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Zostera Marina Population Genetics in Barnegat Bay, New Jersey, and Implications for Grass Bed Restoration

James Campanella
Montclair State University, campanellj@mail.montclair.edu

Paul Bologna
Montclair State University, bolognap@montclair.edu

Stephanie M. Smith
Montclair State University

Eric B. Rosenzweig
Montclair State University

John V. Smalley
Bergen Community College

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ORIGINAL ARTICLE

Zostera marina population genetics in Barnegat Bay, New Jersey, and implications for grass bed restoration

James Joseph Campanella · Paul A. X. Bologna · Stephanie M. Smith · Eric B. Rosenzweig · John V. Smalley

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Abstract Within Barnegat Bay, New Jersey, *Zostera marina* populations have declined by 62% over the last 20 years, and restoration efforts have met with mixed success. We have completed a microsatellite-based genetic investigation of eight populations of *Z. marina* within Barnegat Bay to determine whether the genetic stock origins of the plants used in management projects may affect restoration success. Additionally, we assessed the genetic diversity of *Z. marina* in Barnegat Bay to better understand its population structure. Clonal diversity ranged from 0.70 to 0.95 for the populations studied. Individually, Barnegat Bay populations are not genetically diverse, and there is also little divergence among populations. The Atlantic populations had mean Hobs values (0.20–0.34) that were far lower than the Hexp values (0.69–0.83). Also, the F_{IS} values in all of the eastern populations indicate a surfeit of homozygotes over heterozygotes, suggesting a low degree of outcrossing in the Barnegat Bay populations. Six of the ten populations studied (Ham Island, Manahawkin Bay, Shelter Island, Marsh Elder, Harvey Cedar Sedge, and Long Island) show evidence of historical bottlenecks. Mean estimated F_{ST} values would suggest that most alleles are undergoing moderate genetic differentiation, with values that range from 0.06 to 0.13. Oyster Creek and Sedge Island demonstrate the largest estimated effective population sizes and may be the most appropriate populations for use in future eelgrass restoration projects.

Keywords Eelgrass ecology · Genetic diversity · Historical bottlenecks · Microsatellites · Restoration ecology · *Zostera marina*

J. J. Campanella (✉) · P. A. X. Bologna · S. M. Smith · E. B. Rosenzweig
Department of Biology and Molecular Biology, Montclair State University, 1 Normal Avenue, Montclair, NJ 07043, USA
e-mail: james.campanella@montclair.edu

J. V. Smalley
Department of Science and Technology, Bergen Community College, Paramus, NJ 07652, USA

Introduction

Both terrestrial and aquatic ecosystems are prone to attack and loss from environmental disturbances; severe weather, limited resources, and human activities have decimated ecosystems the world over and disturbed their balances (Emlen et al. 1948; Ploetz et al. 1988; Braeutigam and Elmqvist 1990; Pace et al. 1999; Jackson et al. 2001; Orth et al. 2006). The intent of restoration ecology is to return ecological balance to disturbed systems by re-introducing local species that may have been lost over time. The process of restoration is dependent on replacing lost species with genetically diverse populations of the same species (McKay et al. 2005; Falk et al. 2006). Genetic diversity is an important factor in restoration for a number of reasons. First, genetic diversity helps organisms to cope with environmental variability. Without this diversity, the restored organism may fall prey to a single environmental change that could destroy the entire population. Enough diversity must be present for the population to have a "genetic buffer" from which it may draw as needed to survive. For example, diverse genotypes are needed to support disease resistance in flowering plants (Schoen and Brown 1993). Second, increased diversity reduces the chances of inbreeding among close relatives. Reproduction in small or isolated populations often leads to diminished heterozygosity and inbreeding depression, which results in

reduced overall survival. Third, genetic diversity is the basis for physical phenotypic diversity in form and function. Genes regulate body size, behavioral traits, reproductive characteristics, physiological systems, colonizing capacity, dispersal ability, tolerance to environmental extremes, disease resistance, and other traits. In short, genetic variation is an essential force that molds the ecology of living organisms (Falk et al. 2006).

Seagrass communities are globally common in coastal tropical and temperate regions as well as portions of the Subarctic. *Zostera marina*, in particular, is one of the most widely distributed seagrass species in the world. It serves as an essential fish habitat and provides stability to coastal systems by reducing water velocity, increasing wave attenuation, and stabilizing sediments (Fonseca and Fisher 1986; Almasi et al. 1987). Seagrasses are sensitive indicators of long-term water quality and can be used as a barometer of coastal ecosystem health (Dennison et al. 1993). Changes in the vitality and distribution of these vascular plants generally signal a decline in water quality. Seagrass decline has become a common occurrence in many shallow temperate and tropical regions of the world (Short et al. 1988; Walker and McComb 1992; Short and Burdick 1996; Valiela et al. 2000; Hauxwell et al. 2003; Orth et al. 2006). For *Z. marina*, the wasting disease epidemic of the 1930s caused massive worldwide declines in spatial coverage (den Hartog 1987). As such, the genetic diversity of this plant may have undergone significant declines and populations that have recovered may have had limited regenerative genetic stock with which to repopulate coastal regions. In some cases, localized losses of *Z. marina* can occur on the scale of months (Bologna et al. 2001), with the subsequent recovery taking several years (Bologna et al. 2007). Due to periodic die-offs from natural and anthropogenically induced disturbances, the population structure of these plants may undergo significant founder effects and genetic bottlenecks during recovery.

Zostera marina is a vital member of coastal ecosystems, but major *Z. marina* populations along the western Atlantic coast of the United States have suffered significant declines (Orth and Moore 1983; Short and Burdick 1996; Lathrop et al. 2001; Hauxwell et al. 2003; Keser et al. 2003). The complete destruction of these populations would have detrimental ecological impacts. Restoration efforts for *Z. marina* have been quite successful (Fonseca et al. 1998), but within New Jersey efforts have had varying levels of success (Reid et al. 1993; Bologna and Sinnema 2005, 2006). Bologna and Sinnema (2005) were successful at establishing live transplants at thirteen sites in Barnegat Bay, New Jersey, with varying degrees of planting unit survival (6–100% survival), while five sites showed no success at all. Mitigating factors in the relative successes of sites included ice scour, storm activity, and brown-tide blooms. Additionally, their limited genetic stock incorporated only four donor populations in the bay, and most sites received transplants from only one or two donor populations. However, this restoration work was completed prior to any genetic stock analysis of *Z. marina* in New Jersey.

It has been proposed in other restoration efforts that these differences in survival rates may be linked to the genetics and stock origin of the plants. Williams and Orth (1998) presented evidence that transplants retain donor stock genetic identity, which may have limited the success of prior efforts through reduced genetic diversity. Williams (2001) demonstrated that elevated genetic diversity increased transplant survival, while Hughes and Stachowicz (2004) showed that genetic diversity can contribute to the resistance of *Z. marina* populations to physical disturbance. Also, Reusch et al. (2005) demonstrated greater regrowth of experimental plots with greater genotypic diversity after extreme summer temperature spikes. One potential way to maximize genetic diversity in restoration would be to collect individuals from as many populations as possible to maximize heterozygosity and allele frequency. This is most easily accomplished by mixing seeds collected from numerous locations. As such, the primary research objective for this project was to assess the population genetic structure of *Z. marina* in New Jersey and to determine how genetic diversity and effective population size may impact future restoration activities. Additionally, we wanted to identify potential populations that maintain higher genetic diversity for prospective restoration projects in Barnegat Bay.

Materials and methods

Plant collection

Individual *Zostera marina* plants were collected at eight sites within Barnegat Bay, New Jersey (Fig. 1). These eight sites included Oyster Creek (39°50′N 74°08′W), Barnegat Bay Inlet (39°46′N 74°08′W), Harvey Cedar Sedge (39°42′N 74°09′W), Manahawkin Bay (39°38′N 74°12′W), Ham Island (39°36′N 74°13′W), Marsh Elder (39°35′N 74°14′W) Shelter Island (39°34′N 74°15′W), and Sedge Island (39°33′N 74°16′W). Sites were chosen based on their use as donor sites for previous restoration activities (Bologna and Sinnema 2005) or are part of long-term monitoring sites for eelgrass community structure (Bologna PAX, unpublished data). The only exception is Oyster Creek, which was located near the outfall of a nuclear power plant. *Zostera marina* samples were also collected from Peconic Bay, New York (40°56′N 72°26′W, Brad Peterson, SUNY, Southampton, New York), Chesapeake Bay, Virginia (37°13′N 76°23′W, Ken Moore, Virginia

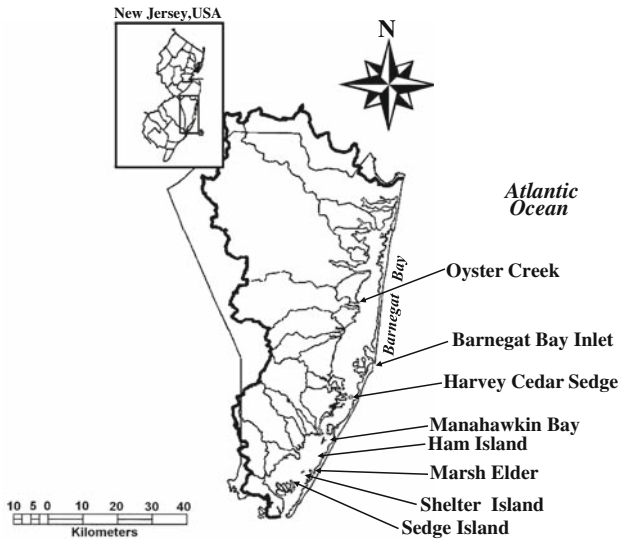


Fig. 1 The geographic collection sites for the populations studied in New Jersey

Institute of Marine Science, Virginia), and Egg Harbor, Alaska (59°38′N 151°32′W, Rick Foster, Kachemak Bay Research Reserve, Alaska). To ensure that we were not gathering clonal samples, plants were collected 5 m apart within expansive eelgrass beds. For all samples, sites were located and then plants were collected in an pattern radiating out from the boat, ensuring that collected individuals were separated by at least 5 m. Tissue samples were shipped or transported on ice to Montclair State University from all locations. Samples were then separated, numerically labeled, and stored at −80°C until DNA extraction.

DNA extraction and microsatellite amplification

Total DNA was extracted from 0.3 to 0.5 g of *Zostera marina* leaf tissue using the DNeasy DNA extraction kit according to the manufacturer's directions (Qiagen Corporation, Valencia, CA, USA). DNA was extracted from 20 individuals within each population. DNA concentration was determined by UV absorbance on a Nanodrop ND-1000 UV Spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA) and samples were stored at −80°C until polymerase chain reaction (PCR) amplification was performed.

The PCR was used to amplify seven microsatellite loci from the extracted *Z. marina* DNA. Primers for these seven amplified loci were developed by Reusch et al. (1999): ZosmarGA2 (AJ009900), ZosmarGA3 (AJ009901), ZosmarCT3 (AJ009898), ZosmarCT12 (AJ249303), ZosmarCT17 (AJ249307), ZosmarCT19 (AJ249304) and ZosmarCT20 (AJ249306). Primers were fluorescently labeled with either FAM or HEX dyes (Invitrogen Corp., Carlsbad, CA, USA).

Reactions were carried out using 10 ng DNA in RNase/DNase-free 0.2 μL tubes with 15–30 nmol labeled primers. Reaction mixtures were all kept at 4°C until 10 μL of Choice Taq Mastermix DNA Polymerase (Denville Scientific, Inc., Denville, NJ, USA) was added. Amplification was performed in a Mastercycler gradient thermocycler (Eppendorf, Inc., Hamburg, Germany). The PCR program employed consisted of a 1 min denaturing step at 95°C, followed by 30 cycles of the following durations and temperatures: 15 s at 95°C, 15 s at 55°C and 30 s at 72°C. Amplified PCR products were then stored at −20°C until later analysis.

Microsatellite allele size analysis

Allele sizes of microsatellite PCR products were determined using an ABI Prism 310 DNA Sequencer (Applied Biosystems Corp., Foster City, CA, USA). The PCR products were diluted 1:10 with sterile water. 0.5 μL of the diluted product was added to an aliquot of 30 μL of formamide and 0.5 μL of the molecular weight standard ROX 500 (Applied Biosystems Corp.). Samples were analyzed for allele sizes on the sequencer for 30 min using POP4 polymer (Applied Biosystems Corp.) and the D Filter setting. GeneMarker v1.51 software (SoftGenetics Corp., State College, PA, USA) was used to evaluate the microsatellite allele sizes from raw data and to score loci for homo/heterozygosity.

Statistical analysis of data

Clonal diversity (C) was determined using the method of Olsen et al. (2004) and was calculated by the number of genets detected divided by the number of ramets sampled, based on all seven loci, with the spatial scale between each ramet sampled being approximately 5 m. Redundant multilocus genotypes were removed from all further data analyses.

Microsat 2.0 (Minch E, 1995, Stanford University) was utilized to calculate allelic heterogeneity and to generate genetic distance matrices based on the allele size data. A modified delta mu distance (delta mu*) was calculated to compensate for small populations (Minch E, personal communication). PHYLIP's subroutines Neighbor and Consense (Felsenstein J, 1989, University of Washington) were employed to evaluate the Microsat genetic distance matrix and construct the neighbor-joining relationships between *Z. marina* populations using 1000 bootstrap iterations. These neighbor-joining data were employed with Treeview (Page RDM, 1996, University of Glasgow) to generate the final graphic trees. In addition, mean estimated gene flow values (N_m), as well as observed (Hobs) and expected (Hexp) heterozygosities were calculated with POPGENE32 under the codominant marker settings (Yeh

FC, 1997, University of Alberta). The F_{ST} values were calculated using Microsat. These were not pairwise F_{ST} values but were calculated at each allele in each population. Mean F_{ST} values were calculated by determining the average F_{ST} at all alleles in a population.

The relationship between genetic and geographic distance was analyzed utilizing the isolation by distance web service using the raw data setting for diploid genotypes with genetic distance F_{ST} (Jensen JL, 2005, Stanford University). The program BOTTLENECK was used to estimate the likelihood of population bottlenecks (Piry S, 1999, French Institut National de la Recherche Agronomique). In the bottleneck analysis, the two-phase mutation model (TPM) (DiRienzo et al. 1994) was employed, as opposed to the stepwise mutation or the infinite allele models of microsatellite changes. The two-phase model assumes that microsatellites mutate at a constant rate without respect to their repeat lengths. Moreover, there is no bias in TPM toward expansion or contraction, so microsatellites grow or contract unconstrained over time. This model was chosen because most microsatellite data sets fit the TPM better than the stepwise mutation model or infinite allele model (DiRienzo et al. 1994; Luikart and Cornuet 1998).

The two-tailed Wilcoxon test (Luikart and Cornuet 1997) was employed because it assumes a "two-tailed" distribution over a population, is relatively powerful, and can be used with as few as four polymorphic loci and any number of individuals. It also provides a more conservative alpha of 0.025 compared to the one-tailed test with an alpha of 0.5. Since it was unclear when and if bottlenecks had taken place in our populations, it seemed wiser to use the more conservative alpha of the two-tailed test and an initial null hypothesis of Hexp equal to Hobs.

Effective population sizes (N_e) were calculated based on linkage disequilibrium by NeEstimator (Peel D, 2004, Queensland Government, Department of Primary Industries and Fisheries, Brisbane). Hill (1981) demonstrated that for neutral loci unlinked with selected loci in a randomly mating isolated population, linkage disequilibrium would come exclusively from genetic drift and could be used to estimate N_e.

Results

Allele frequency data and diversity

More than 200 ramets were sampled from the Barnegat Bay and nearby regions and analyzed with seven microsatellite loci, revealing a total of 180 genets (Table 1). Among the populations studied, Alaska appeared to have the highest clonal diversity ($C = 0.95$) and Long Island the lowest ($C = 0.55$) (Table 1).

Table 1 Clonal diversity in the *Zostera marina* populations studied

Population	No. of ramets	No. of genets	Clonal diversity (C)
Oyster Creek	20	18	0.90
Barnegat Bay Inlet	20	14	0.70
Ham Island	20	16	0.80
Manahawkin Bay	20	18	0.90
Sedge Island	20	18	0.90
Shelter Island	20	15	0.75
Marsh Elder	20	19	0.95
Harvey Cedar Sedge	20	15	0.75
Long Island	20	11	0.55
Chesapeake Bay	20	17	0.85
Alaska	20	19	0.95

Clonal diversity (C) was determined as the number of ramets sampled and the number of genets detected based on all the loci employed

The total number of alleles per locus ranged from 4 to 18 (Table 2). Across all populations, the CT12 locus had the largest mean number of alleles (13.2), while the CT17 locus had the fewest (5.3). Across all loci, the Chesapeake Bay population seemed to have the largest mean number of alleles (10.8). The expected number of heterozygotes (Hexp) was consistently higher for each locus than the observed number of heterozygotes (Hobs) (Table 2). The only exception to this observation was the Alaskan outgroup that demonstrated higher values of Hobs for GA3 (0.95) and CT19 (0.90) than Hexp; additionally, the mean Hobs (0.46) for Alaska was the highest for any of the populations. Long Island and Harvey Cedar Sedge had the lowest average Hobs values at 0.24.

We calculated the coefficient of local inbreeding (F_{IS}) (Nei 1977) in order to further examine the hypothesis that the Barnegat populations are only outcrossing to a small extent (Table 2). At all loci and in all populations except Alaska, it was found that the calculated F_{IS} was a positive value, indicating an excess of homozygotes in the populations. This result supports low levels of diversity in the populations studied. The Long Island population seems to be particularly affected by a lack of heterozygotes because it had high F_{IS} values of 1.00 in the loci GA3, CT3, and CT12. Alaska was the only population to exhibit larger numbers of heterozygotes, with two loci GA3 (−0.02) and CT17 (−0.01) having negative values for F_{IS} (Table 2).

The fixation index (F_{ST}) was also calculated to examine the overall genetic differentiation and the heterogeneity of gene frequencies in the populations studied (Nei 1977). Marsh Elder has the lowest mean F_{ST} value at 0.06, with five loci out of seven below an F_{ST} of 0.05 (GA2, GA3, CT3, CT12, and CT19) (Table 2). Based on Wright's (1978) qualitative guidelines, these low F_{ST} values would suggest little population differentiation within the Marsh

Table 2 Within-population genetic diversity in all of the populations of eelgrass examined in this study

Populations	GA2	GA3	CT3	CT12	CT17	CT19	CT20	Mean
Oyster Creek								
a	14	11	13	17	10	5	4	10.5
Hobs	0.45	0.1	0.4	0.2	0.45	0.3	0.05	0.28
Hexp	0.91	0.87	0.82	0.79	0.81	0.73	0.53	0.78
F_{IS}	0.46	0.87	0.49	0.72	0.42	0.61	1	0.63
F_{ST}	0.03	0.06	0.06	0.11	0.03	0.16	0.43	0.12
Barnegat Bay Inlet								
a	11	8	8	5	2	5	9	6.8
Hobs	0.4	0.25	0.1	0	0.2	0.4	0.55	0.2
Hexp	0.89	0.8	0.81	0.18	0.61	0.76	0.79	0.69
F_{IS}	0.62	0.83	0.91	1	0.75	0.44	0.55	0.69
F_{ST}	0.04	0.13	0.15	0.42	0.83	0.1	0.03	0.24
Ham Island								
a	11	10	10	12	6	8	10	9.5
Hobs	0.7	0.2	0.3	0.1	0.45	0.25	0.35	0.34
Hexp	0.89	0.79	0.68	0.73	0.84	0.77	0.71	0.77
F_{IS}	0.23	0.71	0.55	0.83	0.42	0.6	0.56	0.55
F_{ST}	0.07	0.11	0.26	0.12	0.09	0.08	0.13	0.12
Manahawkin Bay								
a	7	8	10	14	9	6	7	8.7
Hobs	0.45	0.2	0.25	0	0.65	0.35	0.15	0.29
Hexp	0.81	0.82	0.71	0.88	0.9	0.8	0.64	0.79
F_{IS}	0.47	0.79	0.61	1	0.31	0.59	0.74	0.64
F_{ST}	0.13	0.14	0.22	0.09	−0.07	0.03	0.21	0.1
Sedge Island								
a	13	10	10	16	11	6	8	10.5
Hobs	0.5	0.25	0.2	0.15	0.35	0.3	0.35	0.3
Hexp	0.92	0.82	0.79	0.86	0.93	0.58	0.73	0.8
F_{IS}	0.46	0.72	0.71	0.81	0.59	0.47	0.61	0.63
F_{ST}	0.04	0.17	0.17	0.03	−0.07	0.26	0.13	0.1
Shelter Island								
a	9	9	7	13	3	6	5	7.4
Hobs	0.45	0.4	0.15	0	0.35	0.35	0.15	0.26
Hexp	0.9	0.79	0.82	0.63	0.87	0.74	0.66	0.77
F_{IS}	0.48	0.44	0.92	1	0.57	0.65	0.7	0.66
F_{ST}	0.08	0.13	0.11	0.06	0.24	0.11	0.18	0.13
Marsh Elder								
a	13	12	11	17	5	7	8	10.4
Hobs	0.45	0.1	0.1	0.05	0.75	0.4	0.2	0.29
Hexp	0.92	0.92	0.86	0.67	0.94	0.85	0.68	0.83
F_{IS}	0.6	0.88	0.88	0.92	0.22	0.5	0.7	0.66
F_{ST}	0.03	0.02	0.04	0.03	0.17	0.008	0.14	0.06
Harvey Cedar Sedge								
a	9	12	13	18	4	5	5	9.4
Hobs	0.25	0.2	0.25	0	0.6	0.3	0.05	0.24
Hexp	0.92	0.92	0.89	0.66	0.96	0.71	0.76	0.83
F_{IS}	0.78	0.79	0.78	1	0.31	0.43	0.91	0.71
F_{ST}	0.06	0.02	0.01	0.01	0.2	0.31	0.07	0.09
Long Island								
a	8	7	8	10	3	4	8	6.8
Hobs	0.25	0	0	0	0.4	0.65	0.35	0.24
Hexp	0.75	0.79	0.88	0.6	0.9	0.73	0.82	0.78
F_{IS}	0.69	1	1	1	0.48	0.06	0.46	0.67
F_{ST}	0.23	0.12	0.05	0.09	0.3	0.14	−0.02	0.13
Chesapeake Bay								
a	15	13	14	17	3	3	11	10.8
Hobs	0.5	0.25	0.4	0	0.35	0.15	0.15	0.26
Hexp	0.94	0.88	0.92	0.62	0.95	0.44	0.89	0.81
F_{IS}	0.5	0.67	0.55	1	0.63	0.65	0.81	0.67
F_{ST}	0.03	0.06	0.005	0.01	0.28	0.4	−0.11	0.09
Alaska								
a	13	17	8	7	3	12	4	9.1
Hobs	0.5	0.95	0.15	0.05	0.4	0.9	0.3	0.46
Hexp	0.92	0.92	0.71	0.14	0.47	0.89	0.63	0.67
F_{IS}	0.49	**−0.02**	0.78	0.66	**−0.01**	0.003	0.47	0.3
F_{ST}	0.04	0.02	0.19	0.57	0.81	−0.06	0.26	0.26

Multiply sampled ramets have been excluded from these calculations. Bold values indicate loci with a putative heterozygous excess

a, Allele number; *Hobs*, observed heterozygotes; *Hexp*, expected heterozygotes; F_{IS}, coefficient of local inbreeding; F_{ST}, fixation coefficient

Elder plants compared to the other populations, as well as increased gene flow in those populations. However, the Alaska or Barnegat Bay Inlet populations with mean F_{ST} values of 0.26 and 0.24, respectively, have greater genetic differentiation and reduced gene flow as compared with other populations. Most of the populations have mean F_{ST} values that fall into the "moderate" range of Wright's (1978) genetic differentiation values (0.06–0.15), indicating a reasonable level of gene flow among most of the populations. Three loci (CT17, CT19, and CT20) showed negative F_{ST} values, which indicate a limited role of the loci in the genetic differentiation of these populations (Table 2).

Genetic distance calculations

A cladogram was constructed using the modified delta mu* (Ddm*) statistic for small populations (Fig. 2) (Minch E, personal communication). The cladogram was rooted using Alaska as the geographic outgroup and bootstrapped 1000 times. Chesapeake Bay naturally emerged as the second most distant geographic ecotype. A large "southern" Barnegat Bay clade emerged containing Sedge Island, Manahawkin Bay, Harvey Cedar Sedge, and Marsh Elder, with Oyster Creek and Ham Island in a nearby cluster (Fig. 2). The relatively low bootstrap value (58.0%) on the main branch

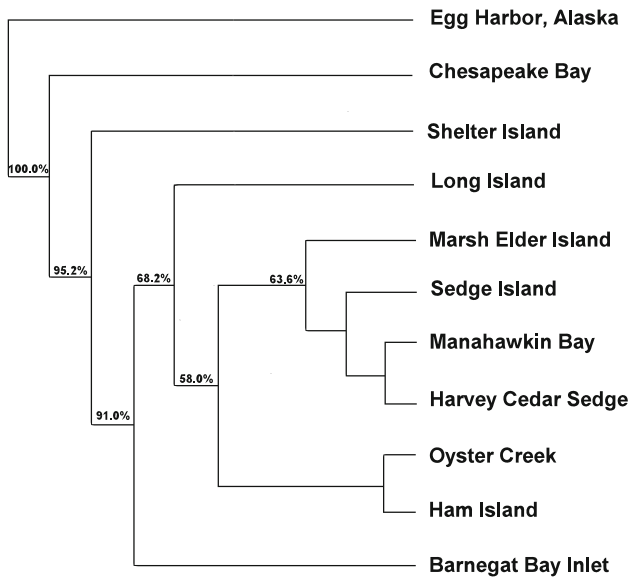


Fig. 2 Rooted neighbor-joining cladogram of eight *Zostera marina* populations from Barnegat Bay, New Jersey, with neighboring populations of Long Island and Chesapeake Bay; Alaska acts as the root and geographic outgroup. This neighbor-joining tree was produced using the distance matrices generated by the modified delta mu genetic distance between alleles. 1000 bootstrap iterations were performed in the generation of this tree. Multiply sampled ramets have been excluded from these calculations

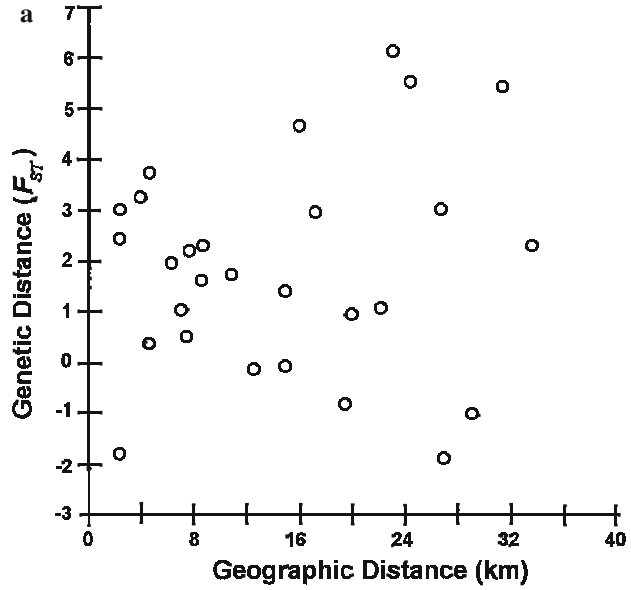
leading into the main Barnegat clade suggests that these local populations have not differentiated a great deal and are genetically difficult to distinguish from each other using the stepwise mutation model. The Shelter Island and Barnegat Bay Inlet populations did not fall into any particular clade.

Genetic isolation (distance) by geographic distance

The relationship between genetic distance and geographic distance was determined for populations within Barnegat Bay (Fig. 3a) and the mid-Atlantic populations (Fig. 3b). When the Barnegat Bay populations were analyzed alone, the R^2 value was found to be 0.0159 ($P = 0.280$), indicating no significant correlation between geographic and genetic distance within the confines of the bay (Fig. 3a). The R^2 value increased to 0.720 ($P = 0.0060$) when the mid-Atlantic populations were included in the analysis (Fig. 3b), suggesting that there was significant genetic isolation between the Barnegat populations and the more geographically distant ones.

Population bottlenecks and effective population size (N_e)

The presence of historical population bottlenecks was calculated using the two-tailed Wilcoxon test with the TPM (DiRienzo et al. 1994) (Table 3). Since a two-tail test was



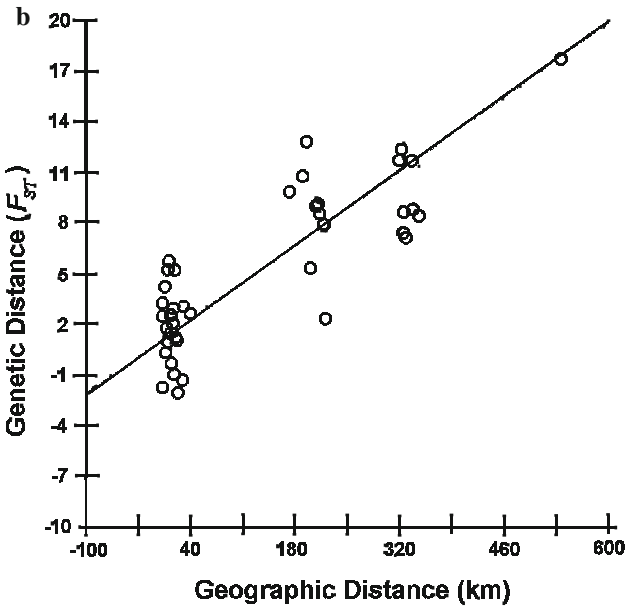


Fig. 3 Linear relationship between genetic isolation and geographic distance for **a** Barnegat Bay populations and **b** Barnegat Bay, Long Island and Chesapeake Bay populations. All geographic distances were measured in kilometers. Multiply sampled ramets have been excluded from these calculations

applied, an α-value of 0.025 was used to designate a cut-off value for the significance of bottlenecks. Widespread evidence of bottlenecks was found in several of the New Jersey populations examined, including Ham Island, Manahawkin Bay, Shelter Island, Marsh Elder, Harvey Cedar Sedge, and Long Island (Table 3). All of these populations have historically been under environmental stress (Gastrich et al. 2004).

The effective population sizes (N_e) with 95% confidence intervals were estimated using linkage disequilibrium for

Table 3 Bottlenecks and effective population size (N_e)

Populations	Bottleneck probability	Effective population size (N_e)	95% Confidence interval
Oyster Creek	0.296	∞	118–∞
Barnegat Bay Inlet	0.070	56.9	25–214.4
Ham Island	**0.007**	92.4	41.6–446.1
Manahawkin Bay	**0.015**	133.6	49.2–597.6
Sedge Island	0.296	4058.0	97.1–5233.0
Shelter Island	**0.023**	61.3	28.3–240.0
Marsh Elder	**0.007**	82.9	44.4–399.0
Harvey Sedge	**0.007**	57.8	31.6–233.4
Long Island	**0.007**	32.3[a]	18.2–100.3
Chesapeake Bay	0.078	63.7	36.9–191.3
Alaska	0.054	78.6	40.2–572.0

The probability of population bottlenecks was determined using the two-tailed Wilcoxon test and the TPM (values below the α-value of 0.025 (bold) support the occurrence of bottlenecks). Values of "∞" indicate a value that was too large to calculate. Multiply sampled ramets have been excluded from these calculations

[a] Lowest effective population size

all populations in order to better characterize their genetic diversity (Table 3). The Long Island population ($N_e = 32.3$) had the lowest value observed, with a 95% confidence interval of 18.2–100.3 individuals. Sedge Island had the highest calculable effective population size ($N_e = 4058$). Oyster Creek demonstrated the highest N_e value, infinity, indicating that the linkage disequilibrium method could not discern the very large N_e from infinity. The program can distinguish an upper limit for N_e. If a value of infinity is obtained, all that can be said is that the N_e estimate is greater than the limit. We can only state that the estimated N_e value for Oyster Creek is larger than the 5233 upper limit calculated for Sedge Island.

Discussion

Zostera marina population genetics in Barnegat Bay

Our research assessed the microsatellite-based genetic differences of *Zostera marina* populations within Barnegat Bay, New Jersey. These populations vary with respect to environmental stressors such as temperature elevations, macroalgal blooms (Bologna et al. 2001), and recurrent brown-tides (Gastrich et al. 2004). Based on a preponderance of evidence, these populations are not genetically diverse. This conclusion is supported by the paucity of observed versus expected heterozygotes. All of the Atlantic populations had mean Hobs (0.20–0.34) that were far lower than the Hexp values (0.69–0.83) (Table 2). Additionally, the F_{IS} values in all of these populations are positive, again indicating a surfeit of homozygotes over heterozygotes (Table 2). In fact, the CT12 allele shows complete homozygosity in several populations, with F_{IS} values of 1.00 (Barnegat Bay Inlet, Manahawkin Bay, Shelter Island, Harvey Cedar Sedge, Long Island and Chesapeake Bay) (Table 2). These results support a lack of genetic diversity and suggest a low degree of outcrossing in the Barnegat Bay populations.

The clonal diversity (C) values (Table 1) suggest that some of the populations with few heterozygotes are more genetically diverse than is initially apparent. Marsh Elder (0.95), Oyster Creek (0.90), Manahawkin Bay (0.90), Sedge Island (0.90), and Alaska (0.95) have high clonal diversity, which appears to contradict the conclusions drawn from the heterozygosity data. At the same time, the low clonal diversity of Long Island (0.55) supports a conclusion of low genetic diversity. However, it is worth noting that high clonal diversity values are not necessarily at odds with high levels of inbreeding if all of the distinct genets are in fact closely related. Although the ratio of genets to ramets has been used in the literature to examine genetic diversity in clonal populations (Ellstrand and Roose 1987), the more accepted metrics in the population genetics literature are F_{IS} and Hobs/Hexp, because they have greater predictive power (Reusch et al. 1999; Olsen et al. 2004).

There is evidence for historical bottlenecks in six of the ten populations studied (Table 3). This bottleneck evidence would support the hypothesis that these populations have been adversely affected by major environmental stresses over a period of years. In 1998, macroalgal blooms caused rapid declines (<4 months) in *Z. marina* in the southern portion of Barnegat Bay and wiped out both above- and belowground biomass (Bologna et al. 2001). Similar declines have been observed in Long Island (Dennison et al. 1989; Keser et al. 2003) and the Chesapeake Bay (Orth and Moore 1983). Therefore, environmental stresses may have induced bottlenecks and resulted in low effective population sizes in the sites affected (Table 3). In particular, the Long Island population appears to have undergone substantial population declines, inducing a population bottleneck, showing low genetic diversity based on a mean F_{IS} (0.67), and the lowest N_e (32.3) of any population studied (Tables 2, 3). Keser et al. (2003) examined *Z. marina* populations at three locations in the Long Island Sound from 1985 to 2000. Statistically significant declines in eelgrass abundance were observed at all three locations, which could create the genetic bottlenecks observed in our data.

The mean fixation index values (F_{ST}) observed in the populations studied would suggest that most loci are undergoing "moderate genetic differentiation" (Fig. 3). This increased populational differentiation translates into reduced gene flow. The F_{ST} is the proportion of the total

genetic variance contained in a subpopulation (the S subscript) relative to the total genetic variance (the T subscript) (Wright 1978). Values of F_{ST} can range from 0 to 1. The higher the F_{ST}, the greater the implied differentiation in a population from other populations. Wright (1978) defined "moderate differentiation" as being F_{ST} values between 0.05 and 0.15; values < 0.05 were defined as evincing low levels of genetic differentiation and "higher" levels of gene flow. Based on these assumptions, Alaska (mean $F_{ST} = 0.26$) and Barnegat Bay Inlet (mean $F_{ST} = 0.24$) seem to manifest "very great" differentiation from the other populations based on Wright's (1978) estimates. The increased genetic fixation in the Barnegat Bay Inlet plants may be due to the fact that their habitat is the most dynamic site in Barnegat Bay, with a constant tidal flux, making it physically difficult for gene flow to occur with other Barnegat Bay populations. Marsh Elder, with a mean F_{ST} of 0.06, may have the highest gene flow of any of the populations examined.

Marsh Elder, Sedge Island, Manahawkin, Harvey Cedar Sedge, Oyster Creek, and Ham Island share an ill-defined clade with a low bootstrap value. The Shelter Island and Barnegat Bay Inlet populations appear to be slightly more genetically distant; the positions of these populations in the tree are more heavily supported, with bootstrap values of greater than 90%. Alaska is the most genetically "distant" population.

Management implications

Because *Zostera marina* restoration efforts have had mixed success rates (Williams and Davis 1996; Fonseca et al. 1998; Bologna and Sinnema 2005, 2006; Tay Evans and Short 2005), one purpose of our research was to suggest how genetic variation may help to promote restoration success.

Although Oyster Creek has a surfeit of homozygotes (mean $F_{IS} = 0.63$), suggesting that it may not be genetically diverse, other data support a diverse population (Table 2). A value of infinity for N_e means that employing linkage disequilibrium as the method of calculation will not give a numerical answer, since the value is too high to estimate (Wright 1978). Although we could not obtain a value for N_e for Oyster Creek, it had the largest effective population size of any group studied (Table 3). High effective population size is considered to occur concomitantly with high genetic variation in natural populations (Wang 2005). Oyster Creek demonstrated a mean F_{ST} of 0.12, showing moderate genetic differentiation, and little evidence of any historical bottlenecks (Table 2). Additionally, Oyster Creek appears to have a large number of alleles (Table 2) and measurable gene flow with many of the other populations in Barnegat Bay (data not shown).

One interesting feature of the Oyster Creek population is that it is found near the exit plume of the Oyster Creek nuclear power plant and is under considerable heat stress (>30°C, Bologna PAX, unpublished data). The Oyster Creek population may flourish despite the temperature disturbance because of its high effective population size and genetic diversity. Reusch et al. (2005) showed that a six-genotype treatment had greater shoot recovery after extreme summer temperatures compared to low genotype diversity and monocultures in field manipulated experiments. Additionally, Hughes and Stachowicz (2004) previously demonstrated that genetic diversity can contribute to the resistance of *Z. marina* populations to various physical disturbances. We surmise, therefore, that Oyster Creek may be a good candidate population to use as the stock origin of eelgrass plants in prospective restoration projects in Barnegat Bay due to its potential resilience and resistance to thermal stress and disturbance. At the same time, we realize that Oyster Creek may be a poor choice of donor population for the same reasons mentioned. It is possible that it may be acclimated to a warm environment and may not grow well away from its adapted home. Unfortunately, we can only test its qualities by experimentation and transplantation. Other populations may be employed that may be more successful. Marsh Elder, with its high gene flow, may be another candidate among the populations studied here.

While elevated genetic diversity from a single population may result in increased restoration success, this has never been experimentally tested. Prior work has shown that the inclusion of multiple donor populations increases restoration success, but adequate genetic analyses of these survivors has not been performed (Williams 2001; Hughes and Stachowicz 2004). If the elevated diversity is related to the ability of transplants to survive under new environmental conditions, then it is possible that only a single or a few donor populations contribute to the survival of the transplants. This would lead to the establishment of the bed, but may doom it in the long run if only a few genotypes survive. Ultimately, the application of seed stock from multiple populations over several years may help to alleviate the potential minimizing phenomenon of effective population size and founder effects on genetic survival. Orth et al. (1994, 2003, 2006) and Pickerell et al. (2005) have demonstrated that the use of seed-based restorations is highly successful, and that seeds broadcast to the benthos remain very close to their broadcast point. As such, the use of seed stocks would allow us to experimentally manipulate the ratios of donor populations and effective genetic population size to assess how population genetics impact the survival of *Z. marina*.

This research is essential if we intend to be successful in our restoration efforts in coming decades. However, it is important that we keep in mind the reasons that many

seagrass beds have undergone losses. Unfortunately, the greatest peril to these communities is human activity. Eutrophication and urbanization of coastal systems continues to be the greatest threat to existing and restored habitats. If these overarching factors are not assessed and addressed in management plans then many restoration efforts may fail, regardless of elevated genetic diversity or the work of the individuals involved.

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