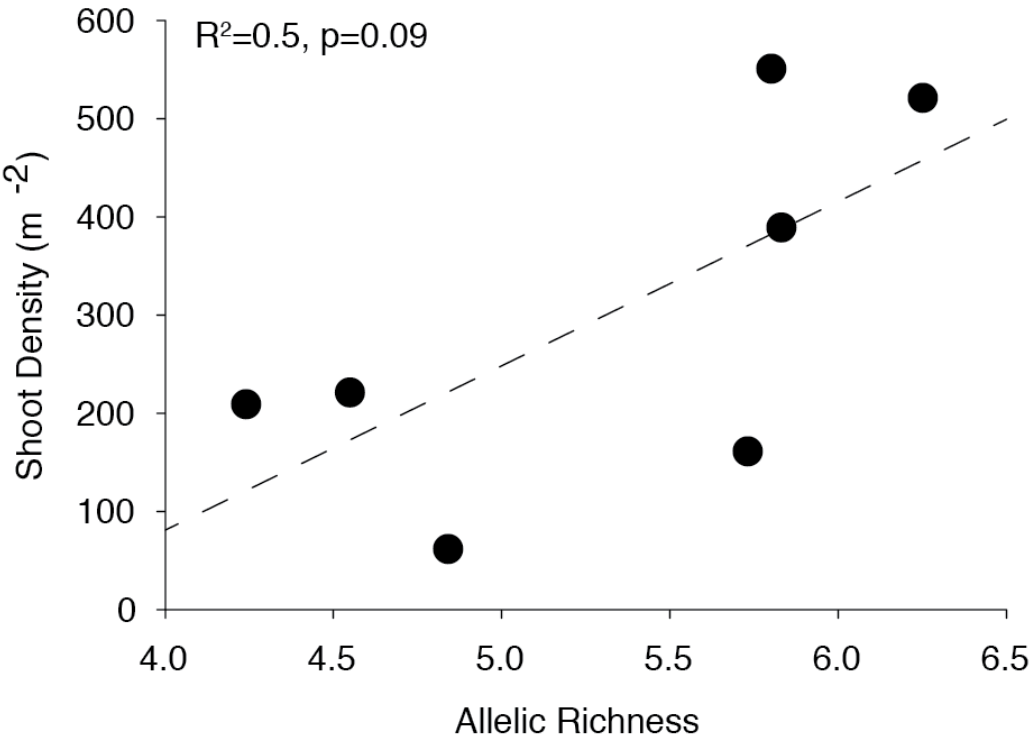

Fig. 2. Regional relationship of genetic diversity and shoot density.

Zostera marina shoot density was compared with plant genetic diversity measured using microsatellites. Each dot represents a meadow, and the meadows are distributed along the Virginia coastline and vary in meadow age, water temperature, and nutrient loading.

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Chapter 8: Synthesis and Significance

The goal of this dissertation was to use genetic tools to describe the seagrass populations in Virginia, to explore the ecological processes and anthropogenic activities responsible for the observed genetic structure and variability, and to investigate the ecological implications of the observed genetic structure and variability. I found that genetic diversity in both the Virginia coastal bays and in the Chesapeake Bay was high and that disturbance played a role in maintaining that high diversity. Further, the high diversity of donor meadows was passed on to restored meadows since the technique of restoring via seed maintained genetic diversity. My experiments showed that across all levels of stress gradient (from shallow water where temperatures were high and stressful to moderate depths where stress was minimal, or deeper depths where plants were chronically stressed by low light), high genetic diversity was associated with higher seagrass density and higher amounts of ecosystem services (nutrient cycling, habitat, and primary productivity) provided. Finally, I demonstrated that the magnitude of the relationship between genetic diversity and seagrass density was comparable to parameters known to influence density, including nutrient concentrations, light availability, and temperature variation.

In Chapter 2, I describe both the donor meadows in Chesapeake Bay and the restored meadows in the Virginia coastal bays as being genetically diverse, and among the most diverse in the world. This was contrary to my hypothesis that Chesapeake Bay meadows would be genetically depleted because of high rates of disturbance and repeated reductions in areal cover. This surprising finding suggests that the theory that disturbance causes population bottlenecks should be re-evaluated in clonal systems where sexual reproduction is also high. Also in contrast to my hypothesis, I find that restored meadows

that were planted using seeds had no significant reduction in diversity compared to donor and no apparent selection for specific genotypes. My findings suggest that the large success of the seagrass restoration in the Virginia coastal bays may be attributed partially to a greater level of ecosystem resilience often associated with high levels of genetic diversity. Based on these findings, to maintain genetic diversity, seeds should be used in lieu of adult plants for restoration when seeds are available and harvesting is practical.

In Chapter 3, I explore the mechanisms that result in the high rates of genetic diversity in eelgrass meadows found in Chesapeake Bay. I found that over 3 gradients from stress to disturbance (a spatial gradient incorporating depths where light was limiting within a single meadow, a temporal gradient where entire meadows experience die-back due to warm temperatures, and a regional gradient where plants were stressed by temperature), genetic diversity increased with disturbance, in contrast with the theory that disturbance results in genetic bottlenecks. It has been proposed in terrestrial systems that when clonal plants also reproduce sexually at high rates, disturbance opens space and diminishes competition between seedlings and clones, allowing sexual reproduction to dominate and increase diversity. My results suggest that a similar mechanism may be responsible for the patterns seen in the seagrass systems, and follow up research on the mechanism is warranted.

In Chapter 4, I used metapopulation models to demonstrate that natural recruitment into the Virginia coastal bays originated from meadows to the north, likely as the result of rafting reproductive shoots. Because several natural meadows differ slightly in genetic makeup, I suggest that recruitment came from a variety of sources and may be relatively rare, explaining why the natural recovery process is slow. Additionally, these

natural events result in a smaller seed addition than the restoration in the coastal bays, and thus the natural patches have lower genetic diversity than the plots that resulted from restoration. A first-order calculation suggested that the achieved by active restoration in only 10 years would take 157–185 years to achieve by means of natural recruitment (which has been occurring since seagrasses disappeared in 1933).

In Chapter 5, I evaluated the success of the restoration in terms of the economic value of selective ecosystem services provided. Considering only the ecosystem service of nutrient cycling, I conservatively estimated that the nutrient cycling provided by these restored seagrass meadows is currently valued at over $16 million USD yr^{-1}, over 8 times the amount invested in the restoration over the last decade. Natural recruitment from outside the region could have eventually restored this region, however, restoration accelerated the recovery. The increased time of seagrass coverage is worth nearly $400 million USD in increased nutrient cycling. While these are first-order estimates and therefore contain significant uncertainty, they demonstrate that ecosystem restoration can be a sound economic investment.

In Chapter 6, I explored the idea that incorporating genetic diversity into restoration would increase overall restoration success, measured by the provision of ecosystem services. Studies have shown that plots with higher genetic diversity are more dense and provide more ecosystem services (habitat and nutrient cycling) during and after large-scale disturbances, suggesting that restoration with a genetically diverse source may create meadows that are more resistant to disturbance (Hughes & Stachowicz 2004, Reusch et al. 2005). However, the application of these literature results to other systems is difficult because experimental diversities used in the studies were low (compared to

baseline diversity in Chesapeake Bay and the Virginia coastal bays), and because the levels of disturbance, after which results were seen, were extreme and potentially more severe than a typical restorated habitat would experience. I conducted an experiment that was realistic in the sense that I used seeds that were collected for large-scale restoration, a site that where large-scale restoration was occurring, and a technique of planting seeds which was being used for large-scale restoration of the region. Plots were planted over a depth gradient from shallow warm water, where plants were temperature stressed, to moderate water depth, where plants were relatively unstressed, to deeper water where plants eventually died presumably from chronic light stress. At each of these depths, plots with a higher genetic diversity, measured by allelic richness, were more dense and provided more ecosystem services (habitat, productivity, and nutrient retention). These results expand our understanding of the importance of genetic diversity and suggest that ecosystem restorations will significantly benefit from obtaining sources of restoration materials (transplants or seeds) with high genetic diversity and from restoration techniques that can maintain that genetic diversity.

Finally, in Chapter 7, I compared the importance of genetic diversity with other environmental factors in controlling seagrass density which is a proxy for the provision of ecosystem services (habitat, productivity, and nutrient retention). Within one basin, plant density was significantly correlated with water depth, genetic diversity, and sediment nitrogen. While water depth was the most important predictor, when only depths where seagrass meadows were stable (0.6–1.4 m depth mean sea level) were considered, genetic diversity was the best predictor of density. Further, in a regional survey using meadows that were nutrient stressed, meadows that were temperature

stressed, and meadows that were relatively unstressed, I showed that genetic diversity explained 50% of the variance in seagrass density, suggesting that the magnitude of this relationship is comparable to such important parameters as nutrient concentrations, light availability, and temperature variation. Therefore, monitoring, conservation, and restoration plans, which aim to maximize seagrass density and the provision of ecosystem services, should include genetic diversity as well as more traditional environmental parameters.