Stony Brook University

Genetic Diversity and Gene Flow in *Zostera marina* Populations Across the Long Island Sound and South Shore Estuaries

A Thesis Presented

by

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to

The Graduate School

in Partial Fulfilment of the

Requirements

for the Degree of

Master of Science

in

Marine and Atmospheric Science

Stony Brook University

May 2010

Stony Brook University

The Graduate School

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Abstract of the Thesis

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The dominant species of seagrass in NY, *Zostera marina*, has experienced several historical die-offs and is currently under heavy stress due to water quality and other anthropogenic problems. The consequences of these events on the genetic diversity and population structure of the remaining grass beds are unknown. This thesis addresses questions regarding the genetic diversity of extant populations, and how this information can aid current conservation and restoration efforts. Plant morphometrics and genetic samples of *Zostera marina* were collected at sites across Great South Bay, Shinnecock Bay, Peconic Bay and the Long Island Sound. Each individual was genotyped at 8 different microsatellite loci. Analysis of microsatellite alleles was used to examine the genetic diversity, population structure and gene flow between meadows within and between bays. Moderate levels of clonal and genetic diversity were exhibited across all study areas. No evidence of local inbreeding or of a severe population bottleneck was found. With the exception of individuals sampled from around Fishers Island in the Long Island Sound, connectivity is high within and between the major Long Island estuaries examined in this thesis. These results suggest the existence of an abundance of potential donor material from Great South Bay, Shinnecock Bay and the Peconics suitable for transplant within or between any of the three bays based on genetic criteria. However, continued monitoring of genetic diversity and additional documentation and small-scale sampling of future restoration efforts is important in maintaining current levels of genetic diversity.

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Acknowledgements

I would like to thank the following people who made the completion of this thesis possible: My advisor, Dr. Bradley Peterson as well as my other readers, Dr. Christopher Gobler and Dr. Eric Bricker. I would like to acknowledge the hard work of all my lab-mates and co-workers including Tom Behling, John Carroll, Liam Dolgin, Brad Furman, Maggi Mars, Brooke Rodgers, Joanne Siskidis, Amber Stubler and Chuck Wall. I'd also like to thank Blaine Kopp and Hilary Neckles of the USGS and the financial supporters of this research, the National Park Service and The Nature Conservancy. Finally I'd like to thank my friends and family for all of their support and love.

INTRODUCTION

Seagrasses are an ecologically successful group of marine angiosperms (Waycott et al., 2006) that provide structural habitat, primary production, oxygenation and sediment stabilization for shallow marine ecosystems. Due to this provision of ecosystem services and their role as a foundation for extraordinarily productive ecosystems, seagrasses are among the most valuable (Costanza et al., 1997) and at the same time, vulnerable ecosystems on the planet (Orth et al., 2006, Waycott et al., 2009). This means that seagrasses and the ecosystems they support are prime targets for conservation and restoration. The International Union for Conservation of Nature has identified genetic diversity as one of three forms of biodiversity deserving conservation. Temperate seagrass meadows are generally dominated by a single species, meaning that genetic diversity in these systems is present primarily in a single species at the genetic and genotypic levels (Procaccini et al., 2007).

Globally, seagrasses are a group in crisis. The majority of seagrass species are in decline. Where seagrasses are in decline, that decline has been increasing in pace (Orth et al., 2006). New York's coastal waters are no exception. In New York State, seagrass meadows are dominated by one species, *Zostera marina*. Recognizing the importance of seagrasses and our coastal ecosystems, New York State has made controlling losses of seagrasses a management priority. With recent advances in conservation biology and population genetics, it is becoming clear that ecologists and conservationists can no longer afford to consider ecological and genetic processes separately when attempting to preserve the biodiversity of an ecosystem. In a meta-analysis of 170 species and computer simulations, Speilman et al. (2004) showed that most species are not driven to

extinction before genetic factors impact them. Because seagrasses typically exhibit low effective population sizes and large clonal spreads with relatively low genotypic diversity, seagrasses, including *Z. marina* are particularly susceptible to genetic degradation under poor environmental conditions (Procaccini et al., 2007).

Zostera marina populations in New York and the surrounding waters have already been under environmental stress for quite some time. These populations experienced a severe die-off during the wasting disease of the 1930's that resulted in a >80% loss (Rasmussen, 1977). The persistence of brown tide blooms in New York waters during the 1980's resulted in another loss of 40% of the seagrass in the south shore estuaries (Cosper et al., 1987; Dennison et al., 1989). These combined events suggest that the *Z. marina* populations in New York may have low genetic diversity. One objective of this thesis is to determine if these severe die-offs have had a significant impact on the genetic diversity of *Z. marina* in New York State.

Because of this potential vulnerability of local *Z. marina* populations to genetic degradation, it is vital for any management strategy employed to take genetic factors into account. Attempting to control or reverse seagrass losses in New York State by solely examining and regulating environmental and ecological conditions without regard to genetic diversity may prove to be ineffective. Restorative strategies that attempt to increase population size through transplantation must take into account that using a single genotype as donor material may actually negatively impact the fitness of the population by decreasing the overall genetic diversity (Procaccini et al., 2007). Using donor genotypes that are not locally adapted could also lead to increased mortality (Williams, 2001) and lower the fitness of local populations through outbreeding depression.

This thesis will begin to address several questions relevant to the problems faced by those engaged in *Z. marina* conservation and restoration efforts in New York and elsewhere. The only previous genetic work carried out on *Z. marina* in New York State suggested that local populations may be suffering from low diversity, a high level of inbreeding and possible evidence of a severe genetic bottleneck (Campanella et al., 2010a & b). However, that work was of extremely limited scope and questionable usefulness. What is the current level of genetic diversity in *Z. marina* beds in New York State? Have challenges like wasting disease, brown tide and poor water quality impacted the genetic diversity or population structure of *Z. marina*? Due to its ability to reproduce both sexually and asexually, *Z. marina* is known to exist in large meadows composed of a single clone or a large number of genetically distinct individuals (Procaccini et al., 2007). Is the prevailing mode of reproduction in New York's *Z. marina* meadows sexual or asexual? Previous studies have found that population structure in temperate seagrasses like *Z. marina* can exist on scales of kilometers (Muñiz-Salazar et al., 2006) to hundreds of kilometers (Ferber et al., 2008). Is there a significant genetic structure apparent in populations of *Z. marina* across New York State? Finally, how do the answers to all of these questions potentially impact local *Z. marina* conservation and restoration efforts?

METHODS

Sampling Strategy

A stratified random sampling design (hexagon tessellation) was used to identify 200 sites within Fire Island National Seashore in Great South Bay (Figure 1). Extant *Z. marina* meadows in Shinnecock, The Peconics and in the area of Fishers Island in the Long Island Sound were also identified as potential targets for genetic sampling (Figure 2).

Four sub-stations were set-up at least two meters apart at each site in Great South Bay. Keeping each sub-station at least two meters apart was done to help avoid collecting ramets that were physically connected by rhizome runners. If present, plant material from each sub-station was taken for genetic analysis. Up to four whole shoots were taken from each quadrat. Whole shoots were stored in individually labeled gas impermeable plastic bags at -20°C immediately after collection until they could be returned to the lab. Once sampled shoots had been returned to the lab, they were rinsed in fresh water, cleaned of epiphytes by gentle scraping with a straight razor, gently dried with a paper towel and the youngest two blades were placed in 20ml plastic scintillation vials and covered with silica bead desiccant for storage until they were ready for DNA extraction and genotyping.

Plants from Shinnecock Bay, the Peconics and around Fishers Island in the Long Island Sound were also sampled for inclusion in this study. Two transects of approximately 75 meters in length each were established in a known seagrass bed in Shinnecock bay. A diver swam along each transect and collected a randomly selected shoot approximately every five meters (Figure 3). These shoots were immediately

cleaned of epiphytes using a straight razor, placed in 20ml glass scintillation vials and covered with silica bead desiccant until ready for DNA extraction and genotyping.

Cornell Cooperative Extension personnel collected samples from the Peconics and around Fishers Island/Long Island Sound during routine seagrass monitoring trips in 2008. Personnel visited 19 different beds across the Peconics and Long Island Sound (Figure 4) where ten shoots were randomly collected at least one meter apart, cleaned of epiphytes and placed in gas impermeable plastic bags. These bags were then stored at -20º C until ready for DNA extraction and genotyping.

Genotyping of Samples

DNA was extracted from the dry (in the case of individuals from Great South Bay and Shinnecock Bay) or frozen (in the case of individuals from the Peconics or Long Island Sound) genetic samples using a mortar and pestle and a modified Qiagen DNeasy plant mini kit (Qiagen Pty. Ltd. Valencia, CA). The extracted DNA was processed in two separate multiplex polymerase chain reaction (PCR) amplifications in a MJ Research PTC-200 using 8 fluorescently labeled polymorphic microsatellite markers specifically developed for *Z. marina*. These primers were developed by Reusch et al. (1999) and were chosen for use in this project based on their extensive use in previous projects involving microsatellite genotyping of *Z. marina* (Provan et al., 2008, Muñiz-Salazar et al. 2006, Ferber et al., 2008, Reusch et al., 2000, others).

PCR amplifications were performed in 20 µL reactions containing 30 ng of template DNA, 0.5 µL of Bioline Immolase DNA Taq (Bioline Pty. Ltd. CA), 1.5 µL 10x Bioline Immobuffer (160 mM $(NH_4)_2$ SO_4, 670 mM Tris-HCl pH 8.3, 0.1% Tween-20),

2.5 mM MgCl, 0.133 mM each dNTP, BSA at a concentration of 0.1 μg/μL, and 0.33 mM fluorescently labeled (FAM, HEX, or TET) forward and reverse microsatellite primers. Thermal cycling protocols consisted of a 7-minute denaturing step followed by 30 cycles at 50°C. For all cycles denaturing steps were 94°C and extension temperature was 72°C (Bricker, pers. comm.).

PCR amplification products were analyzed using a GE MegaBACE 1000 capillary sequencer with filters and software configured for microsatellite genotyping. GE Fragment Profiler™ software was used to automatically score the fluorescence peaks generated by the amplified fragments and assign alleles based on number of base pairs for each sample at every loci (Figure 5). After human proofreading, these scores were exported into a Microsoft Excel™ spreadsheet for use with population genetics analytical software packages.

Statistical Analyses

Basic population genetics tests were run using GenAlEx 6.2 software (Peakall & Smouse, 2006). GenAlEx 6.2 was used to detect clones and calculate clonal diversities (the proportion of sampled individuals that are genetically unique at at least one locus) for each sampling area (Great South Bay, Shinnecock Bay, the Peconics and Long Island Sound). Multiple clones were excluded from further allele frequency based analyses.

GenAlEx 6.2 was also used to calculate Hardy-Weinberg expected and observed heterozygosities, allelic richness and diversity. A Mantel test to determine genetic isolation by distance (IBD) was used to look for any correlation between the geographic and genetic distances between sampled individuals in Great South Bay. Analysis of

molecular variance (AMOVA) was used to compare the distribution of molecular variance within Great South Bay to the distribution of molecular variance found across all sampled individuals.

GenAlEx 6.2 was also used to run multiple two-way population assignment tests based on Nei's genetic distance. In the first series of population assignment tests, samples from Great South Bay were broken into three groups based on their geographic distribution (Figure 7). These tests plotted the Nei's genetic distance of each individual against the two geographic subpopulations being tested. The second series of six separate population assignment tests were between all possible pairings of Great South Bay as a whole, Shinnecock Bay, The Peconics and Long Island Sound.

The presence of null alleles was tested for using FreeNA (Chapuis & Estoup 2007) software. FreeNA was used to estimate potential null allele frequencies at each locus and calculate revised F_{ST} values based on those predictions (Weir 1996). FreeNA was also used to create a pairwise F_{ST} matrix between all sampled areas (Cavalli-Sforza & Edwards 1967).

The program BOTTLENECK (Cornuet & Luikart 1997) was used to test each sampled area for the potential presence of a recent and/or severe genetic bottleneck. BOTTLENECK calculates heterozygous surpluses relative to allelic diversity by locus and attempts to determine if any significant heterozygous surpluses may be an indication of a recent and/or severe population bottleneck. BOTTLENECK was run using a two-tailed Wilcoxon test and a two-phase model of mutation with 10,000 replications for all loci from all sampled areas.

RESULTS

Clonal Diversity

There were very few individuals sampled that possessed identical sets of alleles at all 8 loci (potential clones/genets). Out of a total of 290 individuals sampled, 274 were genetically distinct at all eight loci. This was an overall clonal diversity of 0.95. The clonal diversities of the geographic areas sampled ranged from 0.96 in Great South Bay and the Peconics to 0.85 in the Long Island Sound (Table 4). When clones were detected they were always from within the same small-scale sampling plot (within 2-5 meters of each other) and never from separate areas within that bay or among other bays.

Allelic Diversity

Analysis shows a moderate level of allelic diversity within *Z. marina* meadows in all of the study areas. All loci were 100% polymorphic and exhibited moderate to high numbers of alleles and effective alleles (Figure 6). Allelic diversity (total number of alleles divided by samples loci) was 13.4.

Hardy-Weinberg Expectations

The vast majority of loci were not significantly out of Hardy-Weinberg equilibrium (Table 1). Every geographic subpopulation except for the Peconics was found to have an overall excess of heterozygotes compared to Hardy-Weinberg equilibrium predictions. These excesses are so small that they are most likely not significant different from zero (figure 8).

In accordance with these findings, all of the geographic areas had negative inbreeding coefficients with the exception of the Peconics, which had an F value of 0.055 (Table 1). Figure 9 shows a graphic representation of this by plotting F averaged across all loci by geographic area. F_{ST} values were negative or low to moderate (<0.1) when averaged across populations for all loci with the exception of CT19 (Table 2). This locus shows unusual homozygous excess in the Peconics (Table 1).

Local Inbreeding (F_{IS})

There was no evidence of local inbreeding in any of the sampled areas as F_{IS} values were negative or quite low for all loci (Table 2). Values ranged from -0.115 at locus CT3 to 0.022 at GA6 with a mean of -0.034. Negative F_{IS} values can probably be interpreted as not being significantly different than zero.

Null Alleles & F_{ST}

FreeNA was used to calculate estimated null allele frequencies for all sampled loci. Table 3 shows these calculated null allele frequencies, which were all very low. The Peconics had the highest estimate of null allele frequency at 0.02542. Although this would seem to indicate that any null alleles that may be present almost certainly did not affect our allele frequency based analyses, we used Chapuis & Estoup's (2007) ENA and INA methodologies to incorporate estimated null allele frequencies into our analyses of F_{ST}.

Figure 11 shows global F_{ST} values calculated with and without Chapuis & Estoup's ENA correction. Based on these global F_{ST} values, loci GA2, GA6 and CT19

are responsible for the majority of the genetic differentiation seen between the sampled areas. Table 5 shows pairwise F_{ST} values between all populations. Based on F_{ST} values alone, there is little to no variation between sampled areas. However, there does appear to be "moderate differentiation" as defined by Wright (1978) between individuals from Fisher's Island/Long Island Sound and all other areas. Both Shinnecock Bay and the Peconics have pair-wise FST values ≤0.040 when compared with Great South Bay.

Genetic Isolation by Distance

A Mantel test was conducted using GenAlEx 6.2 (Peakall & Smouse 2006) to determine if there was any genetic isolation by distance (IBD) between individuals sampled in Great South Bay. This analysis showed no significant correlation between geographic and genetic distance between sampled individuals (Figure 15). Because the sampling coordinates of individuals from other bays were not recorded with the same degree of precision as those from Great South Bay, and the differing sampling scales used in different systems, Mantel tests for genetic IBD were not applied to any other study areas.

Analysis of Molecular Variance

Two analyses of molecular variance (AMOVA) were carried out using GenAlEx 6.2 (Figure 16). AMOVAs of individuals from Great South Bay and of all sampled individuals were compared to illustrate the differences in the distribution of molecular variance between *Z. marina* in Great South Bay and across the entire sampled Long Island & Long Island Sound region. Great South Bay was divided into three regions

based on the geographic distribution of *Z. marina* for AMOVA analysis; Western, Central and Eastern Great South Bay (Figure 7). AMOVA of individuals from these three areas of Great South Bay showed that none (0%) of the molecular variance within the bay occurred among the geographic subpopulations (Figure 16a). AMOVA of all individuals genotyped revealed that a substantially larger fraction (11%) of molecular variance occurred among geographic subpopulations (Figure 16b).

Population Assignment

The population assignment tests between the three areas of Great South Bay (Figure 7) showed that individuals from these zones were indistinguishable based on their Nei's genetic distances (Figures 12-14).

The second series of six separate population assignment tests still showed overlap between individuals from Great South Bay, Shinnecock Bay and the Peconics. However, there appeared to be a greater divergence in genetic distances between paired areas across these three areas than between individuals from within Great South Bay only (Figures 17-19). Individuals from the Long Island Sound area appeared to be distinct from all other groups based on their population assignment tests (Figures 20-22).

Bottleneck Analysis

The bottleneck index and *p* value for each area were generated using the program BOTTLENECK (Table 6). Large positive bottleneck index values would indicate a possible bottleneck. None of the sampled areas showed a relative excess of heterozygosity that might suggest the occurrence of a recent severe bottleneck. Great

South Bay showed a relative deficiency of heterozygotes in relation to the expected level of heterozygosity predicted by BOTTLENECK based on allelic diversity.

DISCUSSION

This thesis set out to address several questions regarding *Z. marina* in New York waters. What is the clonal and genetic diversity of local *Z. marina*? Is there a discernable population structure within the state? Have recent ecological challenges to the species had an observable effect on the genetic diversity or population structure of New York's *Z. marina*? Finally, how can we apply this information to local endeavors to conserve and restore *Z. marina* meadows in coastal New York waters?

Genetic Diversity

In species that have a capacity for both sexual and asexual reproduction, genetic diversity is dependent primarily on clonal & allelic diversity and heterozygosity (Procaccini et al., 2007). These characteristics are important to consider because of the negative effects their degradation can have, especially on small fragmented groups exhibiting low effective population sizes (Frankham et al., 2002). The levels of allelic richness, diversity and heterozygosity (Table 4, Figures 6 & 8) observed all seem to indicate a moderate level of genetic diversity and a very high level of sexual reproduction. Accordingly, there is no evidence of any significant inbreeding in any of the sampled areas at this time (Tables 1 & 2, Figures 8-10).

Clonal diversities approaching 1.0, high allelic diversities and low F_{IS} values are quite common and have been found in both Atlantic and Pacific *Z. marina* meadows (Ferber at al., 2008, Muniz-Salazar et al, 2006, Procaccini et al., 2007). While the results presented here are far from unprecedented they are important pieces of information to consider when addressing issues of conservation and restoration. Of all the threats faced

by *Z. marina* on Long Island, loss of genetic diversity does not seem to be critical, at least not in the short term. Widespread genetic diversity also means that as far as genetic criteria is concerned, there is a large pool of suitable donor material that can be utilized in a very flexible manner in future restoration efforts.

Population Structure & Connectivity

While previous studies carried out in similar physical settings (Muñiz-Salazar et al., 2006) and the complex geographic structure of the estuary systems in the study area (Figure 2) may have suggested the possibility of highly structured subpopulations of *Z. marina* in New York State, our findings suggest otherwise. While all of the geographic areas examined exhibited moderate genetic diversity, this diversity was divided more or less proportionately across sampled individuals regardless of their geographic area, suggesting high connectivity (Figures 12-19). The exceptions to this were individuals from the Long Island Sound in the area of Fishers Island (Table 5, Figures 20-22). There appears to be a history of very free and extensive flow of genetic material throughout Great South Bay and to a slightly lesser extent between Great South Bay, Shinnecock Bay and the Peconics. Individuals from around Fishers Island in the Long Island Sound appear to be members of a subpopulation that is genetically somewhat distinct from the grass in Long Island estuaries. Although more detailed future analysis will hopefully begin to reveal some of the processes responsible for these patterns, based on our knowledge of sexual reproduction in marine angiosperms widespread dispersal of seeds or 'rafting' of reproductive shoots would certainly be possible explanations for the level of connectivity observed between Great South Bay, Shinnecock Bay and the Peconics

(Reusch et al., 1999, Reusch, 2001, Kornelis van Dijk et al., 2009). *Zostera marina* from around Fishers Island may not be able to exchange seeds or floating wrack with areas of Long Island due to tidal currents, prevailing winds or other characteristics of the Long Island Sound. It will be important to further investigate the roles of processes like seed dispersal, viability, seed banks, rafting of reproductive shoots and others in maintaining the current level of diversity and connectivity.

Bottleneck Analysis

Bottleneck analysis using the program BOTTLENECK (Cornuet &Luikart, 1997) showed no evidence of a recent severe genetic bottleneck at any of our sampled areas (Table 6). Plants from Fishers Island/Long Island Sound, the Peconics and Shinnecock did not exhibit any significant divergence from expected heterozygosity relative to allelic diversity. Both eastern and western Great South Bay seem to exhibit significant deficiencies in heterozygotes, the opposite of what would be expected following a severe bottleneck. Cornuet & Luikart (1997) suggest that under the two-phase model of mutation, two possible explanations for this might be the introduction of rare alleles by migrants or recent expansion in population in those areas. There is an extensive anecdotal history of *Z. marina* restoration on Long Island dating back to the original outbreak of wasting disease, much of which is characterized by poor or a total absence of documentation. The introduction of plants from geographically and therefore possibly genetically distant sources into Great South Bay during this time could be one explanation for the results of our bottleneck analysis. It's also possible that any population bottleneck events that may have occurred were not severe enough to be

detected, or that we tested an insufficient number of loci or picked poor markers for the test. Provan et al. (2008) found that evidence of a genetic bottleneck caused by wasting disease in *Z. marina* in Northern Ireland was present in chloroplast microsatellite loci and nuclear DNA sequence information but not apparent when testing nuclear microsatellite loci. This suggests that nuclear microsatellites might not be an ideal marker for exploring possible population bottlenecks in *Z. marina*.

Implications for Conservation & Restoration

There are several considerations that must be kept in mind when interpreting these results, especially in regards to conservation and restoration. One is the disparate sampling scale used in different areas (Figures 1, 3, 4 & 7). A large number of individuals from a variety of sources were included in this thesis in an effort to maximize its effectiveness and impact. In some cases this meant that individuals were collected at different times (see appendix C) and using various experimental designs. The analyses used were selected and interpreted with the objective of controlling for these temporal and spatial inconsistencies in mind. Regardless of the sampling scale, all areas exhibited high levels of clonal diversity (Table 4), meaning few individuals were disqualified from inclusion in allele frequency based analyses and bolstering the validity of those analyses. This apparent lack of a correlation between sampling scale and clonal diversity hopefully indicates that our measures of clonal and genetic diversity were not biased by discrepancies in scale.

A further limitation of this study is our hindered ability to examine any changes in genetic diversity through time. Even a relatively dynamic set of genetic markers like

microsatellite loci operate on evolutionary timescales. Recent challenges faced by *Z. marina* on Long Island, including episodes of wasting disease (Rasmussen, 1977) and brown tide (Cosper et al., 1987; Dennison et al., 1989) have only occurred within the last 80 years or so, hence their full effects may not yet have manifested themselves in the population genetics of local meadows. Even factors like sea level change and geologic history can have a significant impact on the genetic diversity and structure of eelgrass populations and must be considered (Procaccini et al., 2007). The estuaries and bays of Long Island are not only quite dynamic but also young when considered on an evolutionary timescale. Long Island in its current form is no more than 10,000 years old (Merguerian & Sanders, 1990) meaning that the genetic diversity and structures of extant populations of *Z. marina* is dependant on how the plants established themselves in the area following the last glacial retreat as well as all of the evolutionary significant events that have occurred since then. Some measures, like allelic diversity can quickly reflect changes over a very short period of time, while clonal diversity, F-statistics and many others may change at a much slower rate (Frankham et al., 2002). This variation in the speed at which various population genetics processes function is actually the basis for the bottleneck analysis used here (Cornuet & Luikart, 1997).

The uniformly high level of sexual reproduction exhibited by these plants across Long Island waters may be an indication of extreme environmental stress. As sessile organisms, preferential sexual reproduction given the option of both sexual and asexual reproduction may represent an attempt to escape poor local conditions (Procaccini et al., 2007). This high level of sexual reproduction may increase diversity in the short-term, but unrelenting stressful conditions may eventually lead to a loss of genetic diversity

through selection and certainly do not bode well for any attempted conservation or restoration efforts (Alexandre et al., 2005).

The only other examination of local *Z. marina* population genetics was published while this study was underway (Campanella et al., 2010a & b) and concluded that Long Island *Z. marina* was suffering from low diversity and inbreeding as a result of a recent and severe population bottleneck. The analyses of *Z. marina* on Long Island presented in these papers used a total of only 20 plants taken from a very small area in a single location. As they report a clonal diversity of 0.55 for this group of plants, the majority of their analyses on this group of individuals were carried out with an *n* of 11. These Long Island individuals may have been suitable for use as an 'out-group'; rooting the analyses of other larger datasets, but were probably not suitable for supporting the conclusions regarding the genetic diversity, population structure and recent population history of *Z. marina* on Long Island made by Campanella et al. The results presented here directly contradict these conclusions, and are based on much more robust data and analyses.

Seagrass meadows are at once the most valuable (Costanza et al., 1997) yet vulnerable ecosystems on the planet (Orth et al., 2006). Most species of seagrasses, including *Z. marina*, generally exhibit low effective population sizes and genotypic diversity. Because of this, species like *Z. marina* can be susceptible to genetic degradation, especially when subjected to increased levels of environmental stress (Procaccini et al., 2007). It is becoming clear that ecologists and conservationists can no longer afford to consider ecological and genetic processes separately when attempting to preserve the biodiversity of an ecosystem. Managing genetic diversity can be vital to successful conservation and restoration efforts (Frankham et al., 2002). Recognizing the

importance of seagrasses and their vital role in our coastal ecosystems, New York State has made controlling losses of seagrasses a management priority. This thesis provided an unprecedented evaluation of the genetic diversity and population structure of *Z. marina* in New York waters that will aid in these efforts.

This research clearly demonstrates that at present, *Z. marina* from all of the areas examined exhibit moderate and widespread levels of genetic diversity, no evidence of inbreeding, high levels of connectivity (with the exception of Fishers Island/Long Island Sound) and no evidence of a recent and/or severe population bottleneck. Although continuing to monitor the genetic diversity of these plants over time is important, especially considering the severe anthropogenic environmental stress they are subject to, these results suggest that there is currently a relatively high level of flexibility in selecting donor material and target sites for restoration based on genetic factors. Because of the connectivity between Great South Bay, Shinnecock Bay and the Peconics, there is a relatively high level of diversity being shared more or less equally across all these systems. Eelgrass from any given bed, if carefully selected, should neither contribute to inbreeding nor suffer from a lack of local adaptation in when transplanted to any other bed. Given the importance of small-scale genetic diversity and local adaptation on the success of *Z. marina* transplants (Williams, 2001), small scale surveys of potential donor and target sites would be prudent in future restoration efforts. Detailed examination of allele frequencies from eelgrass actually being transplanted and from target beds would aid in the success of transplant efforts.

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APPENDIX A - TABLES

Table 1. Number of alleles, observed heterozygosity, expected heterozygosity and fixation index by population and locus. Bold numbers indicate values resulting from a significant divergence from Hardy-Weinberg equilibrium at that locus.

Populations	*GA2*	*GA3*	*GA6*	*CT3*	*CT17H*	*CT19*	*CT20*	*CT35*	*Mean*
Western GSB									
Na	8	8	9	9	23	5	8	19	11.1
H_{OBS}	0.643	0.196	0.705	0.554	0.929	0.598	0.536	0.652	0.602
H_{EXP}	0.623	0.200	0.717	0.505	0.923	0.503	0.532	0.689	0.587
F	-0.031	0.018	0.016	**-0.095**	-0.006	**-0.189**	-0.007	**0.054**	-0.030
Eastern GSB									
Na	10	7	8	9	22	4	8	17	10.6
H_{OBS}	0.613	0.280	0.747	0.387	0.907	0.627	0.520	0.573	0.582
H_{EXP}	0.661	0.262	0.761	0.398	0.914	0.474	0.535	0.599	0.576
F	0.072	-0.068	0.019	0.027	0.008	-0.321	0.028	0.043	-0.024
Peconics									
Na	8	7	5	9	19	2	8	13	8.9
H_{OBS}	0.700	0.333	0.600	0.617	0.867	0.383	0.500	0.733	0.592
H_{EXP}	0.747	0.360	0.579	0.545	0.912	0.483	0.590	0.783	0.625
F	0.063	0.073	**-0.036**	-0.131	**0.050**	0.207	0.153	**0.063**	0.055
LI Sound									
Na	3	2	2	7	10	2	2	4	4.0
H_{OBS}	0.583	0.333	0.250	0.917	0.917	0.167	0.500	0.750	0.552
H_{EXP}	0.569	0.278	0.219	0.788	0.875	0.153	0.444	0.587	0.489
F	-0.024	-0.200	-0.143	-0.163	-0.048	-0.091	-0.125	-0.278	-0.134
Shinnecock									
Na	7	2	4	6	11	2	2	8	5.3
H_{OBS}	0.857	0.071	0.571	0.643	1.000	0.286	0.071	0.786	0.536
H_{EXP}	0.758	0.069	0.663	0.559	0.883	0.245	0.069	0.793	0.505
F	-0.131	-0.037	**0.138**	-0.151	-0.133	-0.167	-0.037	0.010	-0.063

Table 2. Seawall Wright's F-Statistics over all populations for each locus as calculated using GenAlEx 6.2 (Peakall & Smouse 2006).

	GA2	*GA3*	*GA6*	*CT3*	*CT17H*	*CT19*	*CT20*	*CT35*	*Mean*
F_{IS}	-0.011	-0.039	0.022	-0.115	-0.025	-0.109	0.020	-0.013	-0.034
F_{IT}	0.075	-0.001	0.111	-0.062	0.005	0.195	0.098	0.035	0.057
F_{ST}	0.086	0.037	0.091	0.048	0.029	0.274	0.080	0.047	0.086

Table 3. Estimated null allele frequencies for each sampling area as predicted by FreeNA (Chapuis & Estoup 2007).

	Western GSB	***Eastern GSB***	***Peconics***	***LI Sound***	***Shinnecock***
Estimated pNA	0.00015	0.00251	0.02542	0.00004	0.00226

Table 4. Ramets, Genets and Clonal Diversity (C) for each geographic population. Clonal diversity is the proportion of all sampled individuals that were genetically distinct at at least one locus.

Population	***Ramets***	***Genets***	***C***
GSB	187	179	0.96
Shinnecock	31	28	0.90
Peconics	52	50	0.96
LI Sound	20	17	0.85
Total	**290**	**274**	**0.95**

Table 5. Pairwise population FST values. Grey shading indicates FST values between 0.05 and 0.15, indicating "moderate differentiation" (0.05≤FST≤0.15) according to Wright (1978).

	Peconics	***LI Sound***	***GSB***
Peconics	-		
LI Sound	0.060	-	
GSB	0.022	0.089	-
Shinnecock	0.040	0.135	0.019

Table 6. Results from the program BOTTLENECK (Cornuet & Luikart 1997) indicating the significance of any heterozygous excess or deficiency detected. The □ symbol indicates populations with significant heterozygous deficiencies.

Populations	***Bottleneck index***	***p-value***
Western GSB	-2.33925□	0.020
Eastern GSB	-2.792375□	0.039
Peconics	-0.903	0.383
LI Sound	0.268	0.195
Shinnecock	-0.371	0.461

APPENDIX B - FIGURES

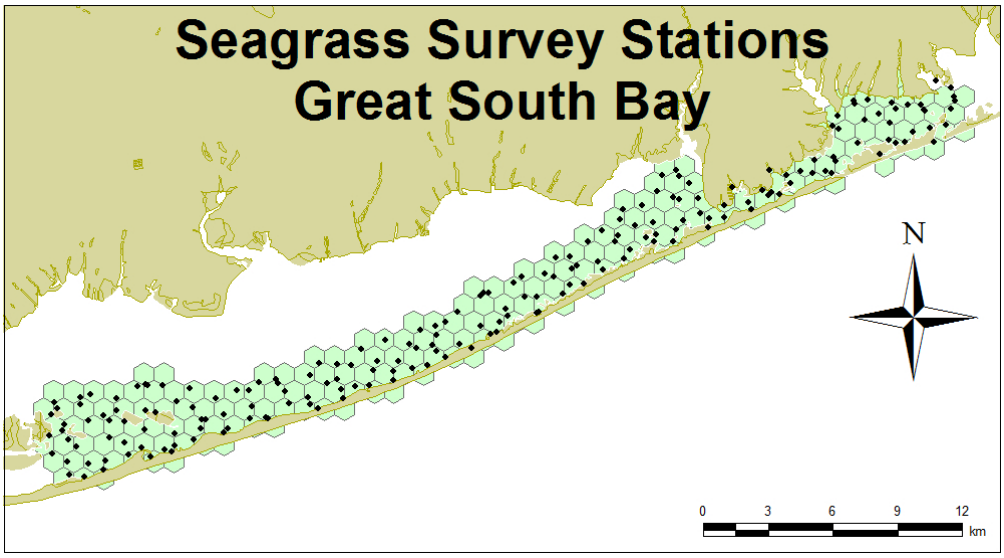


Figure 1. Sampling design schematic of Fire Island National Seashore. This area of Great South Bay was divided into 200 tessellated hexagons shown in green above. The black dots show the random positions chosen within each hexagon as sampling sites. All 200 sites were visited during the summer of 2009.

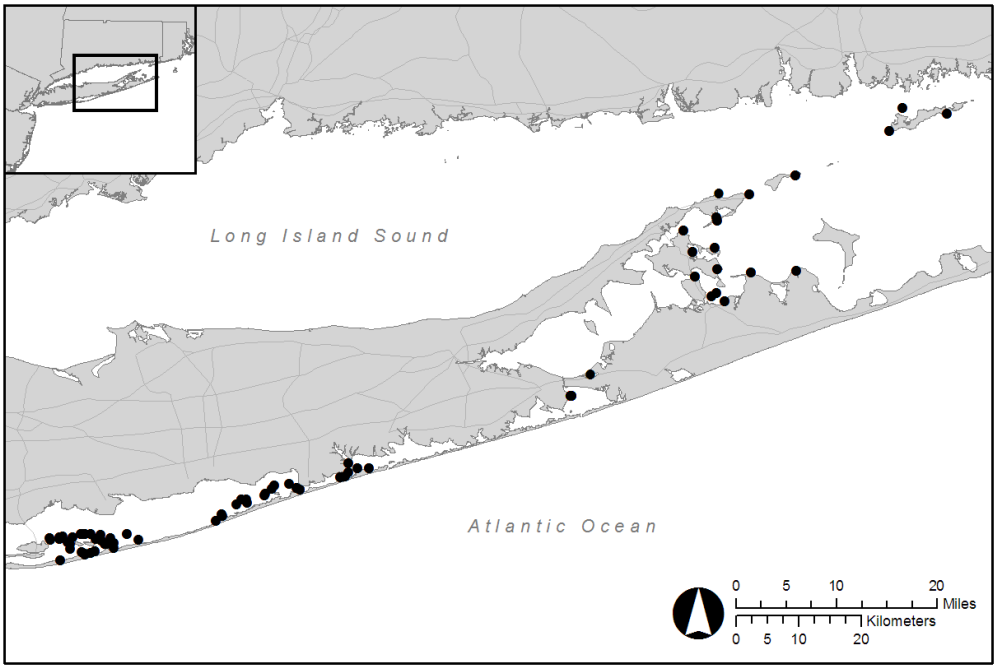


Figure 2. Locations of all sampling sites across Long Island south shore estuaries and the Long Island Sound. Black dots indicate sites where grass was found and genetic samples were taken.

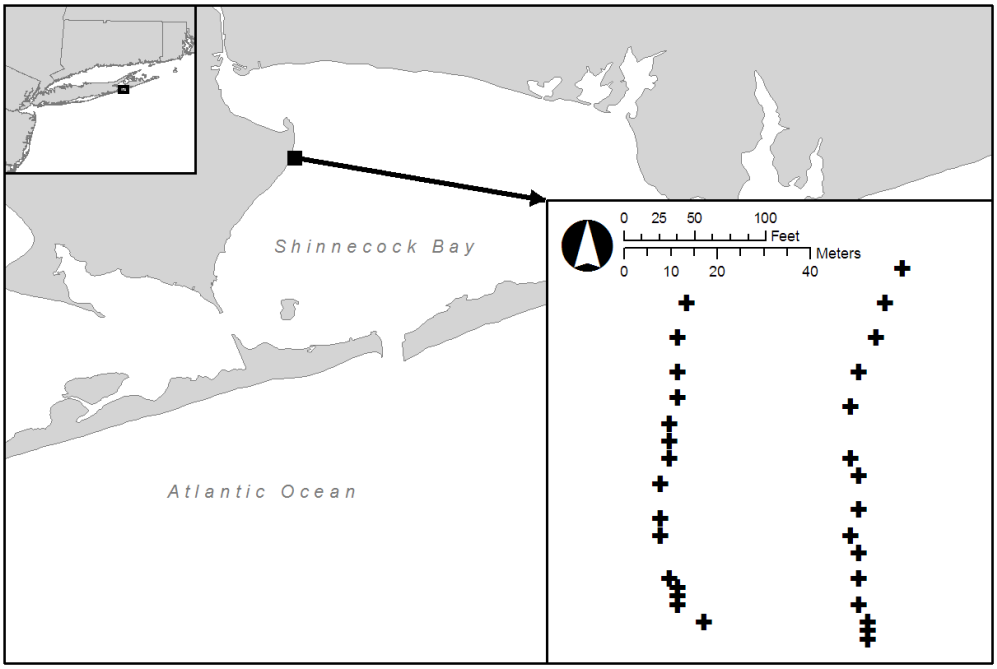


Figure 3. Locations of sampled shoots in Shinnecock Bay, NY. Two 75-meter transects were established running approximately parallel to the shoreline. Fifteen plants were collected at random intervals along each transect in July of 2009.

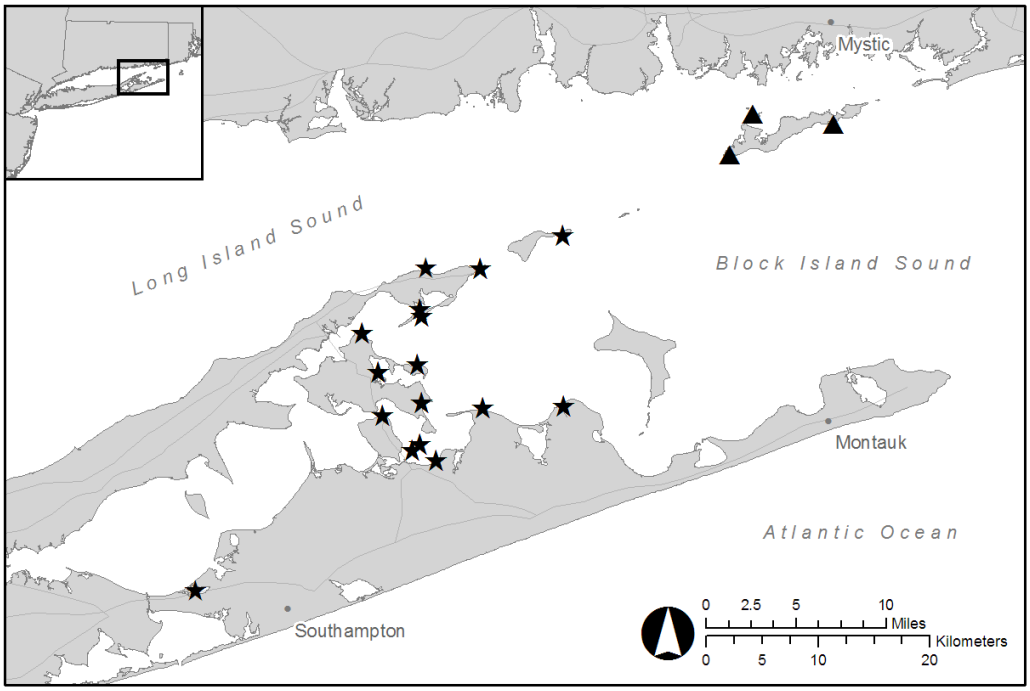


Figure 4. Locations of sampling sites across the Peconics and Long Island Sound. Stars indicate sampling locations within the Peconics while triangles indicate sampling sites across the Long Island Sound near Fishers Island.

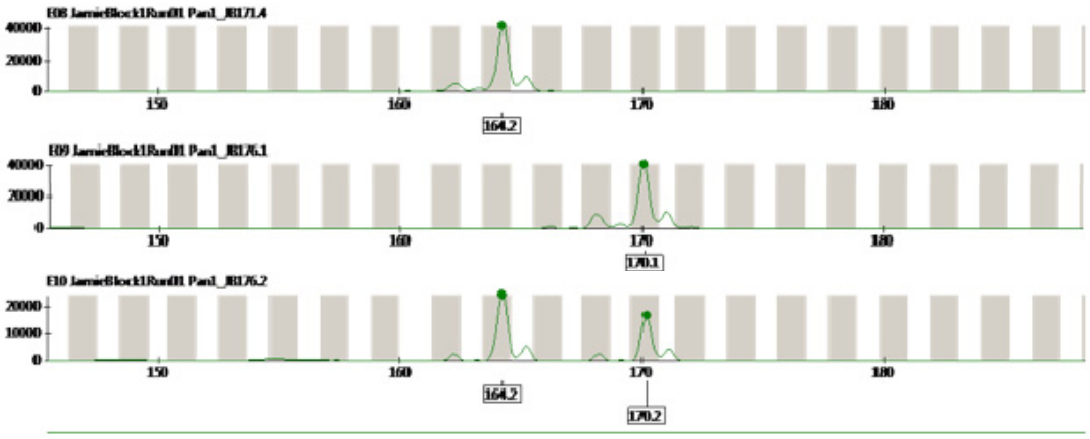


Figure 5. Example of results from microsatellite genotyping. These traces represent the fluorescence of marked primer molecules that have been incorporated into target microsatellite loci during PCR. The top trace represents a homozygous individual with two 164 base pair alleles at this locus. The middle trace is another homozygous individual, this time with two 170 base pair alleles. The bottom trace is heterozygous individual with one 164 and one 170 base pair allele at this locus.

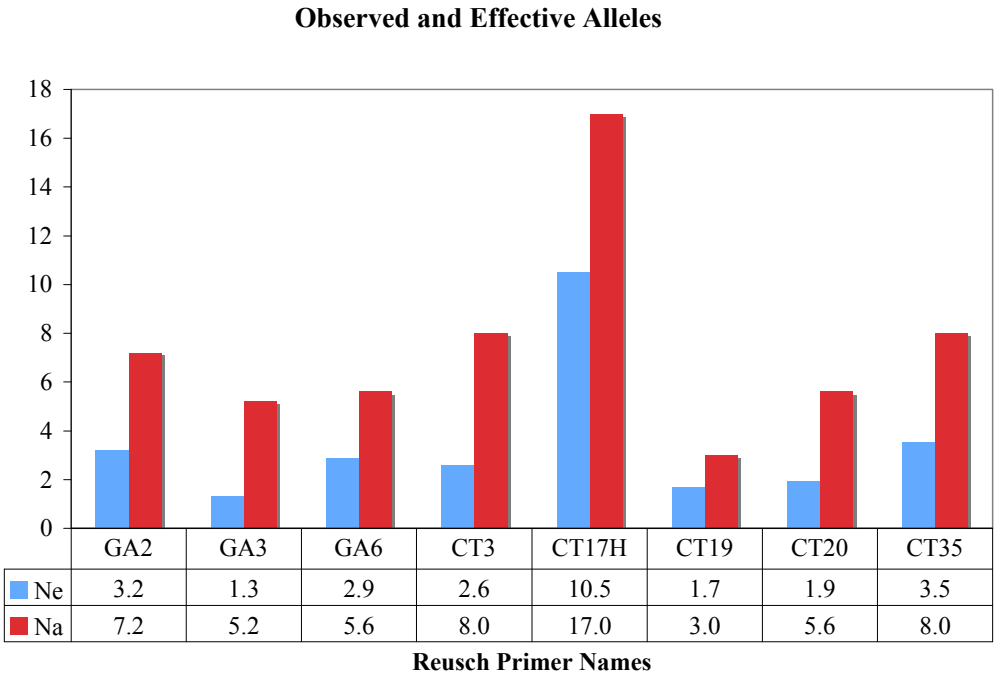


Figure 6. Observed (Na) and effective (Ne) alleles for all loci averaged across populations. Ne is an adjustment of the total number of alleles based on the frequency distribution of the different alleles at a given locus.

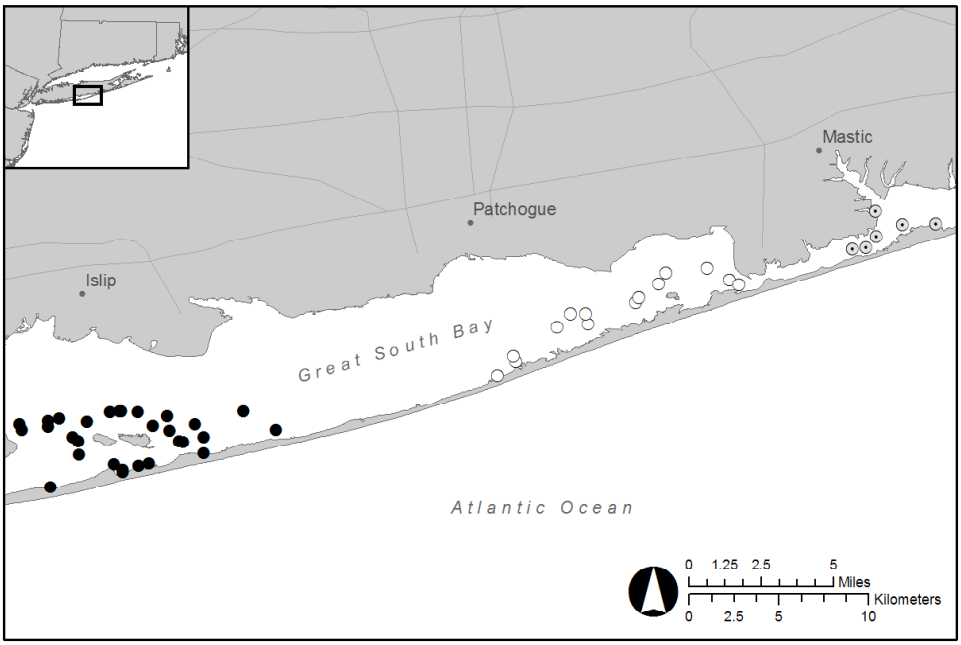


Figure 7. Locations of sampling sites possessing *Z. marina* in Great South Bay showing the geographic distinction between different areas of the bay. Black circles represent the 'western GSB' sites, white circles represent 'central GSB' sites and dotted circles represent 'eastern GSB' sites.

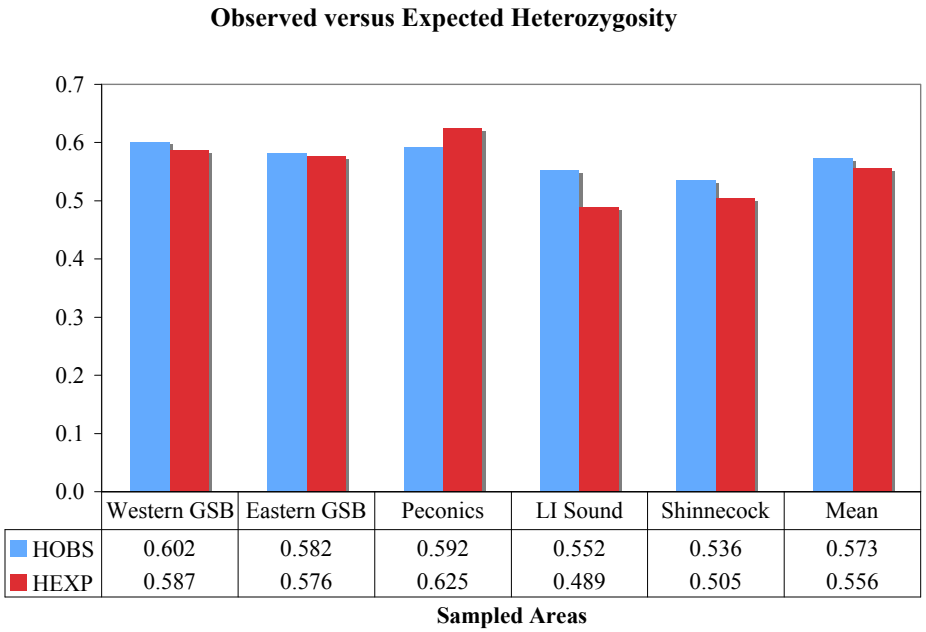


Figure 8. Observed heterozygosity (H_{OBS}) and Hardy-Weinberg expected heterozygosity (H_{EXP}) averaged across all loci for the different sampling areas.

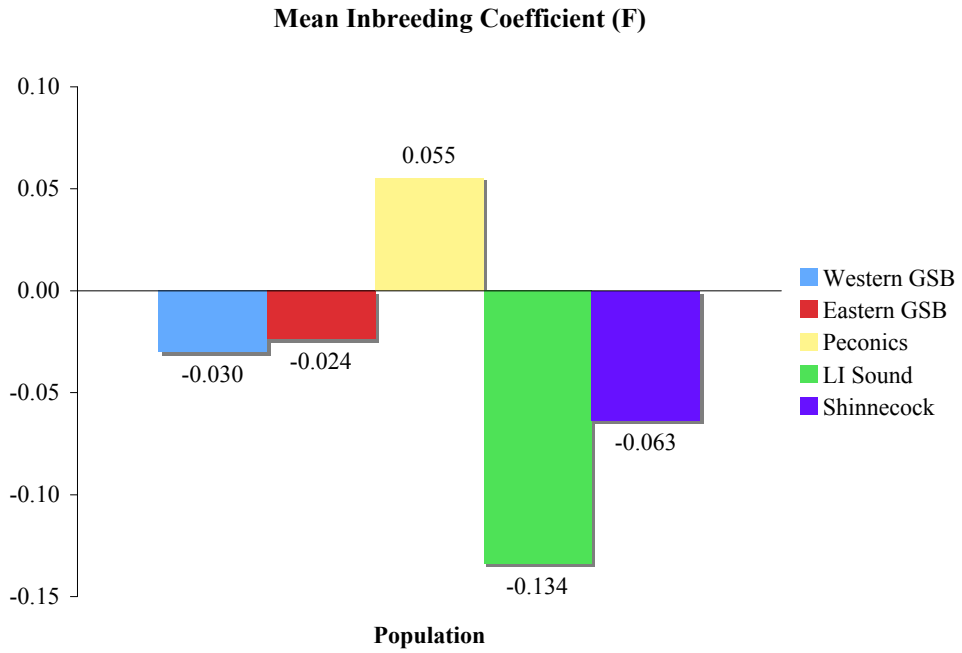


Figure 9. Inbreeding coefficient averaged across all loci by sampling area. This is the probability that any two individuals sharing an allele at a locus inherited that allele from a common ancestor, based on Hardy-Weinberg equilibrium.

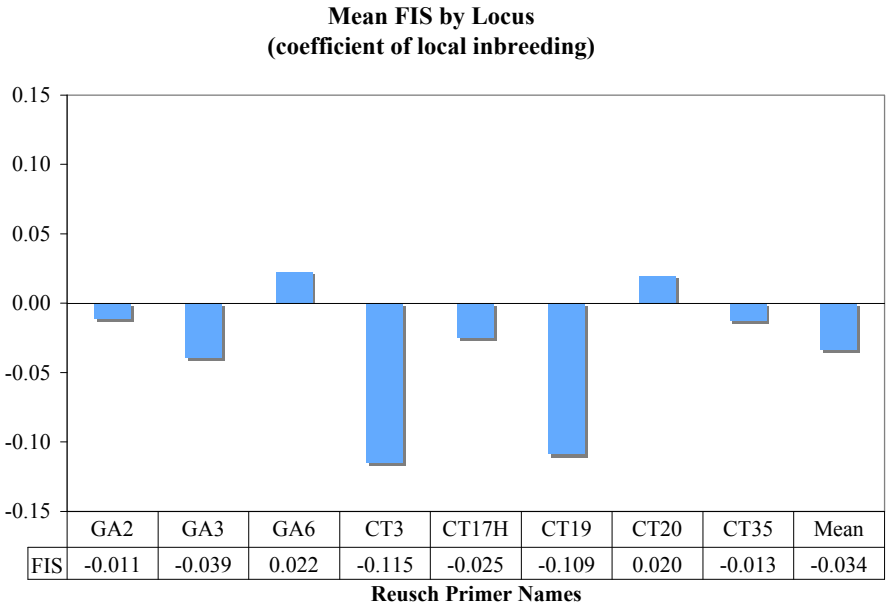
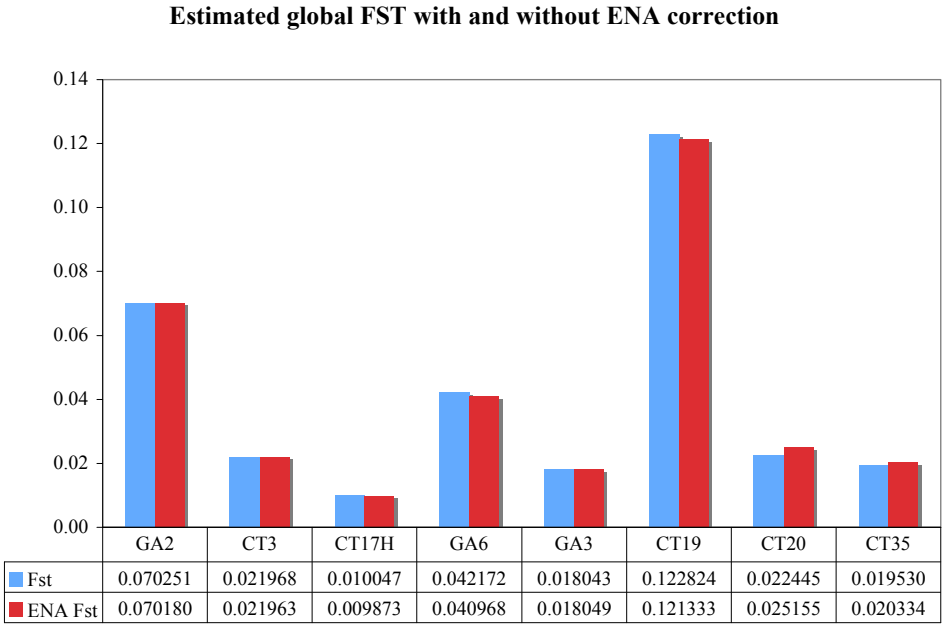


Figure 10. Mean F_{IS} values by loci. This is a proportion from zero to one that reflects the distribution of heterozygosity between individuals and subpopulations. F_{IS} values less than zero are likely not significant.



	GA2	CT3	CT17H	GA6	GA3	CT19	CT20	CT35
Fst	0.070251	0.021968	0.010047	0.042172	0.018043	0.122824	0.022445	0.019530
ENA Fst	0.070180	0.021963	0.009873	0.040968	0.018049	0.121333	0.025155	0.020334

Figure 11. F_{ST} values before and after correcting for estimated null allele frequencies using Chapuis & Estoup's ENA method (2007). This is a proportion between zero and one showing the distribution of heterozygosity between subpopulations and all sampled individuals.

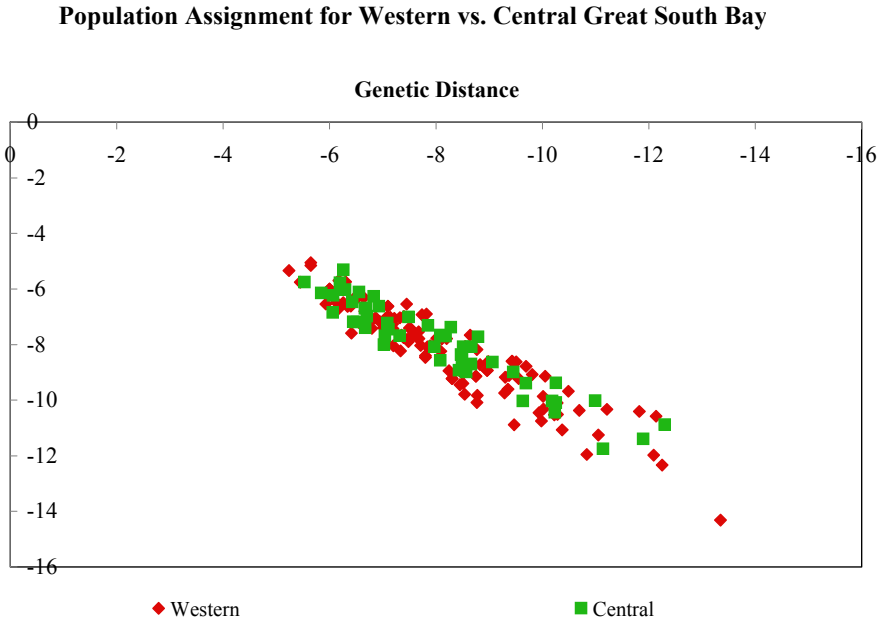


Figure 12. Population assignment scatter plot between Western and Central Great South Bay. Individuals from both areas show no difference in genetic distances from either area.

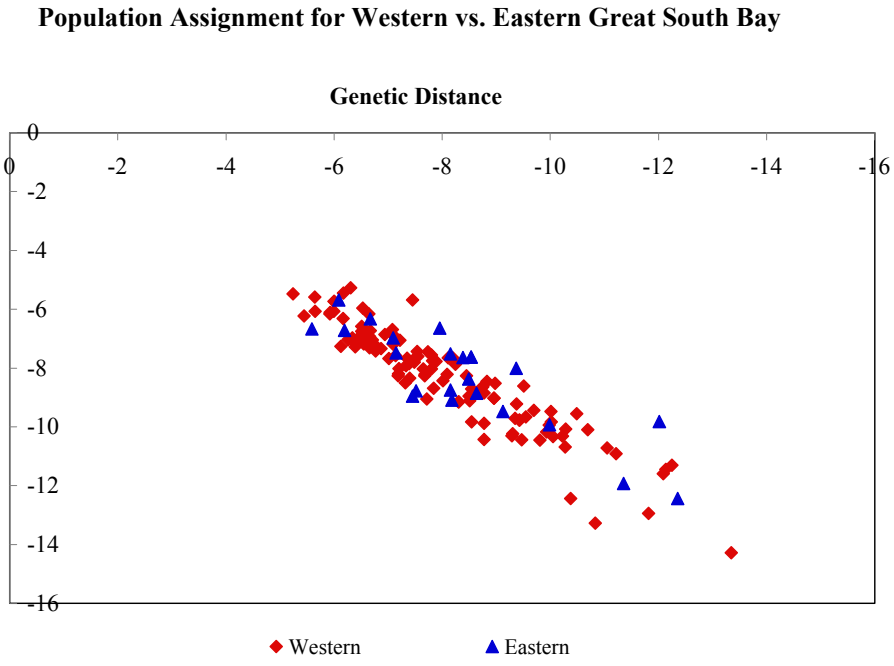


Figure 13. Population assignment scatter plot between Western and Eastern Great South Bay. Individuals from both areas show no difference in genetic distances from either area.

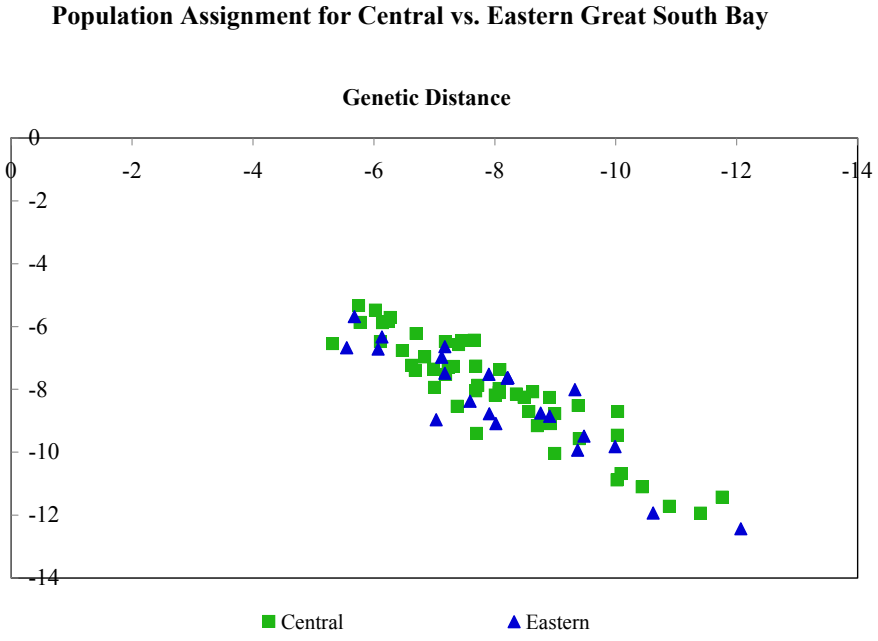


Figure 14. Population assignment scatter plot between Central and Eastern Great South Bay. Individuals from both areas show no difference in genetic distances from either area.

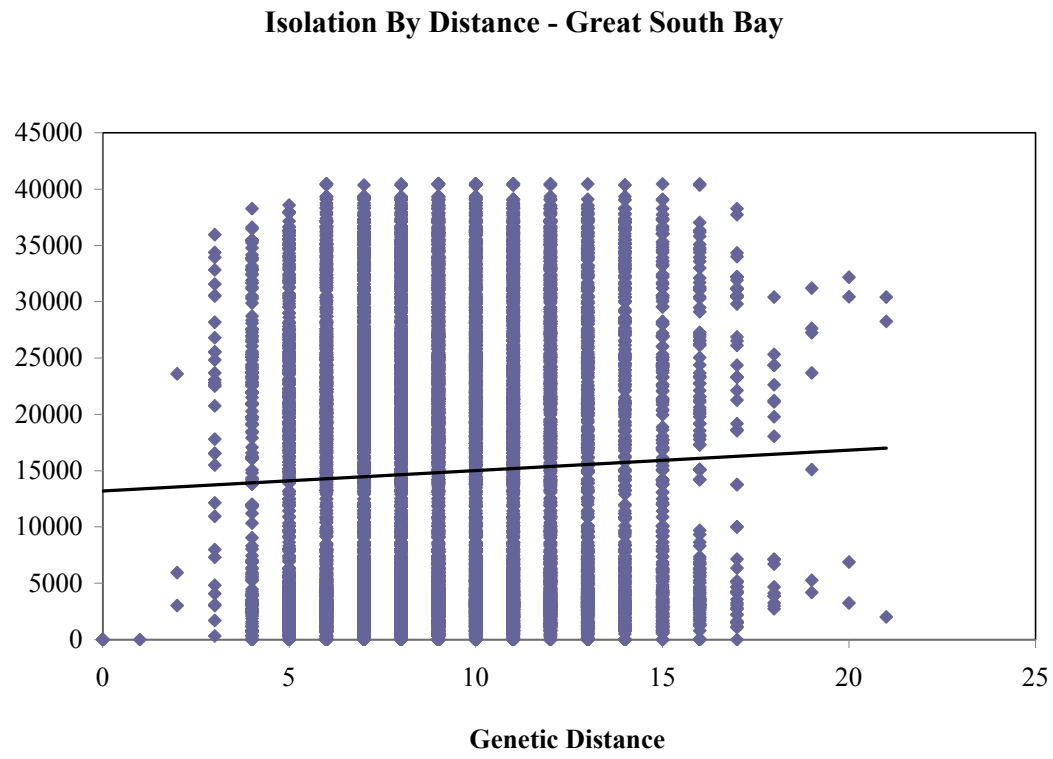


Figure 15. Mantel analysis of genetic isolation by distance of individuals from Great South Bay. There is no significant correlation between geographic and genetic distances of individuals.

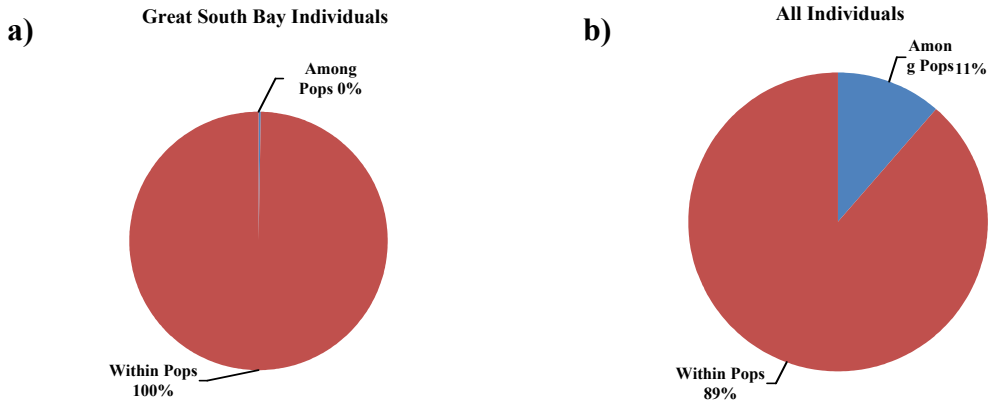


Figure 16. Analyses of molecular variance within Great South Bay (a) and across all sampled areas (b). In Great South Bay, all molecular variance is between individuals, rather than between different areas of the bay. When individuals from Shinnecock Bay, the Peconics and Long Island Sound are introduced into the analysis, 11% of all molecular variance is found between sampled areas rather than between individuals.

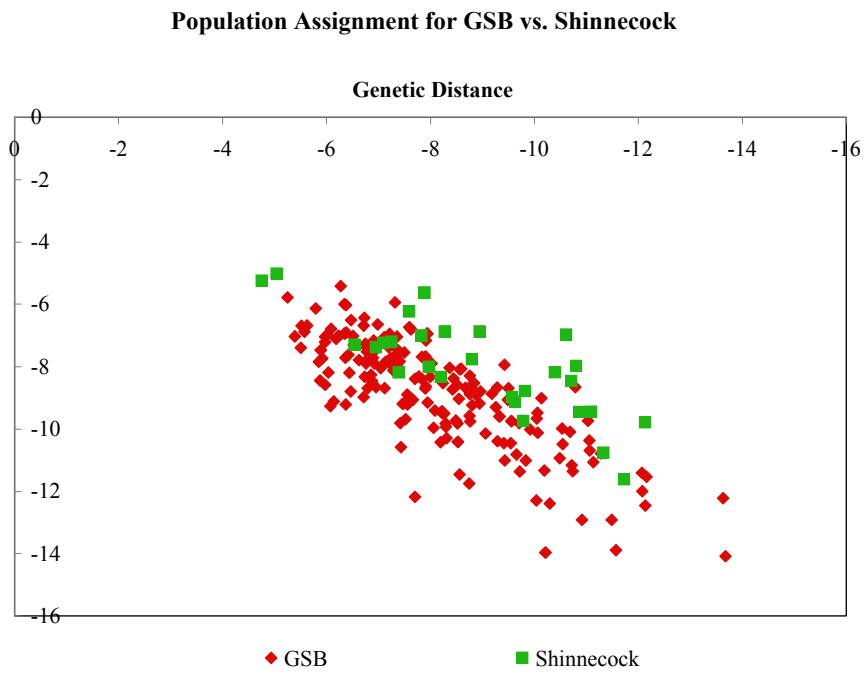


Figure 17. Population assignment scatter plot between all of Great South Bay and Shinnecock Bay. While there is overlap between the two regions individuals identify more closely with other individuals from their respective areas.

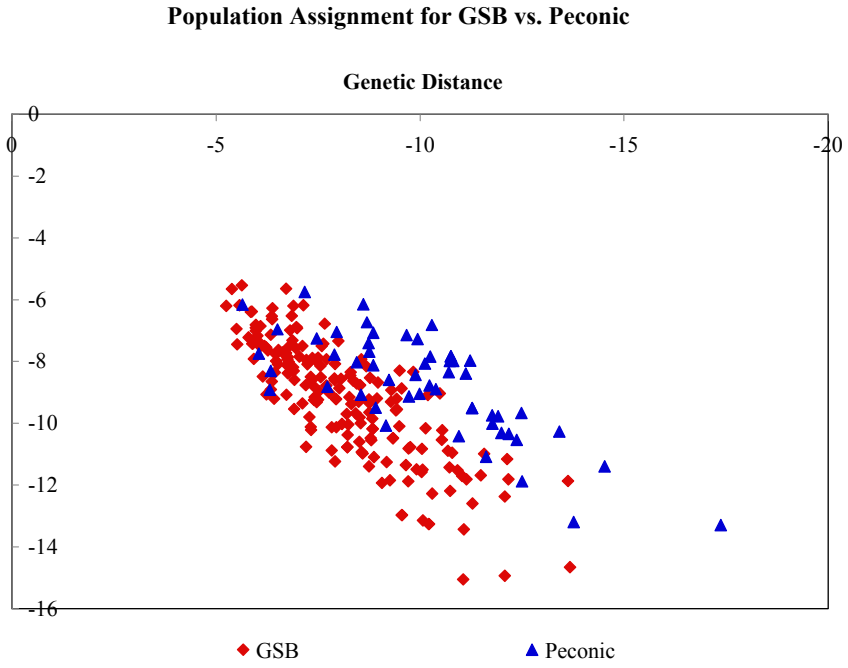


Figure 18. Population assignment scatter plot between Great South Bay and Peconics. While there is overlap between the two regions individuals identify more closely with other individuals from their respective areas.

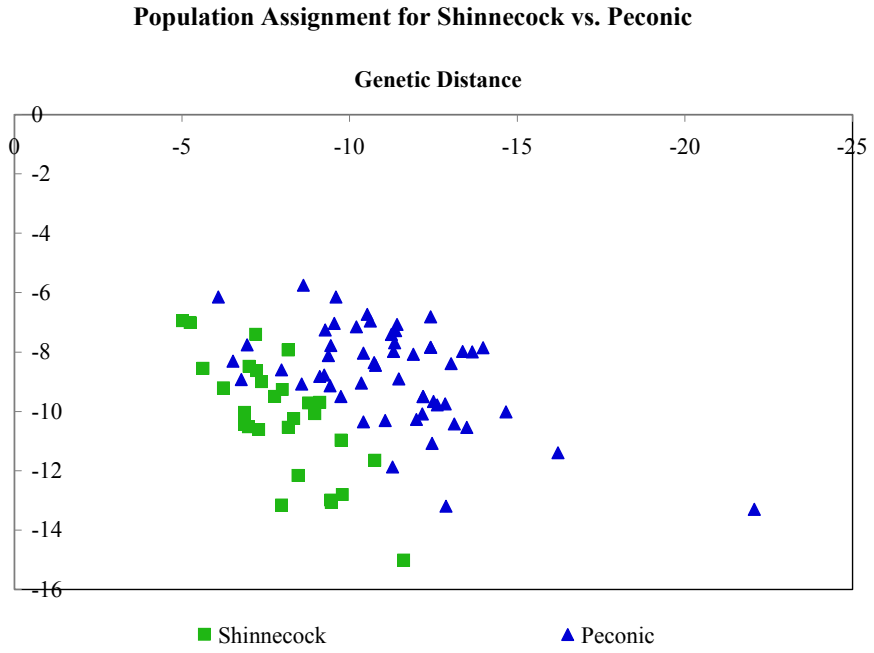


Figure 19. Population assignment scatter plot between Shinnecock and Peconic Bays. While there is overlap between the two regions individuals identify more closely with other individuals from their respective areas.

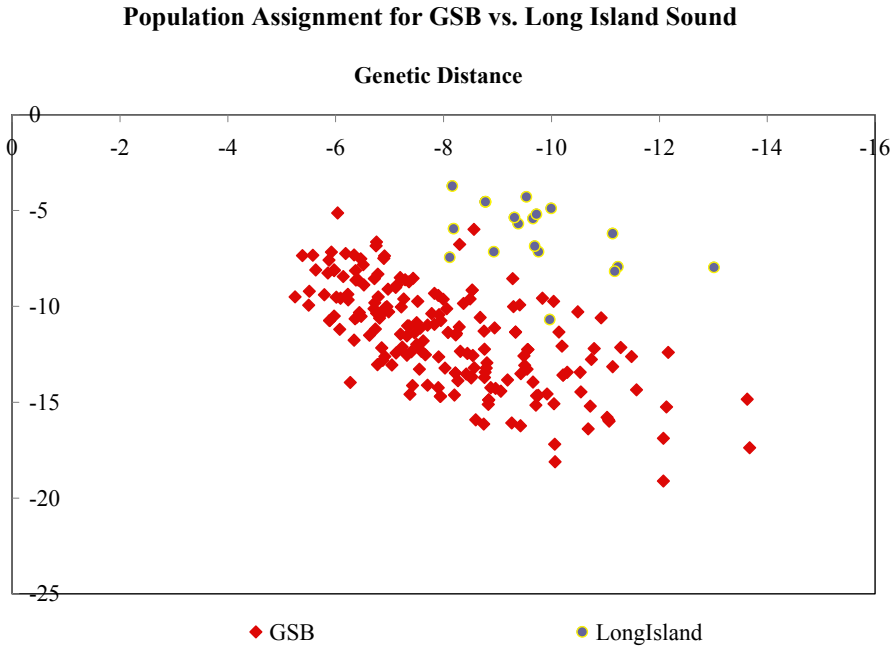


Figure 20. Population assignment scatter plot between Great South Bay and Long Island Sound. Individuals from Long Island Sound show clear distinctions from other areas.

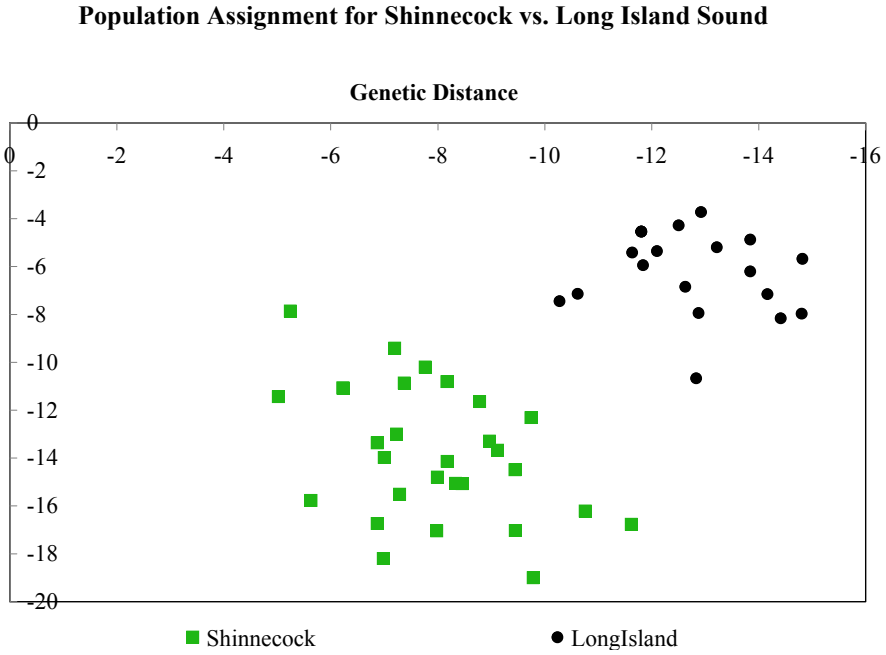


Figure 21. Population Assignment scatter plot between Shinnecock Bay and Long Island Sound. Individuals from Long Island Sound show clear distinctions from other areas.

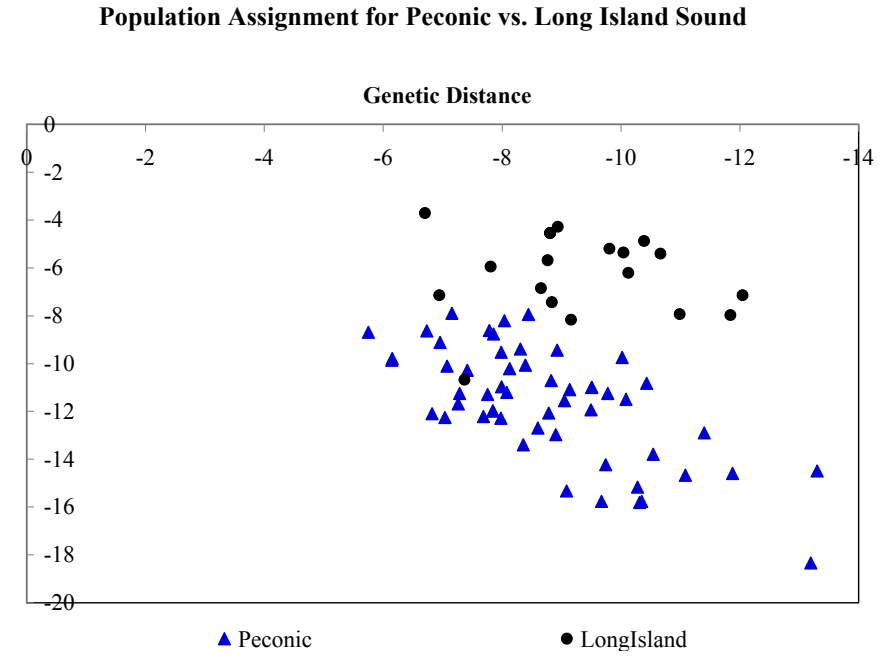


Figure 22. Population assignment scatter plot between Peconic Bay and Long Island Sound. Individuals from Long Island Sound show clear distinctions from other areas.

APPENDIX C - LIST OF SAMPLES

Sample	*Region*	*Latitude*	*Longitude*	*Date Sampled*
1	Great South Bay	40.632400	73.225750	6/10/09
2	Great South Bay	40.632400	73.225750	6/10/09
3	Great South Bay	40.632400	73.225750	6/10/09
4	Great South Bay	40.632400	73.225750	6/10/09
5	Great South Bay	40.641167	73.189550	7/8/09
6	Great South Bay	40.641167	73.189550	7/8/09
7	Great South Bay	40.641167	73.189550	7/8/09
8	Great South Bay	40.641167	73.189550	7/8/09
9	Great South Bay	40.640150	73.189633	7/8/09
10	Great South Bay	40.640150	73.189633	7/8/09
11	Great South Bay	40.640150	73.189633	7/8/09
12	Great South Bay	40.648867	73.211583	6/17/09
13	Great South Bay	40.648867	73.211583	6/17/09
14	Great South Bay	40.648867	73.211583	6/17/09
15	Great South Bay	40.644083	73.194050	7/8/09
16	Great South Bay	40.644083	73.194050	7/8/09
17	Great South Bay	40.644083	73.194050	7/8/09
18	Great South Bay	40.643150	73.181533	7/17/09
19	Great South Bay	40.643150	73.181533	7/17/09
20	Great South Bay	40.643150	73.181533	7/17/09
21	Great South Bay	40.643150	73.181533	7/17/09
22	Great South Bay	40.644683	73.176150	7/17/09
23	Great South Bay	40.644683	73.176150	7/17/09
24	Great South Bay	40.644683	73.176150	7/17/09
25	Great South Bay	40.644683	73.176150	7/17/09
26	Great South Bay	40.649567	73.148883	7/24/09
27	Great South Bay	40.649567	73.148883	7/24/09
28	Great South Bay	40.649567	73.148883	7/24/09
29	Great South Bay	40.655717	73.211733	6/17/09
30	Great South Bay	40.655717	73.211733	6/17/09
31	Great South Bay	40.655717	73.211733	6/17/09
32	Great South Bay	40.655717	73.211733	6/17/09
33	Great South Bay	40.655367	73.159017	7/17/09
34	Great South Bay	40.655367	73.159017	7/17/09
35	Great South Bay	40.655367	73.159017	7/17/09
36	Great South Bay	40.655367	73.159017	7/17/09
37	Great South Bay	40.661100	73.240067	6/10/09
38	Great South Bay	40.661100	73.240067	6/10/09

Sample	*Region*	*Latitude*	*Longitude*	*Date Sampled*
39	Great South Bay	40.661100	73.240067	6/10/09
40	Great South Bay	40.662750	73.227133	6/17/09
41	Great South Bay	40.662750	73.227133	6/17/09
42	Great South Bay	40.662750	73.227133	6/17/09
43	Great South Bay	40.662750	73.227133	6/17/09
44	Great South Bay	40.657700	73.214617	7/8/09
45	Great South Bay	40.657700	73.214617	7/8/09
46	Great South Bay	40.657700	73.214617	7/8/09
47	Great South Bay	40.657700	73.214617	7/8/09
48	Great South Bay	40.660717	73.165867	7/17/09
49	Great South Bay	40.660717	73.165867	7/17/09
50	Great South Bay	40.660717	73.165867	7/17/09
51	Great South Bay	40.660717	73.165867	7/17/09
52	Great South Bay	40.655500	73.161050	7/17/09
53	Great South Bay	40.655500	73.161050	7/17/09
54	Great South Bay	40.655500	73.161050	7/17/09
55	Great South Bay	40.655500	73.161050	7/17/09
56	Great South Bay	40.657517	73.148667	7/24/09
57	Great South Bay	40.657517	73.148667	7/24/09
58	Great South Bay	40.657517	73.148667	7/24/09
59	Great South Bay	40.657517	73.148667	7/24/09
60	Great South Bay	40.661267	73.112733	7/3/09
61	Great South Bay	40.661267	73.112733	7/3/09
62	Great South Bay	40.661267	73.112733	7/3/09
63	Great South Bay	40.663933	73.241300	6/17/09
64	Great South Bay	40.663933	73.241300	6/17/09
65	Great South Bay	40.663933	73.241300	6/17/09
66	Great South Bay	40.663933	73.241300	6/17/09
67	Great South Bay	40.665733	73.227067	6/17/09
68	Great South Bay	40.665733	73.227067	6/17/09
69	Great South Bay	40.665733	73.227067	6/17/09
70	Great South Bay	40.665733	73.227067	6/17/09
71	Great South Bay	40.666967	73.221500	6/17/09
72	Great South Bay	40.666967	73.221500	6/17/09
73	Great South Bay	40.666967	73.221500	6/17/09
74	Great South Bay	40.665117	73.207233	6/17/09
75	Great South Bay	40.665117	73.207233	6/17/09
76	Great South Bay	40.665117	73.207233	6/17/09
77	Great South Bay	40.665117	73.207233	6/17/09
78	Great South Bay	40.669850	73.195917	7/8/09
79	Great South Bay	40.669850	73.195917	7/8/09

Sample	*Region*	*Latitude*	*Longitude*	*Date Sampled*
80	Great South Bay	40.669850	73.195917	7/8/09
81	Great South Bay	40.669850	73.195917	7/8/09
82	Great South Bay	40.670267	73.190233	7/8/09
83	Great South Bay	40.670267	73.190233	7/8/09
84	Great South Bay	40.670267	73.190233	7/8/09
85	Great South Bay	40.670267	73.190233	7/8/09
86	Great South Bay	40.663200	73.174217	7/8/09
87	Great South Bay	40.663200	73.174217	7/8/09
88	Great South Bay	40.663200	73.174217	7/8/09
89	Great South Bay	40.663200	73.174217	7/8/09
90	Great South Bay	40.668183	73.167300	7/8/09
91	Great South Bay	40.668183	73.167300	7/8/09
92	Great South Bay	40.668183	73.167300	7/8/09
93	Great South Bay	40.668183	73.167300	7/8/09
94	Great South Bay	40.664217	73.153167	7/17/09
95	Great South Bay	40.664217	73.153167	7/17/09
96	Great South Bay	40.664217	73.153167	7/17/09
97	Great South Bay	40.670483	73.191183	7/8/09
98	Great South Bay	40.670483	73.191183	7/8/09
99	Great South Bay	40.670483	73.191183	7/8/09
100	Great South Bay	40.670483	73.191183	7/8/09
101	Great South Bay	40.669950	73.181867	7/8/09
102	Great South Bay	40.669950	73.181867	7/8/09
103	Great South Bay	40.669950	73.181867	7/8/09
104	Great South Bay	40.670367	73.129033	7/24/09
105	Great South Bay	40.670367	73.129033	7/24/09
106	Great South Bay	40.670367	73.129033	7/24/09
107	Great South Bay	40.670367	73.129033	7/24/09
108	Great South Bay	40.688367	73.001567	6/25/09
109	Great South Bay	40.688367	73.001567	6/25/09
110	Great South Bay	40.688367	73.001567	6/25/09
111	Great South Bay	40.688367	73.001567	6/25/09
112	Great South Bay	40.695550	72.992183	6/11/09
113	Great South Bay	40.695550	72.992183	6/11/09
114	Great South Bay	40.695550	72.992183	6/11/09
115	Great South Bay	40.698467	72.993483	6/11/09
116	Great South Bay	40.698467	72.993483	6/11/09
117	Great South Bay	40.698467	72.993483	6/11/09
118	Great South Bay	40.698467	72.993483	6/11/09
119	Great South Bay	40.712950	72.971467	6/16/09
120	Great South Bay	40.712950	72.971467	6/16/09

Sample	*Region*	*Latitude*	*Longitude*	*Date Sampled*
121	Great South Bay	40.712950	72.971467	6/16/09
122	Great South Bay	40.714600	72.956200	6/16/09
123	Great South Bay	40.714600	72.956200	6/16/09
124	Great South Bay	40.714600	72.956200	6/16/09
125	Great South Bay	40.719717	72.964933	6/16/09
126	Great South Bay	40.719717	72.964933	6/16/09
127	Great South Bay	40.719717	72.964933	6/16/09
128	Great South Bay	40.719717	72.964933	6/16/09
129	Great South Bay	40.719717	72.957400	6/16/09
130	Great South Bay	40.719717	72.957400	6/16/09
131	Great South Bay	40.719717	72.957400	6/16/09
132	Great South Bay	40.725467	72.932183	6/23/09
133	Great South Bay	40.725467	72.932183	6/23/09
134	Great South Bay	40.725467	72.932183	6/23/09
135	Great South Bay	40.725467	72.932183	6/23/09
136	Great South Bay	40.727917	72.930367	6/23/09
137	Great South Bay	40.727917	72.930367	6/23/09
138	Great South Bay	40.727917	72.930367	6/23/09
139	Great South Bay	40.734700	72.920667	7/2/09
140	Great South Bay	40.734700	72.920667	7/2/09
141	Great South Bay	40.734700	72.920667	7/2/09
142	Great South Bay	40.734700	72.920667	7/2/09
143	Great South Bay	40.736583	72.885117	7/7/09
144	Great South Bay	40.736583	72.885117	7/7/09
145	Great South Bay	40.736583	72.885117	7/7/09
146	Great South Bay	40.736583	72.885117	7/7/09
147	Great South Bay	40.734333	72.880350	7/7/09
148	Great South Bay	40.734333	72.880350	7/7/09
149	Great South Bay	40.734333	72.880350	7/7/09
150	Great South Bay	40.734333	72.880350	7/7/09
151	Great South Bay	40.740300	72.917033	7/7/09
152	Great South Bay	40.740300	72.917033	7/7/09
153	Great South Bay	40.740300	72.917033	7/7/09
154	Great South Bay	40.740300	72.917033	7/7/09
155	Great South Bay	40.742450	72.896217	7/7/09
156	Great South Bay	40.742450	72.896217	7/7/09
157	Great South Bay	40.742450	72.896217	7/7/09
158	Great South Bay	40.751950	72.822917	7/23/09
159	Great South Bay	40.751950	72.822917	7/23/09
160	Great South Bay	40.751950	72.822917	7/23/09
161	Great South Bay	40.751950	72.822917	7/23/09

Sample	*Region*	*Latitude*	*Longitude*	*Date Sampled*
162	Great South Bay	40.752700	72.816017	7/23/09
163	Great South Bay	40.752700	72.816017	7/23/09
164	Great South Bay	40.752700	72.816017	7/23/09
165	Great South Bay	40.758483	72.811167	7/23/09
166	Great South Bay	40.758483	72.811167	7/23/09
167	Great South Bay	40.758483	72.811167	7/23/09
168	Great South Bay	40.764283	72.797883	7/23/09
169	Great South Bay	40.764283	72.797883	7/23/09
170	Great South Bay	40.764283	72.797883	7/23/09
171	Great South Bay	40.764283	72.797883	7/23/09
172	Great South Bay	40.771383	72.811233	7/23/09
173	Great South Bay	40.771383	72.811233	7/23/09
174	Great South Bay	40.771383	72.811233	7/23/09
175	Great South Bay	40.771383	72.811233	7/23/09
176	Great South Bay	40.764750	72.781150	6/24/09
177	Great South Bay	40.764750	72.781150	6/24/09
178	Great South Bay	40.764750	72.781150	6/24/09
179	Great South Bay	40.764750	72.781150	6/24/09
180	The Peconics	40.898611	70.463889	6/9/08
181	The Peconics	40.898611	70.463889	6/9/08
182	The Peconics	40.898611	70.463889	6/9/08
183	The Peconics	40.898611	70.463889	6/9/08
184	The Peconics	41.075630	72.316110	6/10/08
185	The Peconics	41.075630	72.316110	6/10/08
186	The Peconics	41.075630	72.316110	6/10/08
187	The Peconics	41.075630	72.316110	6/10/08
188	The Peconics	41.040890	72.312230	6/10/08
189	The Peconics	41.040890	72.312230	6/10/08
190	The Peconics	41.040890	72.312230	6/10/08
191	The Peconics	41.040890	72.312230	6/10/08
192	The Peconics	41.012370	72.288320	6/10/08
193	The Peconics	41.012370	72.288320	6/10/08
194	The Peconics	41.012370	72.288320	6/10/08
195	The Peconics	41.012370	72.288320	6/10/08
196	The Peconics	41.081600	72.284320	6/10/08
197	The Peconics	41.081600	72.284320	6/10/08
198	The Peconics	41.081600	72.284320	6/10/08
199	The Peconics	41.081600	72.284320	6/10/08
200	The Peconics	41.017310	72.282160	6/10/08
201	The Peconics	41.017310	72.282160	6/10/08
202	The Peconics	41.017310	72.282160	6/10/08

Sample	*Region*	*Latitude*	*Longitude*	*Date Sampled*
203	The Peconics	41.017310	72.282160	6/10/08
204	The Peconics	41.121060	72.280760	6/10/08
205	The Peconics	41.121060	72.280760	6/10/08
206	The Peconics	41.121060	72.280760	6/10/08
207	The Peconics	41.121060	72.280760	6/10/08
208	The Peconics	41.051210	72.280760	6/10/08
209	The Peconics	41.051210	72.280760	6/10/08
210	The Peconics	41.051210	72.280760	6/10/08
211	The Peconics	41.051210	72.280760	6/10/08
212	The Peconics	41.004620	72.269550	6/10/08
213	The Peconics	41.004620	72.269550	6/10/08
214	The Peconics	41.004620	72.269550	6/10/08
215	The Peconics	41.004620	72.269550	6/10/08
216	The Peconics	41.159380	72.234020	6/10/08
217	The Peconics	41.159380	72.234020	6/10/08
218	The Peconics	41.159380	72.234020	6/10/08
219	The Peconics	41.159380	72.234020	6/10/08
220	The Peconics	41.046910	72.231880	6/10/08
221	The Peconics	41.046910	72.231880	6/10/08
222	The Peconics	41.046910	72.231880	6/10/08
223	The Peconics	41.046910	72.231880	6/10/08
224	The Peconics	41.107267	72.328900	6/13/08
225	The Peconics	41.107267	72.328900	6/13/08
226	The Peconics	41.107267	72.328900	6/13/08
227	The Peconics	41.107267	72.328900	6/13/08
228	The Peconics	41.048450	72.166980	6/10/08
229	The Peconics	41.048450	72.166980	6/10/08
230	The Peconics	41.048450	72.166980	6/10/08
231	The Peconics	41.048450	72.166980	6/10/08
232	The Peconics	41.126025	72.282203	6/11/08
233	The Peconics	41.126025	72.282203	6/11/08
234	The Peconics	41.126025	72.282203	6/11/08
235	The Peconics	41.126025	72.282203	6/11/08
236	The Peconics	41.186000	72.167550	6/13/08
237	The Peconics	41.186000	72.167550	6/13/08
238	The Peconics	41.186000	72.167550	6/13/08
239	The Peconics	41.186000	72.167550	6/13/08
240	The Peconics	41.159028	72.279039	6/16/08
241	The Peconics	41.159028	72.279039	6/16/08
242	The Peconics	41.159028	72.279039	6/16/08
243	The Peconics	41.159028	72.279039	6/16/08

Sample	*Region*	*Latitude*	*Longitude*	*Date Sampled*
244	Long Island Sound	41.249967	72.033050	6/13/08
245	Long Island Sound	41.249967	72.033050	6/13/08
246	Long Island Sound	41.249967	72.033050	6/13/08
247	Long Island Sound	41.249967	72.033050	6/13/08
248	Long Island Sound	41.282933	72.014650	6/13/08
249	Long Island Sound	41.282933	72.014650	6/13/08
250	Long Island Sound	41.282933	72.014650	6/13/08
251	Long Island Sound	41.282933	72.014650	6/13/08
252	Long Island Sound	41.274933	71.949650	6/13/08
253	Long Island Sound	41.274933	71.949650	6/13/08
254	Long Island Sound	41.274933	71.949650	6/13/08
255	Long Island Sound	41.274933	71.949650	6/13/08
256	Shinnecock Bay	40.868650	72.490783	7/31/09
257	Shinnecock Bay	40.868683	72.490833	7/31/09
258	Shinnecock Bay	40.868700	72.490833	7/31/09
259	Shinnecock Bay	40.868717	72.490833	7/31/09
260	Shinnecock Bay	40.868733	72.490850	7/31/09
261	Shinnecock Bay	40.868817	72.490867	7/31/09
262	Shinnecock Bay	40.868850	72.490867	7/31/09
263	Shinnecock Bay	40.868917	72.490867	7/31/09
264	Shinnecock Bay	40.868967	72.490850	7/31/09
265	Shinnecock Bay	40.869000	72.490850	7/31/09
266	Shinnecock Bay	40.869033	72.490850	7/31/09
267	Shinnecock Bay	40.869083	72.490833	7/31/09
268	Shinnecock Bay	40.869133	72.490833	7/31/09
269	Shinnecock Bay	40.869200	72.490833	7/31/09
270	Shinnecock Bay	40.869267	72.490817	7/31/09
271	Shinnecock Bay	40.868617	72.490467	7/31/09
272	Shinnecock Bay	40.868633	72.490467	7/31/09
273	Shinnecock Bay	40.868650	72.490467	7/31/09
274	Shinnecock Bay	40.868683	72.490483	7/31/09
275	Shinnecock Bay	40.868733	72.490483	7/31/09
276	Shinnecock Bay	40.868783	72.490483	7/31/09
277	Shinnecock Bay	40.868817	72.490500	7/31/09
278	Shinnecock Bay	40.868867	72.490483	7/31/09
279	Shinnecock Bay	40.868933	72.490483	7/31/09
280	Shinnecock Bay	40.868967	72.490500	7/31/09
281	Shinnecock Bay	40.869067	72.490500	7/31/09
282	Shinnecock Bay	40.869133	72.490483	7/31/09
283	Shinnecock Bay	40.869200	72.490450	7/31/09
284	Shinnecock Bay	40.869267	72.490433	7/31/09

Sample	*Region*	*Latitude*	*Longitude*	*Date Sampled*
285	Shinnecock Bay	40.869333	72.490400	7/31/09