

Genetic diversity and gene flow in *Zostera marina* populations surrounding Long Island, New York, USA: No evidence of inbreeding, genetic degradation or population isolation

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ABSTRACT

Since the 1930s, eelgrass around Long Island, New York, USA, has experienced significant ecological and anthropogenic disturbances reducing areal coverage of the species. Patchiness, low density or isolation of these remaining populations increase susceptibility of this aquatic angiosperm to extinction. The loss of genetic diversity and patch connectivity, may contribute to lower fitness of eelgrass thus affecting recovery potential. Previous studies of eelgrass populations around Long Island report genetically isolated populations with low diversity. In contrast, this study found neither the evidence of inbreeding nor indications of genetic degradation for the same populations. Measures of genetic diversity such as average alleles ($A = 7.59$) and fixation index ($F = 0.02$) suggest no significant impediments to genetic connectivity among populations sampled. Gene flow ($N_m = 4.58$) and bottleneck analyses suggest the major disturbances of the past have not strongly affected population structure in the Long Island system. These findings have significant implications for both management and restoration. Locally, eelgrass populations in Long Island waters are unlikely to decline through genetic erosion or inbreeding processes alone. Plants from within these populations possess adequate genetic diversity to undertake restoration activities. On a larger geographic scale, the ability of these plants to maintain such high levels of genetic diversity and connectivity despite the significant areal losses historically provides optimism for the recovery potential of this species despite recent global losses.

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1. Introduction

Zostera marina L. (eelgrass) is an ecologically and economically important marine angiosperm. The current species range spans the northern hemisphere (Green and Short, 2003) across large physiochemical gradients. The species is important because it controls primary production, oxygenation, sediment stabilization and habitat complexity in shallow coastal ecosystems (Moore and Short, 2006). Over the last century, there have been global losses of seagrass coverage (Waycott et al., 2009), most without indications of recovery (Rasmussen, 1977; Dennison et al., 1987; Rozsa, 1994). In addition, there are increasing concerns that the rates of loss are accelerating (Orth et al., 2006; Waycott et al., 2009). Recognizing

the importance of *Z. marina* in coastal ecosystems, understanding the mechanisms of areal loss and developing potential strategies to mitigate these changes, are of management priority.

Eelgrass (*Z. marina*) populations in the coastal waters of Long Island, New York, USA have experienced a series of ecological disturbances during the past century. During the 1930s, there were catastrophic *Z. marina* population losses due to a wasting disease that decimated eelgrass meadows on both sides of the Atlantic. It is estimated that 80% of the eelgrass coverage was lost along the east coast of North America (Cottam and Munro, 1954). In 1985, Long Island's eelgrass meadows were further impacted by the first recorded bloom of a 'brown tide' forming pelagophyte, *Aureococcus anophagefferens* (Gobler and Sunda, 2012). The annual reoccurrence of these blooms during the subsequent three decades has substantially altered the abundance and distribution of eelgrass on Long Island through presumed light stress brought on by rampant water column production. During this time, these blooms further reduced

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areal coverage of eelgrass meadows by 40% within the Great South Bay and Peconic Estuary (Casper et al., 1987; Dennison et al., 1987). Collectively, these significant losses in *Z. marina* coverage may have severely impacted the capacity of remaining populations to maintain genetic diversity (Frankel and Soulé, 1981; Lande, 1988). Loss of genetic diversity characteristically leads to inbreeding which is particularly problematic through the expression of deleterious alleles that reduce the fitness and the adaptive capacity of the plant. Additionally, low hydrologic connectivity between populations (Wilson et al., 1991) may reduce the dispersal potential, gene flow and long-term recovery in subpopulations of *Z. marina* (Kendrick et al., 2012).

High resolution, co-dominant genetic markers are now widely used tools for determining population genetic structure (Bachmann, 1994), and have been successfully applied to the study of *Z. marina* (Bachmann, 1994; Reusch, 2001). The investigation of eelgrass genetic diversity has been made possible through the availability of numerous microsatellite markers (Reusch et al., 1999). Previous genetic analysis has shown that eelgrass populations across broad geographic ranges have maintained moderate to high levels of genetic diversity even within populations that have endured large scale losses (Ferber et al., 2008; Ort et al., 2012; Wyllie-Echeverria et al., 2010). Across broad geographic scales a diversity of population genetic structure exists, including rare cases of highly clonal populations (Reusch et al., 1999; Olsen et al., 2004).

Given the ecological importance of eelgrass in New York, management agencies have made repeated efforts to restore their populations (South Shore Estuary Reserve, Peconic Estuary Program; New York State Seagrass Taskforce 2009). Unfortunately, the majority of these restoration efforts have been unsuccessful. Choice of restoration strategies, in particular the use of transplanted fragments over broadcast seeds and the selection of donor populations will have a significant impact on total effective population size and genetic diversity in the coming years (Williams and Orth, 1998; Williams, 2001; Reynolds et al., 2012). Long-term success of such efforts will be measured by the ability of restored populations to be self-sustaining and to increase the connectivity of presently fragmented subpopulations (Reynolds et al., 2012). Low genetic diversity, however, will jeopardize both population maintenance and recovery through the deleterious effects of inbreeding. Therefore, it is critical to quantify the current level of genetic diversity and connectivity between populations within this system prior to effective restoration.

Historical eelgrass losses in Long Island waters (Rasmussen, 1977; Casper et al., 1987; Dennison et al., 1987), limited geographic connectivity (Wilson et al., 1991) and Campanella et al. (2010a,b) suggest that eelgrass populations around Long Island may be highly clonal, existing as spatially segregated populations. They suggested that evidence pointed to historical bottlenecks for eelgrass populations in Long Island. This would imply that significant genetic erosion has occurred, making the populations quite vulnerable to ongoing or future disturbances exhibiting low population resilience. Because of the potential vulnerability to genetic degradation, knowledge of population structure is critical to the development of management strategies to protect, maintain, and restore eelgrass populations in this region. The purpose of this study was to evaluate current genetic diversity of eelgrass within Long Island waters.

2. Materials and methods

Two different sampling designs were employed to collect *Z. marina* ramets from meadows throughout Long Island (Fig. 1). The first was a stratified random sampling method (hexagon tessellation) designed to locate and sample 188 sites throughout the Fire

Island National Seashore in Great South Bay. In addition, nineteen other meadows located in Peconic Bay and Long Island Sound were sampled in a similar way. A second methodological design was used in Shinnecock Bay to collect 31 samples from a rectangular spatial array (3 transects) where sequential samples were collected at 5 m intervals. In total, this study sampled genetic material from 293 eelgrass ramets within the South Shore Estuaries, Peconic Estuary and NY waters of Long Island Sound.

Genetic samples were acquired by collecting whole plants and storing them at -20°C in plastic, gas impermeable bags. Upon returning to the lab, the two youngest leaves were removed, cleaned of epiphytes, dried with paper towels, and placed in labeled 40 ml plastic scintillation vials filled with silica bead desiccant. DNA was extracted from these dry tissue samples using manual grinding in a mortar and pestle and a modified Qiagen DNeasy plant mini kit protocol (Qiagen Pty. Ltd., Valencia, CA). Aliquots of extracted DNA were used in two separate multiplex PCR amplifications on an MJ ResearchTM PTC-200 thermocycler using *Z. marina* specific, 5'-fluorochrome (standard labels Hex, Tet, Fam) labeled polymorphic microsatellite markers GA2, CT3, CT35, CT17H, GA6, GA3, CT19, and CT20 (Reusch, 2001). Amplification of the microsatellites was accomplished through 20 μL polymerase chain reactions (PCR) containing approximately 30 ng of template DNA, 0.5 U of Bioline Immolase DNA Taq (Bioline Pty. Ltd., CA), 1.5 μL 10x Bioline Immo buffer (160 mM $(\text{NH}_4)_2\text{SO}_4$, 670 mM Tris-HCl pH 8.3, 0.1% Tween-20), 2.5 mM MgCl_2 , 0.133 mM each dNTP, BSA at a concentration of 0.1 $\mu\text{g}/\mu\text{L}$, and 0.33 mM fluorescently labeled forward (FAM, HEX, or TET) and reverse microsatellite primers. Thermal cycling protocols consisted of a 7 min 94°C denaturing step followed by 30 cycles of at annealing temperature of 50°C . For all cycles denaturing steps were conducted at 94°C and extension temperature was 72°C . PCR products were analyzed using a DNA sequencer MEgaBACETM 1000 (GE Healthcare). The software MEgaBACE Genetic Profiler 2.2 (GE Healthcare) was used to assign alleles for each sample at each locus relative to an internal size standard (MEgaBACETM ET400-R size standard, GE Healthcare, Buckinghamshire, UK).

Estimates of genetic diversity and population genetic structure were conducted using a suite of genetic analysis software packages. Standard tests for expected versus observed heterozygosity (Hardy Weinberg Equilibrium), allelic diversity, population differentiation (F -statistics), gene flow (F_{ST}) and isolation by distance were analyzed using GenAlEx 6.41 (Peakall and Smouse, 2006). Estimates of gene flow based on private alleles were calculated in Genepop 4.0.10 (Raymond and Rousset, 1995; Rousset, 2008) and implemented via Genepop on the web (<http://genepop.curtin.edu.au/index.html>). Clonal identity and the spatial distribution of clones were identified using Geneclone 2.0 (Arnaud-Haond and Belkhir, 2007). Measures of population differentiation as hierarchical f -statistics (Weir and Cockerham, 1984) were assessed using Genodive 2.0b22 (Meirmans and van Tienderen, 2004) using 9999 jackknife replicates across loci to generate standard errors for pairwise population comparisons. Fstat 2.9.3 (Goudet, 1995) was used to calculate allelic richness (A_r) using rarefaction to equivalent population size ($N = 20$; the smallest population size from LIS). The fixation index (F_{ST}) ranges from 0 to 1 where 0 indicates no genetic differentiation among groups (i.e. the groups are acting as a single genetically connected population) and 1 indicating fixed differences between populations.

Small spatial scale genetic structure within and among populations was evaluated using spatial autocorrelation across various equidistant size classes (GenAlEx 6.41; Peakall and Smouse, 2006; Geneclone 2.0; Arnaud-Haond and Belkhir, 2007). In GenAlEx each spatial autocorrelation run consisted of 9999 permutations and 9999 bootstraps. In Geneclone 2.0 each population was run independently using 100 distance classes, genets with resampling,

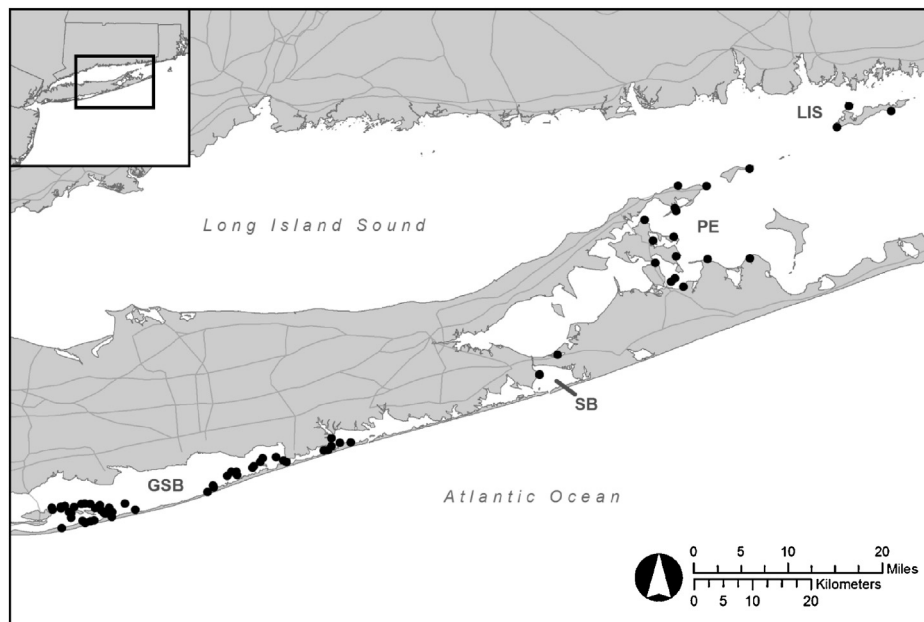


Fig. 1. Location of *Zostera marina* samples taken for genetic analysis in the Long Island region, USA.

and 10,000 bootstrap replicates. Finally, the program BOTTLENECK (Cornuet and Luikart, 1996) was used to test each sampling area for the potential presence of a severe bottleneck. BOTTLENECK was run using the two phase model of mutation with 10,000 replications to determine the significance of any heterozygote excess or deficiency relative to allelic diversity at all loci and sampled locations.

3. Results

Eelgrass population genetics were assessed across three major estuaries of Long Island: Great South Bay (GSB), Shinnecock Bay (SB), Peconic Bay (PB) and Long Island Sound (LIS) (Fig. 1). Some loci departed from Hardy Weinberg Equilibrium expectations in particular populations, however no locus deviated in all populations (Table 1). No evidence of null alleles was detected. Estimates of genetic diversity indicated that the surveyed eelgrass meadows were genetically diverse. One simple indicator of this diversity was the high number of unique genotypes. Among the 293 samples analyzed, nearly all ($n=278$) were genetically unique individuals demonstrating that clonal diversity was high ($[(\text{Genotypes} - 1)/(N - 1)] = 0.96$) ranging between 0.89 and 0.98 within the four sample regions. The average number of alleles ($A = 7.59$) and average heterozygosity ($H_o = 0.56$) did not statically depart from expected values, and there was no indication of inbreeding among the sampled populations ($F = 0.02$).

Most of the 293 samples we screened were found to be unique genotypes, however, all populations had at least one clonal pair of ramets. The clones detected were collected 1–3 m apart. The largest clone detected by this study was located in Shinnecock Bay

where we detected clonal ramets 8 m apart. These results suggest that clonality did not dominate the population structure at the meadow scale. It is likely that clonality may be prevalent within these populations at smaller spatial scales (i.e. <3 m) but an alternative sampling design would be required to detect this.

Variability in allelic diversity was detected across the eight loci (Table 2). The locus with the minimum number of alleles was CT19 ($N_a = 2.75$), while CT17H had the highest ($N_a = 15.75$). GA3 revealed a moderate number of alleles ($N_a = 5.0$), yet calculations revealed a very low number of effective alleles ($N_e = 1.51$). In addition, all loci were polymorphic in all populations and allelic richness was highest for locus CT35 ($A_r = 15.0$) and lowest for GA3 ($A_r = 2.7$). Average allelic richness across loci was moderate ($A_r = 5.8$).

Gene flow between populations is typically measured as the migration rates, N_m , which acts as a measure of the movement of alleles (through either sexual or asexual reproduction) from one population to another. This represents the number of migrants per generation. Across the eight loci, a moderate level of gene flow was detected (Table 3; $N_m = 4.58$, F_{st} method; $N_m = 17.7$, rare allele method). In addition to gene flow between populations, genetic segregation between potentially genetically distinct populations was determined. The average fixation index revealed low population differentiation ($F_{st} = 0.06 \pm 0.02$) among estuaries. There was no evidence of inbreeding for any of the populations sampled ($F_{is} = 0.012 \pm 0.021$). Pairwise F_{st} comparisons identified Shinnecock Bay (SB) and the Fishers Island area (LI) as having the greatest genetic differentiation (Table 3).

To overcome the limiting effect of small sample sizes, an individual genotype comparison approach was adopted to analyze

Table 1

Genetic diversity (mean $\pm$ s.e.) in *Zostera marina* populations from four Long Island estuaries. N , number of samples; G , number of multilocus genotypes (calculated in Genclone 2.0); R , clonal diversity calculated as $[(G - 1)/(N - 1)]$; N_a , number of different alleles; N_e , effective number of alleles; H_o , observed heterozygosity; H_e , expected heterozygosity; H_{eun} , unbiased expected heterozygosity; F , Wrights fixation index (calculated in GenAlix v. 6.41).

Population	N	G	R	N_a	N_e	H_o	H_e	H_{eun}	F
GSB	188	180	0.95	11.13 (1.66)	3.67 (1.34)	0.59 (0.07)	0.58 (0.07)	0.59 (0.59)	−0.02 (0.03)
SB	31	30	0.97	6.63 (1.52)	3.35 (0.97)	0.52 (0.11)	0.58 (0.07)	0.59 (0.07)	0.15 (0.12)
PB	54	53	0.98	8.13 (1.52)	3.65 (1.04)	0.61 (0.06)	0.63 (0.06)	0.64 (0.64)	0.03 (0.04)
LI	20	18	0.89	4.50 (1.21)	2.72 (0.78)	0.52 (0.09)	0.49 (0.09)	0.50 (0.50)	−0.06 (0.04)
All groups	73.25 (12.1)	281	0.96	7.59 (0.83)	3.35 (0.50)	0.56 (.04)	0.57 (0.04)	0.58 (0.04)	0.03 (0.04)

Table 2
Summary estimates of genetic diversity for each of eight microsatellite loci across all four Long Island Sound populations of *Zostera marina* samples. Average population sample size for this data set $N = 73.25 \pm 38.90$ (s.e.). N_a , number of different alleles; N_e , effective number of alleles; A_r , allelic richness (rarefaction to equivalent population size of $N = 20$); H_o , observed heterozygosity; H_e , expected heterozygosity; H_{eun} , unbiased expected heterozygosity; F , Wrights fixation index (calculated in GenAlEx v. 6.41).

	GA2	CT3	CT35	CT17H	GA6	GA3	CT19	CT20
N_a	7.25 (1.65)	7.75 (0.48)	10.50 (2.50)	15.75 (1.65)	5.75 (1.49)	5.0 (1.58)	2.75 (0.75)	6.0 (1.58)
N_e	3.01 (0.40)	2.34 (0.30)	3.23 (0.39)	10.29 (1.02)	2.77 (0.47)	1.51 (0.13)	1.67 (0.20)	1.97 (0.19)
A_r	6.1	6.0	9.8	15.0	5.7	4.3	2.7	5.3
H_o	0.65 (0.06)	0.61 (0.07)	0.66 (0.03)	0.90 (0.01)	0.59 (0.08)	0.23 (0.07)	0.38 (0.10)	0.45 (0.08)
H_e	0.65 (0.04)	0.55 (0.05)	0.68 (0.04)	0.90 (0.01)	0.60 (0.08)	0.32 (0.06)	0.37 (0.08)	0.48 (0.05)
H_{eun}	0.66 (0.04)	0.57 (0.05)	0.69 (0.04)	0.91 (0.01)	0.61 (0.07)	0.33 (0.06)	0.37 (0.08)	0.48 (0.05)
F	0.06 (0.04)	−0.10 (0.03)	0.02 (0.05)	−0.03 (0.02)	0.02 (0.05)	0.22 (0.24)	−0.04 (0.08)	0.08 (0.11)

Table 3
Pairwise F_{st} values (lower diagonal) and migration rates (N_m , rare allele method) (upper diagonal) among *Zostera marina* populations sampled from the Long Island Sound region (USA), all population pairwise F_{st} comparisons were significant ($p = 0.000$, Genodive 2.0b22, Meirmans and van Tienderen, 2004).

Population	GSB	SB	PEC	LI
GSB	–	4.3	5.04	1.64
SB	0.023	–	2.37	0.93
PEC	0.039	0.06	–	1.29
LI	0.137	0.197	0.084	–

pairwise F_{st} values. This was implemented in three ways, first through population assignment (Fig. 2). Population assignment for all four sampling regions revealed very little genetic distinctiveness. Only individuals from LIS displayed any genetic discreteness (Fig. 2). Pairwise population assignment of GSB and SB are clustered together and dispersed across the log likelihood axes indicating no genetic separation (Fig. 2A). In contrast, LIS and SB displayed a clearly divergent clustering pattern of population assignment (Fig. 2B). Principal coordinates analysis corroborated the population differentiation of LIS from the other estuaries (Fig. 3). This analysis also demonstrates the lack of differentiation among the other three Long Island estuaries. Bayesian modeling was used to explore the genetic differentiation of LIS from other populations using an unbiased approach by determining the proportional assignment of each individual to one of four genetic populations. Except for the LIS samples, the genetic model generated by STRUCTURE (Pritchard et al., 2000) had difficulty probabilistically

assigning individual samples to clearly defined genetic populations when treated independent of geography (Fig. 4). This demonstrates that three estuaries (GSB, SB, PB) appear as composites which should be interpreted as undifferentiated populations. The lack of population differentiation suggests that these three estuaries have a high degree of connectivity. However, the LIS population may be distinct.

No significant spatial autocorrelation were detected in any analysis, indicating that neighborhood sizes were unable to be detected using the sampling design adopted in this study. A more structured, nested sampling design would be necessary to determine spatial autocorrelation in these populations. Further, there was no effect of a genetic bottleneck detected.

4. Discussion

Z. marina is critically important to the ecosystem functioning of Long Island estuaries, yet little is known regarding its genetic population structure. Prior studies utilizing a limited data set suggested that eelgrass populations in Peconic Bay displayed signs of inbreeding due to isolation (Campanella et al., 2010a,b). If widely observed across the Long Island region, this would imply a high level of genetic erosion. The capacity of eelgrass in this region to recover and persist might be significantly impeded. Given the efforts to restore eelgrass populations along the whole east coast, and Long Island in particular, this type of genetic analysis is critical to the development of effective restoration strategies. The objective of this study was to answer three specific questions.

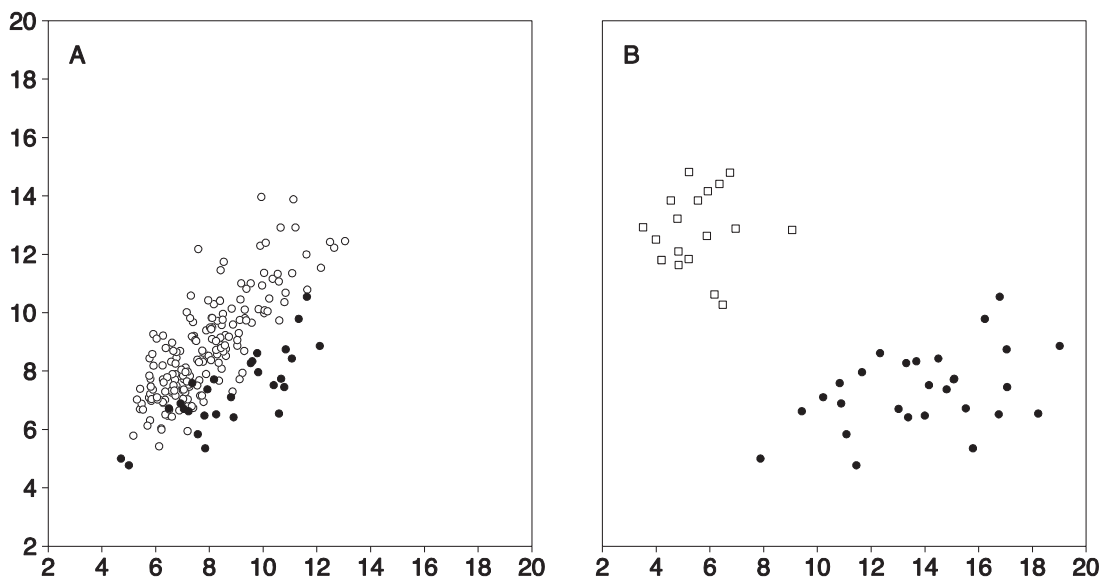


Fig. 2. Log likelihood values for pairwise population assignment of individual *Zostera marina* samples calculated using Nei's genetic distance. (A) Pairwise population assignment of individuals from Great South Bay (GSB) and Shinnecock Bay (SB). (B) Pairwise population assignment of individuals from Shinnecock Bay (SB) and Long Island Sound (LI).

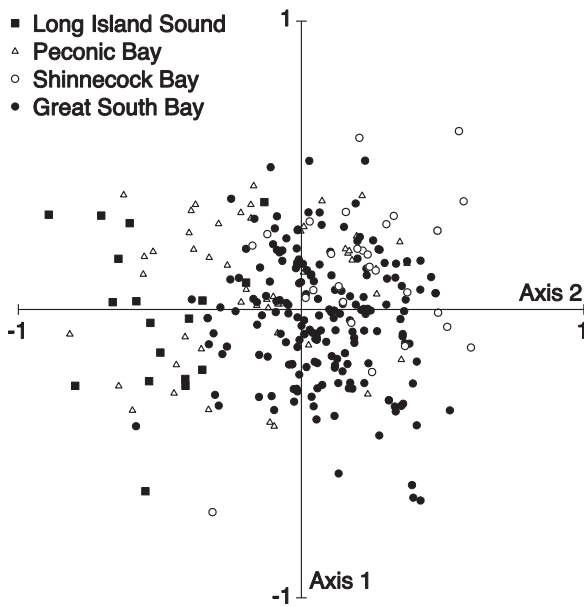


Fig. 3. Principal Coordinates Analysis of genetic distance among individual samples of *Zostera marina* collected from four estuaries in the Long Island Sound region, USA.

4.1. Is there evidence for high levels of clonality in the four target regions of Long Island?

In species that have a capacity for both sexual and asexual reproduction, genetic diversity is dependent primarily on genotypic and allelic diversity (Frankham et al., 2002). The high percentage of unique genotypes provides evidence that genotypic diversity is high and thus clonality does not dominate population structure in these regions. Average allelic richness was equivalent to the higher values measured for this species (Olsen et al., 2004). Clones were detected, but these were nearly all located within 3 m of each other. This does not preclude the possibility that large clones exist in this population at a range of spatial scales but they were undetected because of the large numbers of genetic individuals (genets) present. This might be expected in populations where disturbance occurs at a frequency that enables recruitment to occur.

4.2. Is there evidence of inbreeding in the Long Island *Z. marina* populations?

The observed allelic richness, diversity and heterozygosity indicate moderate to high genetic diversity and supports the existence of significant sexual reproduction contributing to the existing population structure. There was no evidence of significant inbreeding in any of the estuaries sampled. The two different measures of inbreeding estimated, Wrights fixation index (F , in Tables 1 and 2) and F_{IS} , approached zero. These results are by no means unprecedented (Procaccini et al., 2007; Ferber et al., 2008), but they contradicted the only other genetic information for eelgrass in Long Island (Campanella et al., 2010a). Despite the historical pressure of disturbance and anthropogenic stress in this region, the populations are maintaining levels of genetic diversity and outbreeding

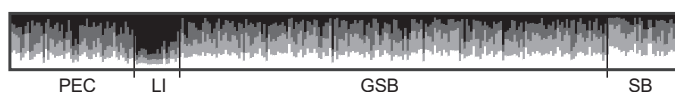


Fig. 4. Model of individual sample assignment to genetic clusters independent of population origin using Bayesian modeling of four genetic clusters implemented in STRUCTURE. Samples from Long Island Sound (LI) are the only samples where STRUCTURE model was able to determine population structure.

that prevent the accumulation of inbreeding. The level of genetic diversity and clonality observed are similar to the population structure of many eelgrass populations reported in Europe (Olsen et al., 2004) and North America (Reynolds et al., 2012, 2013).

Campanella et al. (2010a,b) concluded from a limited sample size within Peconic Bay that Long Island eelgrass populations suffered from low diversity and inbreeding depression as a result of a recent bottleneck. This study, however, found no evidence of a recent severe genetic bottleneck at any of the sampled regions. Even smaller populations (LIS, PB and SB) did not exhibit significant divergence from expected heterozygosity relative to allelic diversity. This is the opposite of what would be expected following a severe bottleneck. The *Z. marina* populations did not have significant deficiencies in heterozygotes. Campanella et al. (2010b) reported average heterozygosity as low as 0.30 from their sample set ($n=20-30$ ramets) and suggested that these deficiencies indicate genetic isolation and inbreeding. In contrast, the sampling effort in this study ($n=293$ ramets) showed no heterozygote deficiencies. Therefore, these Long Island populations are not moving toward fixation.

4.3. What is the scale of genetic connectivity among the major estuaries of Long Island?

Genetic studies of *Z. marina* conducted on estuaries with similar spatial scales (Muniz-Salazar et al., 2006) across similar geographic configurations which found low genetic diversity might lead to the expectation of highly structured subpopulations of *Z. marina* in New York estuaries. However, genetic analysis found strong levels of gene flow among samples areas collected across these aquatic systems. While all of the geographic areas examined exhibited moderate genetic diversity, this diversity was divided proportionately across sampled individuals regardless of their geographic area, suggesting strong connectivity. Using the standard metric for gene flow, N_m , the number of migrants per generation was estimated. When N_m calculations produce values greater than 1, it is accepted that gene flow is occurring between populations or sub-populations at sufficient levels to prevent genetic drift. When analyzed collectively, or restricted to pairwise comparisons, evidence of strong gene flow across all sampled populations was found ($N_m = 17.7$; rare allele method across all samples). Pairwise comparisons between individual regions showed evidence of moderate gene flow among many population pairs $N_m > 2-5$. The notable exception was the population in Long Island Sound. In one case, calculation produced an N_m value below 1 (LIS-SB; $N_m = 0.93$), and pairwise comparison of LIS to the other two regions (SB and PB) is consistently low. However, these values may be biased by the sample size. On the other hand, the genetic isolation may be real and the distance and currents that separate the other Long Island estuaries from Long Island Sound may be acting as a barrier to gene flow. The between-estuary comparison revealed that there was extensive genetic overlap between GSB, SB and PB. Again, this is not unprecedented (Ferber et al., 2008) given the level of sexual reproduction in the area and the reproductive mechanics and seed transport of *Z. marina* (Kendrick et al., 2012).

When gene flow between Long Island's estuaries (GSB, PB, SB) was considered, there was evidence that these eelgrass meadows exist as a series of connected sub-populations. The limited connectivity between these sites and those sampled in Long Island Sound suggest evidence of population genetic differentiation. Population assignment and Bayesian modeling of genetic groups supports this. The Bayesian approach utilized by STRUCTURE (Pritchard et al., 2000) could not successfully assign individuals from GSB, PB and SB into genetically distinct populations, yet did successfully assign LIS to a separate group. This suggests that the LIS populations have low genetic connectivity with the other three estuaries.

Evidence of high genetic diversity in the Long Island estuaries sampled reflects the ability of long distance seed dispersal for *Z. marina* (Erftemeijer et al., 2008; Harwell and Orth, 2002). Erftemeijer et al. (2008) conducted model simulations predicting that detached reproductive shoots with viable seeds could be transported long distances via surface currents and wind-driven advective transport. This mechanism of seed transport was identified as a means of overcoming genetic bottlenecks. Similarly, Harwell and Orth (2002) demonstrated that despite high genetic subdivision among populations in Chesapeake Bay, long distance seed transport allowed for significant gene flow for subpopulations of eelgrass on an ecological time scale. There are significant implications of the results this study to both the management and restoration of eelgrass populations in Long Island waters. First, due to high levels of genetic diversity observed these populations are unlikely to decline through genetic erosion or inbreeding. Second, locally acquired material possesses adequate genetic diversity to undertake restoration activities.

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