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Improvement of genetic health and diversity of *Zostera marina* (eelgrass) in Barnegat Bay, New Jersey ten years after Hurricane Sandy: Support for the "storm stimulus" hypothesis

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ABSTRACT

Hurricane Sandy struck the New York metropolitan region on October 29, 2012. The storm severely impacted the physical state of Barnegat Bay, New Jersey with its heavy storm surge, affecting many forms of benthic life and ripping up extensive beds of *Zostera marina*. Pre-Sandy studies of the genetic status of *Z. marina* in Barnegat Bay indicated low levels of heterozygosity and high levels of inbreeding. This present study examines the long-term effects of Hurricane Sandy on the eelgrass meadows of New Jersey. Heterozygosity analysis (mean Ho= 0.482 ± 0.013 and mean He= 0.498± 0.009) of the five Barnegat populations studied suggest an improvement in diversity from pre-Sandy values of 2008. Mean inbreeding levels (overall Fis = 0.077 ± 0.034) also indicated reduced inbreeding, and the fixation index (overall mean pairwise Fst = 0.064 ±0.006) suggested increased connectivity between populations with low levels of differentiation. Although we found no indication of bottlenecks in the last 2–3 years, by employing m-ratio calculations, there was strong evidence for long-term, historical bottlenecks in all populations, potentially due to the mass wasting disease epidemic in the 1930s. Unexpectantly, the post-Sandy genetic health and diversity of *Z. marina* in Barnegat Bay appears to have improved since it was last surveyed in 2008, supporting the "Storm Stimulus" hypothesis.

1. Introduction

Severe environmental disasters can have serious long-term effects on populations of biological organisms. Plants, being sessile and unable to flee severe weather events, are particularly prone to habitat fragmentation, which can lead to isolation and reduction in population size (Booy et al., 2000; Lienert, 2004). Of course, the loss of reproducing individuals directly results in genetic bottlenecks and deficits in diverse heritable material. Terrestrial plants suffer from fragmentation events, but these occurrences can happen in aquatic environments as well and often lead to isolated sub-populations that are prone to loss (Olivieri, 2000; Kritzer and Sale, 2006).

Seagrasses are particularly disposed to fragmentation and reduced populations (Bell, 2006), because most species lack the ability to disperse seeds over long distances. A majority of seeds, when dispersed, fall through the water column and end up lodging in the soil beneath the plants, creating seed banks (Orth et al., 1994, 2007; Bell, 2006). It is thought that the spread of most isolated seagrass plants occurs through asexual means, i.e. rhizome elongation and dispersal of vegetative fragments, which is advantageous during the growing season when meadows need to expand (Setchell, 1929; Bell, 2006). As long as environmental conditions remain stable with little change in biotic and abiotic factors, grass beds will thrive and continue to expand successfully by vegetative propagation (Reusch et al., 1999a). However, if conditions become challenging for survival (e.g., hurricanes, climate change, wasting disease, etc.) and these asexual processes become the primary means of reproduction, they may cause issues for long-term sustained survival. Loss of population genetic diversity leads to reduced heterozygosity, inbreeding depression, and diminished overall survival.

Damage to seagrass beds along marine coasts is a particularly problematic ecological challenge because these beds provide important ecosystem services that include: support of fisheries, food and shelter for thousands of species, a buffer against ocean acidification by carbon

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sequestration, coastal protection against soil erosion, and reduction of pathogenic marine organisms (Nordlund et al., 2018). This is even more problematic as seagrass beds and many marine coastal habitats have shown severe global declines (Dunic et al., 2021; Turschwell et al., 2021), resulting in loss of those ecosystem services, although rapid recovery is possible through active restoration (Orth et al., 2020).

Barnegat Bay is the only remaining region in New Jersey with substantial *Zostera marina* populations. This system is classified as highly eutrophic (Kennish et al., 2007) and has shown both rapid, short-term losses due to nutrient driven macroalgal blooms (Bologna et al., 2001), as well as long-term declines related to water quality and development (Lathrop et al., 2001; Kennish et al., 2010; Fertig et al., 2013). While active restoration of eelgrass in New Jersey has been successful (Bologna and Sinnema, 2006; Bologna and Sinnema, 2012), depleted genetic diversity among the existing natural populations remains (Campanella et al., 2010b). This critical habitat continues to be subjected to nutrient loading impacts (Tweitmann and Dietl, 2018) and broad declines in fish community structure; both linked to the reduction in seagrass cover (Olson and Vasslides, 2022). Consequently, continued efforts to protect and restore *Z. marina* beds are critical to the long-term survival of this valuable habitat under emerging climate change scenarios (Scalpone et al., 2020).

Hurricane Sandy is one example of a climate change induced environmental event that caused severe ecological harm to *Z. marina* beds in New Jersey, USA. Sandy originated on the west coast of Africa on October 11, 2012 and made its first landfall on October 24, 2012 in Jamaica. The storm intensified as it passed over the warm waters of the Gulf Stream. Storm surges occurred across the entire east coast of the United States, with the highest surges occurring in New York and New Jersey. On October 29, 2012, Hurricane Sandy struck Barnegat Bay, New Jersey. Powerful waves damaged the shore and tides reached 2.66 m above normal levels at the northern end of Sandy Hook Peninsula, New Jersey before the National Ocean Service tide gauge failed and ceased recording, providing indirect evidence that the storm surge may have gone higher (Blake et al., 2013). Some barrier islands of Barnegat Bay and Little Egg Harbor were entirely swamped by the surge and island breaches occurred. Hurricane Sandy caused $50 billion in damages and 147 lives were lost in the United States (Blake et al., 2013). The bottom of Barnegat Bay was scoured by the storm surges and ultra-high tides. This storm had devastating impacts on existing seagrass beds, causing $>$ 70 % declines in both spatial cover and shoot density to grass beds centrally located in the southern part of Barnegat Bay from 2012 to 2013 (Parsons Brinckerhoff, 2013) and disrupting both natural and restored eelgrass beds, causing between 27–69 % loss in spatial cover for robust natural regions between 2012 and 2013 and 13–20 % loss in restored eelgrass sites evaluated in 2010 and 2013 (Bologna et al., 2014; Bologna unpubl. data). While these losses were substantial, they did not completely remove all vegetation from the sediments, leading to potential for increased seed production and germination in the newly generated unvegetated regions.

Eelgrass is an annual or perennial monoecious flowering plant which reproduces underwater with submerged flowers, pollen, and seeds (Ackerman, 2006). Annual populations often occur in shallow intertidal regions (Olesen and Sand-Jensen, 1994) or in areas near their thermal limit (Blok et al., 2018), while perennial populations are common in subtidal regions, including Barnegat Bay. Additionally, mixed annual beds have been described (van Lent et al., 1995; Jarvis et al., 2012) and are important to life history strategies with this species.

Zostera marina has the ability to cross-pollinate with other individuals, but is also capable of self-fertilization due to its self-compatibility (Reusch, 2000). While annual populations flower and sexually reproduce in their first year, seedlings from perennial beds do not flower their first year, leading to a lag in sexual reproduction and potential bed collapse during multi-year stressors (Johnson et al., 2021). Additionally, for established perennial beds, the likely success of seedlings is minimal due to light and space competition from established plants (Johnson et al., 2020); therefore these beds often show clonal growth as the dominant force in replacement of senescent shoots. We estimate, based on *Z. marina's* perennial reproductive capacity and the environment of Barnegat Bay, that maximally seven (7) generations could have been produced from 2008 to 2021; however, the limitations in seedling survival under competition with adult plants make that estimate a generous one.

Our objective in this new study was to determine the present genetic health and diversity of the *Z. marina* populations of Barnegat Bay a decade after Hurricane Sandy decimated the grass beds of the region. Seagrass distribution, coverage, and health have been monitored for over twenty years in Barnegat Bay. Prior to the storm, the Barnegat Bay *Z. marina* populations were in poor genetic health (Campanella et al., 2010b) with low diversity, low connectivity, and high inbreeding. Our initial hypothesis on *Z. marina's* current genetic state was that we would discover even more reduced diversity and connectivity with greater inbreeding than we had observed in our initial 2008 survey (Campanella et al., 2010b). We were surprised to find that although Barnegat Bay grass beds are still problematic and fragmented, they are far more diverse and genetically "healthy" than they were upon our first examination.

Kendall et al. (2004) suggested that massive hurricanes every decade or so are required to induce "Storm Stimulus", leading to pollination and seed dispersal of seagrasses in the Caribbean. We now hypothesize that this "Stimulus" effect can be observed post-Hurricane Sandy in Barnegat Bay and is supported by our recent *Z. marina* population data.

2. Materials and methods

2.1. Sample collection

Zostera marina plants were collected individually at five sites in Barnegat Bay, New Jersey from May to October of 2021. These sites (Fig. 1) included Barnegat Bay Inlet (39.786188 N, −74.148798 W), Oyster Creek (39.804487 N, −74.171949 W), Route 72 South (39.660157 N, −74204361 W), Connective Sedge (39.563911 N, −74.291749 W) and Ham Island (39.600247 N, −74.229049 W). Barnegat Bay Inlet, Connective Sedge, and Ham Island were chosen based on their involvement in long-term ecological monitoring; Route 72 S. was of interest because of its geographic location beneath the Manahawkin Bay Bridge between Long Beach Island and the mainland. Oyster Creek was chosen based on the proximity of the meadow to the outfall of The Oyster Creek Nuclear Generating Station which had been generating thermal stresses in Barnegat Bay for over 50 years. Outgroup *Z. marina* samples were collected in 2018 from Potter Pond, Rhode Island, USA (41.389283 N, −71.531916 W). All populations studied were natural, unrestored meadows.

To ensure that we were not gathering clonal samples, individuals were collected approximately 5 m apart within the beds, using the same technique as in Campanella et al. (2010a, 2010b). Because clonal collection was still possible using this technique, clonality was assessed in the statistical analyses of all populations. Tissue samples were transported to Montclair State University on ice, separated, numbered, and either stored immediately at − 80 °C or dried and then stored at − 20 °C.

2.2. DNA extraction

Total DNA was extracted from *Z. marina* using ~100 mg of leaf tissue from each plant using the Qiagen DNeasy Mericon Food Kit (Qiagen Sciences, Germantown, MD, USA) following the alternate protocol of Abdel-Latif and Osman (2017). DNA was extracted from individuals in each of the six populations studied (population range N = 30–31, Total Plants N = 181).

A Nanodrop ND-1000 UV Spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA) was used to determine DNA concentration

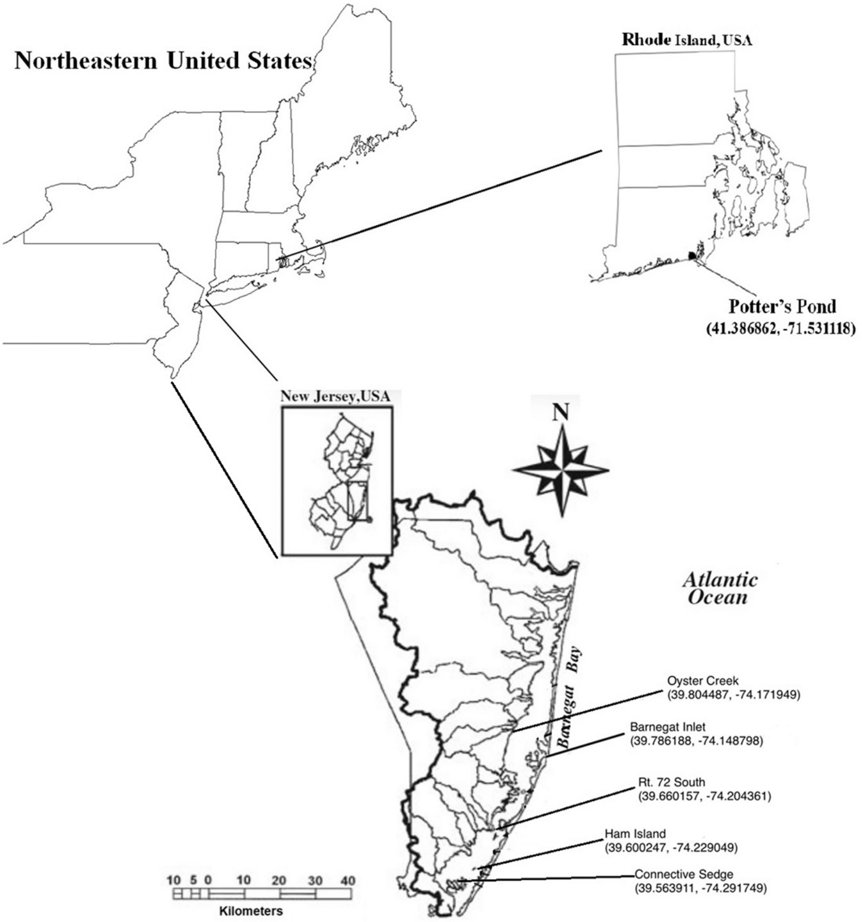

Fig. 1. The geographic collection sites for the *Z. marina* populations studied in New Jersey.

and purity. The genomic DNA extracts were stored at − 20 °C until PCR was performed.

2.3. PCR amplification

We employed thirteen primer sets to amplify thirteen polymorphic microsatellite alleles from the extracted *Z. marina* DNA (Table 1) (as in Campanella et al., 2010a, 2010b). Nine primers (ZmarGA1, ZmarGA2, ZmarGA3, ZmarGA4, ZmarGA6, ZmarCT3, ZmarCT12, ZmarCT17, and ZmarCT19) were developed by Reusch et al. (1999b) and Reusch (1999). Four primers (ZmarGA9, ZmarAC8, ZmarAG8, and ZmarAGC5) were developed by Oetjen et al. (2010). All our primer sets were labelled with either fluorescent FAM or HEX dyes (Table 1).

PCR amplification was carried out in RNase/DNase free 200 ul tubes with 9.5 ul sterile deionized water, 1 ul forward primer (~1.4 ug/ul), 1 ul reverse primer (~1.4 ug/ul), 12.5 ul DreamTaq PCR Mastermix (ThermoFisher Scientific, Waltham, MA, USA), and 1 ul total DNA (~4–100 ug/ul). The PCR reactions were amplified on an Eppendorf Mastercycler EP Gradient S (Eppendorf, Hamburg, Germany) with a 95 °C, 3 min activation step followed by 35 cycles: 30 s at 95 °C, 30 s at varying annealing temperatures depending on the primer set employed (Table 1), and 60 s at 72 °C. The program ended with a 10 min 72 °C extension step. The PCR products were stored at − 20 °C until fragment analysis.

Table 1
PCR primers used to amplify *Z. marina* microsatellite alleles.

Primer	Forward Sequence	Reverse Sequence	Annealing Temp. (°C)	Label	Citation
*Zmar*GA1	5'-TAGTGGTGGTTGTTGGAGTGC-3'	5'-[5-HEX]GCCTCTTCTTCAGACTTCCC-3'	57	HEX	Reusch et al. (1999b)
*Zmar*GA2	5'-AACAGGCAACAGCACAACTG-3'	5'-[6-FAM]ACGTCACATCTTTTCACGACC-3'	55	FAM	Reusch et al. (1999b)
*Zmar*GA3	5'-GTGACGGATTGATCGGAATC-3'	5'-[6-FAM]ACGTCACATCTTTTCACGACC-3'	55	FAM	Reusch et al. (1999b)
*Zmar*GA4	5'-GCGTGGATTCTGGTTTTCG-3'	5'-[5-HEX]GCATATCTCTTCTTTTGCCC-3'	55	HEX	Reusch et al. (1999b)
*Zmar*GA6	5'-AGAAACCCTAATGTGATGAAATG-3'	5'-[5-HEX]TGTTTGGTCTCTTCTAATCTT-3'	55	HEX	Reusch et al. (1999b)
*Zmar*GA9	5'-[6-FAM]GGAATCGTCAAGCAAAAAC-3'	5'-GAACGTTTCCCGGTCATTT-3'	55	FAM	Oetjen et al. (2010)
*Zmar*CT3	5'-AACAGCGACGAAGGATTTTG-3'	5'-[5-HEX]AGACCCGAAAGATACCACCG-3'	57	HEX	Reusch et al. (1999b)
*Zmar*CT12	5'-CGTTCATCTTGTCCTCGTCC-3'	5'-[6-FAM]TTTCATTCCATTTCCCACC-3'	57	FAM	Reusch (1999)
*Zmar*CT17	5'-TCTTTACCAACCGATCTCCG-3'	5'-[6-FAM]AAACACAACAGTTAGTCAG-3'	57	FAM	Reusch (1999)
*Zmar*CT19	5'-CCCAAGAAATATAAAATCGGGG-3'	5'-[6-FAM]CTTCTCCTTCCGCCGCTAC-3'	57	FAM	Reusch (1999)
*Zmar*AC8	5'-[6-FAM]AAACGAATCCTGGTTCCAT-3'	5'-TGCGAGCAGCTAACTAAGTCC-3'	56	FAM	Oetjen et al. (2010)
*Zmar*AG8	5'-GGGGAGGTTTCCGAATACTTT-3'	5'-[5-HEX]TGGAAGATGTTGGACATGGA-3'	55	HEX	Oetjen et al. (2010)
*Zmar*AGC5	5'-GTGGAGGAAAGTGTGGGTGT-3'	5'-[5-HEX]CTTGCATCCAACCTCATTTG-3'	57	HEX	Oetjen et al. (2010)

2.4. Fragment analysis

The allele size of microsatellite loci was determined using an Applied Biosystems 3130 Genetic.

Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) (as in Campanella et al., 2010a, 2010b). Samples were prepared in 8.5 ul of formamide (Fisher, BioReagents, Waltham, MA, USA), 0.25 ul of molecular weight standard ROX 500 (Applied Biosystems, Waltham, MA, USA), and 0.5 ul of PCR product sample. The reaction mixtures were incubated 5 min at 95 ℃, followed by ice for 5 min. Samples were analyzed using POP7 polymer (Applied Biosystems, Waltham, MA, USA) with the D-filter. GeneMarker v1.51 software (SoftGenetics Corp., State College, PA, USA) was used to analyze raw data to determine microsatellite allele sizes and score for hetero/homogeneity.

2.5. Population genetic analysis

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

Observed (Ho) and expected (He) heterozygosities were calculated with GENALEX6 under the codominant marker settings (Peakall and Smouse, 2006). Nei's identity matrix values (Nei and Feldman, 1972) (bootstrapped 1000 times) and a Principal Coordinate Analysis (PCoA) (Gower, 1966) were also determined by GENALEX6. The PCoA allows a multi-coordinate genetic comparison of all individuals in all populations. For the PCoA, program parameters were set to employ a triangular distance matrix and included data labels.

Allelic data was analyzed by Microsat 2.0 (Minch et al., 1995) to generate genetic distance matrices. PHYLP's subroutines Neighbor and Consense (Felsenstein, 1989) were employed to analyze the Microsat genetic distance matrices and generate the final trees using Majority Consensus (Wilkinson, 1996). Three types of distance calculations were employed in matrices: Nei's (Nei, 1972), Chord (Cavalli-Sforza and Edwards, 1967), and Delta Mu distances (DiRienzo et al., 1994; Luikart and Cornuet, 1998). Neighbor-joining cladograms were generated using Treeview 1.6 (Page, 2003). All cladograms were rooted using the Potter Pond *Z. marina* population as the geographic outgroup and bootstrapped 1000 times. These distance calculations were chosen because the Delta Mu and Chord distances generally show higher probabilities of obtaining correct tree topology than other distance measurements (Takezaki and Nei, 1996).

Tests for recent bottlenecks were performed using the program BOTTLENECK (Piry et al., 1999; Cristescu et al., 2010). The Wilcoxon sign-rank tests were conducted for heterozygosity excess (Cornuet and Luikart, 1996) in all populations of *Z. marina*. The Wilcoxon Sign-Rank test is considered a useful bottleneck test because it is robust when used with less than 20 polymorphic loci, but it is only accurate when employed within a couple of generations of a bottleneck; the test allows for the reliable detection of small bottlenecks occurring in natural populations over 2–3 generations.

Long term, historical bottlenecks were detected by the M-ratio (Garza and Williamson, 2001), which was calculated by dividing the observed number of microsatellite alleles by the range of allele sizes ($\text{M-Ratio} = \text{Na}/\text{allele}^{\text{max}} — \text{allele}^{\text{min}}$). The M-ratio defines the proportion between the total number of alleles of a loci divided by the difference between the largest and smallest alleles. Garza and Williamson (2001) found that the value of the M-ratio decreases when a population is reduced in size due to a bottleneck, and the extent of the ratio decrease is directly associated with the severity and duration in the reduction in population size. We calculated the mean M-ratio for each allele and population. Using the program "Critical_M" (Garza and Williamson, 2001), we calculated a threshold for the Critical Value of M (Mc). The Mc is the "cut-off" which indicates whether the calculated M-ratio implies a history of bottlenecks.

The coefficient of local inbreeding (Fis) (Wright, 1950) was determined with Genepop (Yeh et al., 1997). The pairwise Fst (the Fixation Index) (Wright, 1950), and Nei's identity matrix (Nei, 1972) were generated by GENALEX6. The Fis is the probability that alleles at any loci in an individual are identical by descent from the common ancestors. Negative Fis values (−1 to 0) are considered to indicate no inbreeding, while positive Fis values (0−1) suggest mates who are more closely related on average than expected. The Fixation index (Fst) indicates connectivity and differentiation, determined by a measure of the degree of gene separation between populations based on allele frequencies. Values for Fst range from 0 (no genetic divergence) to 1 (complete genetic divergence). Nei's genetic identity is commonly used to assess the level of genetic diversity within a population and to estimate the gene flow occurring between different populations.

We performed an analysis using FreeNA (Chapuis and Estoup, 2007) to determine the impact of null alleles on our data. We directed FreeNA to employ 1000 bootstrap iterations to perform its calculations. Additionally, an estimate was calculated of Weir's (1996) global Fst as well as an Fst value using the excluding null alleles (ENAs) correction described by Chapuis and Estoup (2007).

3. Results

3.1. Diversity, inbreeding, identity, and differentiation/connectivity

181 ramets were sampled from the restored Barnegat Bay populations and analyzed with thirteen microsatellite loci revealing a total of 180 genets using the Microsatellite Toolkit to determine clonality (Park, 2001). Most of the 2021 populations studied had clonal diversity values of 1.0 (*C*). Only Ham Island had a *C*= 0.96.

The total number of alleles per locus ranged from 1 to 16 (Table 2). Across all populations, the CT17 locus had the largest mean number of alleles (14.0 ±0.8, calculated from data in Table 2), while the AG8 locus had the smallest mean number of alleles (1.8 ±0.2). The 2021 Barnegat populations did not differ much for the mean number of alleles observed (Table 2). The numbers of alleles observed were fairly consistent across the board ranging from a mean of 5.615 in Barnegat Inlet to 5.308 in Oyster Creek.

There were twenty-two private alleles spread among the five studied populations; Barnegat Inlet plants possessed the majority with a total of six private alleles (Data not shown).

The overall mean expected number of heterozygotes (He) was higher for all loci than the observed mean number of heterozygotes (Ho) for Barnegat Inlet, Connective Sedge, and Route 72 South (Table 2), indicating a deficit of heterozygotes (Table 2). However, Oyster Creek (mean Ho = 0.514, He = 0.488) and Ham Island (mean Ho = 0.505, He = 0.475) plants had mean Ho values greater than mean He.

We calculated the coefficient of local inbreeding (Fis) (Nei, 1977) in order to further examine the genetic health of the Barnegat populations (Table 2). All Barnegat populations tested, except Ham Island (Fis = −0.026), had mean positive Fis values. These positive values would suggest continued inbreeding; however, Oyster Creek (mean Fis = 0.044) and Connective Sedge (mean Fis = 0.064) have mean values which are close to zero, hence close to Hardy-Weinberg Equilibrium (HWE). Although it appears that Ham Island is the only outbreeding *Z. marina* population, all the 2021 populations have a lower mean Fis value (overall mean Fis = 0.077 ±0.034) (Table 2) compared to those observed in the 2008 populations (overall mean Fis = 0.646±0.010) (Campanella et al., 2010b).

The Pairwise Fixation Index (Fst) was also calculated to examine the overall genetic differentiation and connectivity among the populations studied (Nei, 1977) (Table 3 A). The mean pairwise Fst values for the Barnegat Bay populations range from 0.051 (Oyster Creek) to 0.086 (Rt. 72 S.) (Table 3 A). The Oyster Creek population is the least

Table 2
Within-population genetic diversity in all of the populations of *Z. marina* examined in this study.

Population	Locus	N	Na	Ho	He	Fis	Population	Locus	N	Na	Ho	He	Fis	Population	Locus	N	Na	Ho	He	Fis
Barnegat Inlet							**Oyster Creek**							**Ham Island**						
	GA1	30	12	0.733	0.745	0.032		**GA1**	31	12	0.677	0.684	0.025		**GA1**	30	9	0.7	0.701	0.018
	GA2	30	7	0.733	0.733	0.016		**GA2**	31	7	0.839	0.777	-0.062		**GA2**	30	6	0.667	0.713	0.081
	GA3	30	6	0.233	0.272	0.157		**GA3**	31	6	0.355	0.34	-0.028		**GA3**	30	7	0.433	0.422	-0.009
	GA4	30	3	0.167	0.452	0.641		**GA4**	31	2	0	0.312	1		**GA4**	30	3	0.067	0.065	-0.008
	GA6	30	6	0.633	0.591	-0.054		**GA6**	31	6	0.645	0.605	-0.05		**GA6**	30	6	0.733	0.624	-0.158
	CT19	30	5	0.6	0.618	0.045		**CT19**	31	4	0.645	0.526	-0.21		**CT19**	30	4	0.467	0.463	0.008
	AC8	30	4	0.767	0.497	-0.531		**AC8**	31	3	0.903	0.509	-0.766		**AC8**	30	2	0.933	0.498	-0.871
	CT12	30	4	0.433	0.477	0.107		**CT12**	31	4	0.613	0.53	-0.14		**CT12**	30	3	0.367	0.443	0.188
	AGC5	30	2	0	0.064	1		**AGC5**	31	2	0	0.121	1		**AGC5**	30	4	0.133	0.347	0.625
	CT17	30	13	0.967	0.869	-0.095		**CT17**	31	13	0.871	0.895	0.043		**CT17**	30	16	0.9	0.889	0.004
	CT3	30	6	0.267	0.683	0.62		**CT3**	31	7	0.419	0.582	0.294		**CT3**	30	7	0.367	0.44	0.183
	AG8	30	2	0.033	0.033	0		**AG8**	31	1	0	0	0		**AG8**	30	2	0.033	0.033	0
	GA9	30	3	0.267	0.235	-0.118		**GA9**	31	2	0.71	0.458	-0.538		**GA9**	30	3	0.767	0.543	-0.398
Mean		**30**	**5.615**	**0.449**	**0.482**	**0.14**	**Mean**		**31**	**5.308**	**0.514**	**0.488**	**0.044**	**Mean**		**30**	**5.538**	**0.505**	**0.475**	**-0.026**
S.E.		**0**	**0.959**	**0.086**	**0.073**	**0.11**	**S.E.**		**0**	**1.048**	**0.092**	**0.069**	**0.139**	**S.E.**		**0**	**1.061**	**0.085**	**0.067**	**0.095**
Population	**Locus**	**N**	**Na**	**Ho**	**He**	**Fis**	**Population**	**Locus**	**N**	**Na**	**Ho**	**He**	**Fis**	**Population**	**Locus**	**N**	**Na**	**Ho**	**He**	**Fis**
S. Rt. 72							**Connective Sedge**							**Potter Pond**						
	GA1	30	12	0.933	0.799	-0.151		**GA1**	30	9	0.733	0.698	-0.034		**GA1**	30	6	0.5	0.537	0.085
	GA2	30	7	0.733	0.76	0.052		**GA2**	30	8	0.667	0.752	0.129		**GA2**	30	8	0.7	0.781	0.12
	GA3	30	4	0.267	0.466	0.441		**GA3**	30	6	0.5	0.464	-0.059		**GA3**	30	8	0.467	0.688	0.336
	GA4	30	3	0	0.184	1		**GA4**	30	2	0	0.5	1		**GA4**	30	3	0.067	0.065	-0.008
	GA6	30	6	0.567	0.617	0.097		**GA6**	30	7	0.467	0.503	0.088		**GA6**	30	4	0.533	0.537	0.024
	CT19	30	6	0.667	0.593	-0.106		**CT19**	30	4	0.667	0.586	-0.12		**CT19**	30	4	0.467	0.542	0.155
	AC8	30	4	0.933	0.527	-0.763		**AC8**	30	2	0.867	0.491	-0.757		**AC8**	30	3	0.567	0.452	-0.238
	CT12	30	3	0.367	0.389	0.075		**CT12**	30	3	0.5	0.464	-0.059		**CT12**	30	3	0.067	0.065	-0.008
	AGC5	30	2	0	0.42	1		**AGC5**	30	1	0	0	0		**AGC5**	30	2	0	0.18	1
	CT17	30	12	0.867	0.874	0.025		**CT17**	30	16	0.9	0.909	0.027		**CT17**	30	16	0.8	0.884	0.111
	CT3	30	8	0.5	0.696	0.297		**CT3**	30	8	0.233	0.508	0.552		**CT3**	30	7	0.6	0.665	0.114
	AG8	30	2	0.033	0.033	0		**AG8**	30	2	0.033	0.033	0		**AG8**	30	3	0.433	0.383	-0.115
	GA9	30	2	0.5	0.455	-0.082		**GA9**	30	2	0.367	0.299	-0.208		**GA9**	30	3	0.733	0.476	-0.528
Mean		**30**	**5.462**	**0.490**	**0.524**	**0.164**	**Mean**		**30**	**5.385**	**0.456**	**0.477**	**0.064**	**Mean**		**30**	**5.385**	**0.456**	**0.481**	**0.131**
S.E.		**0**	**0.973**	**0.094**	**0.067**	**0.135**	**S.E.**		**0**	**1.176**	**0.087**	**0.071**	**0.115**	**S.E.**		**0**	**1.055**	**0.072**	**0.071**	**0.086**

N = Plants in population, Na=Total number of alleles, Ho=observed heterozygosity, He=expected heterozygosity, Fis=Coefficient of Inbreeding.

Table 3A
Pairwise Fst Matrix.

	Barnegat Inlet	Oyster Creek	Ham Island	Connective Sedge	Rt. 72 South	Potter Pond
Barnegat Inlet	0.000					
Oyster Creek	0.031	0.000				
Ham Island	0.056	0.021	0.000			
Connective Sedge	0.068	0.054	0.064	0.000		
Rt. 72 South	0.103	0.096	0.099	0.045	0.000	
Potter Pond	0.114	0.110	0.124	0.110	0.122	0.000

differentiated population with a mean Fst value closest to zero. Fst values can range from “0” to “1”, where “0” means complete sharing of genetic material and “1” means that populations do not share any heredity. For populations of plants which belong to the same species, values of Fst greater than 15 % (0.15) are considered to have significant differentiation (Frankham et al., 2002). The mean pairwise Fst for all the Barnegat populations is 0.064 ± 0.006, which suggests high connectivity and low differentiation. The Potter Pond outgroup has the highest level of differentiation from Barnegat Bay and the lowest connectivity with a mean pairwise Fst of 0.116 ± 0.003.

A Nei identity matrix (Nei and Feldman, 1972) was generated for the populations to further examine differentiation (Table 3B). The mean identity among the Barnegat populations ranges from 0.817 to 0.876.

To ensure that our scoring of heterozygotes was accurate, we tested for allelic dropout. Allelic dropout occurs when microsatellites are amplified by PCR and one or both of the allelic copies fail to amplify. The average null allele frequency for all loci and populations was estimated to be 0.044 with mean values ranging between 0.000 (for GA1) and 0.169 (for GA4). We found that the mean global Fst including the null alleles (0.139) differed little from the mean global Fst with the ENA correction (0.132). This result suggests that null alleles are having little effect on our overall analysis.

3.2. *Genetic distance and microsatellite evolutionary models*

We performed genetic distance analyses on all five Barnegat populations and generated rooted, neighbor-joining trees (Fig. 2). The Nei and Chord neighbor-joining trees (Fig. 2A, B) seem to have a similar topology where Ham Island, Barnegat Inlet, and Oyster Creek branch together in one clade while Rt. 72 South and Connective Sedge group in another; this result should be noted since the Ham Island plants are situated geographically between those locations, but appear genetically closer to the more northerly populations.

The Chord tree (Fig. 2B) seems to be the most “accurate” model given the high bootstrap values, while the least accurate distance model is the Delta Mu tree, which not only has a different topology from the other two trees, but also has the lowest bootstrap values (Fig. 2C). The Chord-distance model is mathematically-based, while the other two trees are based on the Infinite Allele (Nei distance) and the Stepwise Mutation Models (Delta Mu distance) of microsatellite evolution.

Table 3B
Nei’s identity matrix.

	Barnegat Inlet	Oyster Creek	Ham Island	Connective Sedge	Rt. 72 South	Potter Pond
Barnegat Inlet	1.000					
Oyster Creek	0.928	1.000				
Ham Island	0.876	0.961	1.000			
Connective Sedge	0.827	0.876	0.871	1.000		
Rt. 72 South	0.770	0.789	0.781	0.928	1.000	
Potter Pond	0.753	0.758	0.758	0.764	0.736	1.000

3.3. *Principal coordinate analysis*

We performed a principal coordinate analysis (PCoA) to determine the genetic relationships between individuals in the various populations (Fig. 3), employing the Potter Pond population as an outgroup. The Potter Pond population distributed individuals into the two right quadrants with no drift at all into the left quadrants. A few Barnegat Bay individuals, however, can be found with a more similar genetic background to Potter Pond in the right quadrants; the majority of these plants appear to be from the Barnegat Inlet population.

3.4. *Recent and historical bottlenecks*

There appears to be no evidence of recent bottlenecks indicated by excess heterozygosity (Table 4) employing the Wilcoxon sign-rank test for heterozygosity excess (Cornuet and Luikart, 1996).

The M-ratio statistic is more utile when detecting both recent and historical bottlenecks. The M-ratio decreases in bottlenecked populations when alleles are randomly lost as a result of genetic drift or population loss over multiple generations. The mean M-ratio among the Barnegat Bay 2021 populations is 0.45 ± 0.06, and all mean M-ratio values are less than the calculated Mc threshold (Fig. 4).

4. Discussion

4.1. *Examination of the “Storm Stimulus” hypothesis*

It is generally believed that environmental catastrophes bring about serious ecological consequences (Ehrlich, 1988; Barbier et al., 2019; Peduzzi, 2019; Walz et al., 2021). This conclusion is sensible in an era when anthropogenic disasters have become so prevalent in engendering the losses of ecosystems and their services. Further, natural disasters (fire, flood, cold, etc.) are considered as destructive to genetic diversity and species survival as any environmental calamities produced by humans. However, do natural disasters inevitably lead to loss of diversity and populations or is survival dependent upon the species and environmental circumstances in which the organisms find themselves?

Annual fires have been shown to stimulate growth in a number of plant species by providing char and smoke that promote seed germination (Nelson et al., 2012). Beaudet et al. (2007) reported that ice storms in northern maple forests led to an increase of light flux levels at almost all understory locations, allowing a high proportion of advanced regeneration. Some plants may even benefit from flooding and water stress; sugarcane, for example, has been reported to benefit from short flooding periods in the form of increased sugar yields (Glaz and Gilbert, 2006; Ray et al., 2009).

Given these examples of “beneficial” natural disasters, it is not hard to hypothesize that seagrasses may benefit from hurricanes by the culling of weakened plants from the soil, the thinning of grass beds to allow new seeds to germinate, and the general increase in light flux that will allow the surviving plants to regenerate to greater heights. Kendall et al. (2004) proposed that Caribbean hurricanes are required to induce “Storm Stimulus”, leading to pollination and seed dispersal of seagrasses in the Caribbean. O’Brien et al. (2018) concurred, finding that seagrass ecosystems are resilient to disturbances that remove or damage biomass, and that this disruption can induce rapid regrowth and expansion after the perturbation subsides. These disturbances can even expose niche regions for invasive species after culling of the primary native species, as was observed with the seagrass *Halophila stipulacea* (Hernández-Delgado et al., 2020), which is now abundant throughout the Caribbean.

In this present *Z. marina* study, we have found further support for the “Storm Stimulus” hypothesis in the aftermath of Hurricane Sandy.

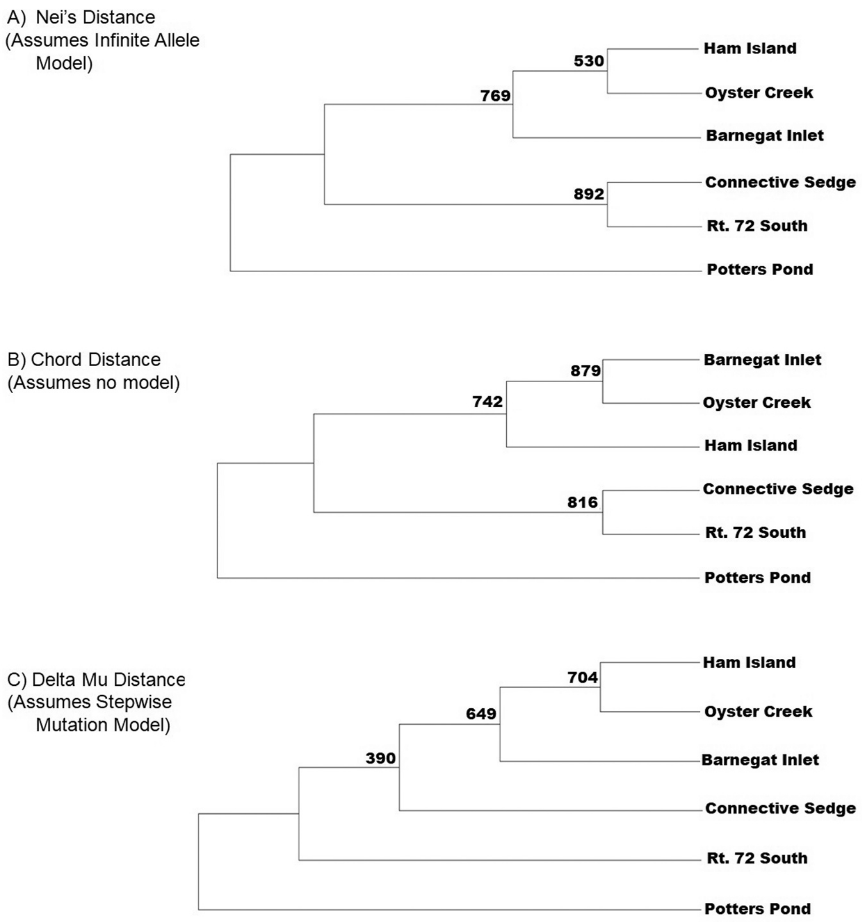

Fig. 2. Neighbor-joining cladograms between the Barnegat Bay *Z. marina* populations with Potter Pond, RI as the geographic outgroup. A) Nei's distance, B) Chord distance, and C) Delta Mu distance. Genetic distances were calculated using Microsat 2.0 with 1000 bootstrap iterations. Final graphic trees were generated with Treeview.

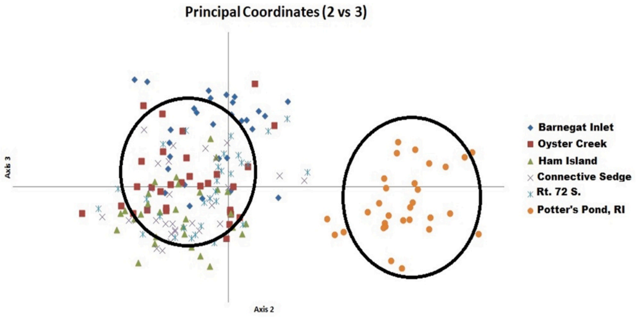

Fig. 3. Principal Coordinate Analysis among 181 *Z. marina* individuals from all the populations examined. A Nei genetic distance matrix was calculated from microsatellite data of thirteen (13) polymorphic loci using GENALEX6, followed by the PCoA analysis. The circles indicate the majority of clustered Barnegat Bay individuals versus the outgroup plants from Potter Pond. Each axis has an Eigen value whose magnitude indicates the amount of variation captured in that axis. The orthogonal axes summarize the variability in the data set and have no units.

Table 4
Recent bottlenecks detected by heterozygosity excess.

		IAM Model			SMM Model		
Population	N	Het_{ex}	Het_{obs}	Prob. Hexcess	Het_{ex}	Het_{obs}	Prob. Hexcess
Barnegat Inlet	30	7.30	1	0.999	7.53	0	1.000
Oyster Creek	31	6.64	6	0.935	6.72	2	0.998
Ham Island	30	7.45	3	0.998	7.70	1	0.999
Connective Sedge	30	6.57	4	0.978	6.78	2	0.999
Rt. 72. South	30	7.20	3	0.965	7.30	2	0.999
Potter Pond, RI	30	7.10	3	0.998	7.54	0	1.000

Almost all genetic factors that we examined suggest that Hurricane Sandy was beneficial to seagrass growth in Barnegat Bay.

All 2021 populations had clonal diversity values much higher than those observed in 2008 (Campanella et al., 2010b) (Table 5). The most striking contrast can be seen in the Barnegat Inlet population, which had a *C*= 0.70 in 2008 (Campanella et al., 2010b), but the same population in 2021 revealed a *C*= 1.0. This population was monitored in 2013 and it showed a 69 % reduction in spatial coverage compared to pre-Hurricane Sandy sampling (Bologna et al., 2014; Bologna unpubl. data), thereby opening the region to recolonization of the site by sexually generated seedlings. This result strongly supports lower levels of asexual reproduction, since clonal diversity is considered such a strong indicator of population diversity (*sec.* Arnaud-Haond et al., 2020).

The observed heterozygosity (Ho) for all 2021 populations was either close to HWE with a slight deficit (Barnegat Inlet, Connective Sedge, Route 72 S.) or had a slight surplus of heterozygosity compared to He (Ham Island, Oyster Creek) (Table 2). Severe deficits were observed in heterozygosity of the 2008 Barnegat Bay populations, which had overall means of Ho = 0.27 ± 0.01 and He = 0.78 ± 0.01 (Campanella et al., 2010b) (Table 5). It appears that this improvement in the

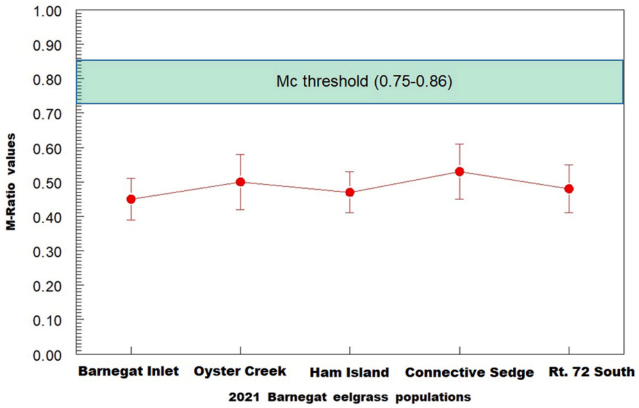

Fig. 4. Bottleneck analysis of Barnegat Bay populations using M-Ratio. The M-ratio was calculated by dividing the observed number of microsatellite alleles by the range of allele sizes. The critical M threshold (Mc) was calculated employing the “Critical_M” program (Garza and Williamson, 2001). The band on the graph indicates Mc threshold calculated between q= 0.01 and q= 10. “q” represents the pre-bottleneck Ne values (250 and 250,000 in this case with 10, 000 simulations calculated using Critical_M).

Table 5
Mean statistics for the Barnegat Bay populations before and after Hurricane Sandy.

	2008	2021
Ho	0.270 ± 0.010	0.482± 0.013
He	0.780 ± 0.010	0.498 ± 0.009
Fis	0.646 ± 0.010	0.077 ± 0.034
Pairwise Fst	0.120 ± 0.010	0.064 ± 0.006
Clonal Diversity	0.831 ±0.032	0.990 ± 0.010

Ho= observed heterozygosity, He=expected heterozygosity, Fis= Coefficient of Inbreeding
2008 data from Campanella et al. (2010b).

diversity has occurred over the last decade since Hurricane Sandy.

All the Barnegat Bay populations, except Ham Island, showed evidence of inbreeding. However, all the inbreeding values calculated were much closer to HWE (overall mean Fis for all 2021 populations = 0.077 ±0.030) (Table 2), than those found in 2008 (overall mean Fis = 0.646 ±0.010) (Campanella et al., 2010b) (Table 5). As suggested by the population studies of Arnaud-Haond et al. (2020), this post-Sandy shift supports increased outbreeding and diversity in all 2021 populations tested.

Our pairwise Fst analysis (Table 3 B) supports this result as well because compared to the older 2008 populations (mean overall 2008 Fst = 0.120 ±0.010), connectivity was increased in the 2021 plants, while differentiation between the populations was reduced (mean overall 2021 Fst = 0.060 ±0.006) (Table 5).

At the same time, additional evidence for increased connectivity can be observed in our Nei Identity Matrix (Table 3 B) where the overall mean identity among the Barnegat populations is 0.841 ± 0.010. The matrix values for Barnegat ranged from 0.817 to 0.876, supporting a strong genetic identity in *Z. marina* there, along with low levels of differentiation. The reduced mean identity for the Rhode Island outgroup population (0.753) suggests the Barnegat Bay populations have not been isolated for a long evolutionary time period and still retain a great deal of commonality. The Pairwise Fst values (Table 3 A) support this result with evidence of reduced connectivity for the Rhode Island population.

Another aspect of the shared genetic heritage among Barnegat Bay plants can be seen in the results of the PCoA study (Fig. 3). This relationship is indicated by the circle around the majority of Barnegat individuals (Fig. 3). The PCoA data suggest that the Barnegat populations examined either a) share genetic backgrounds from pre-Sandy or b) remain connected through interbreeding. Certain individuals are outliers from the core group, but in general, most are genetically similar.

We found it notable that Ham Island seemed to clade more closely with Oyster Creek or Barnegat Inlet than it does with either geographically closer population (Fig. 2A, B). Oyster Creek and Barnegat Inlet already appear to be genetically related due to their northern geographic proximity (Fig. 1). Ham Island may be genetically linked to these populations because currents around the island result in a generally northward particle movement, which could be a source of genetic transfer between these regions (Defne and Ganju, 2015). This northward bulk flow could explain the connectivity of Ham Island by the movement of either floating reproductive shoots or shoots with flowers dispersing seed (Harwell and Orth, 2002). Unfortunately, neither of these phenomena can explain why there seems to be a lower rate of connection with the Rt. 72 population which lies between these areas. However, a more likely pathway could exist as a result of Hurricane Sandy, where storm water entered Barnegat Inlet tearing plant material, sediments, and potential seed banks from these populations. This storm surge was then redirected north and south of the inlet, flushing large quantities of material into adjacent landscapes (Blake et al., 2013). As water flushed southward, a channel constriction at the Rt. 72 site would have accelerated flow, transporting material further south toward Ham Island under this extreme event. In this region, *Z. marina* spatial coverage was reduced by 78 % and shoot density decreased by 75 %, including complete loss of *Z. marina* from 57 % of the 235 sampled stations (Parsons Brinckerhoff, 2013), with substantial erosion taking place within the constricted water way (Bologna et al., 2014). This provides a proximal mechanism connecting Ham Island with the more northerly populations of Barnegat Inlet and Oyster Creek through this major stochastic event. The additional recruitment of plant material from around the bay could therefore account for Ham Island’s lack of inbreeding (Table 2) and relatively low fixation value (Table 3 A).

The Oyster Creek population is also notable because of its unusual “natural” history. This population has been under selective environmental conditions for decades (Campanella et al., 2010b), growing near the hot water outflow of Oyster Creek Nuclear Generating Station (OCNGS) since 1969. Qin et al. (2020) documented differences in *Z. marina* flowering under marine heat waves leading to lower flower and seed production in response to heat. However, Johnson et al. (2021) suggest that an acute excessive heat stress led to reduced survival of *Z. marina* plants, with recovery dependent upon seedling recruitment as opposed to clonal regrowth. While marine heat wave events have been investigated, major chronic thermal stresses have not really been assessed due to the rarity of the situation, but this population could provide a foundation for genetic selection related to climate change. Consequently, the Oyster Creek genetic make-up may reflect this long-term stress through repeated deaths and recovery from seeds, akin to the marine heat wave response observed in the Chesapeake Bay (Johnson et al., 2021), or it may reflect longer-term directional selection forces leading to heat tolerance. The OCNGS was shut down on September 17, 2018 and is being decommissioned. The elimination of this chronic heat stress may have allowed a greater number of seed haplotypes from sexual reproduction to germinate, grow, and expand in that region. These events, along with the Hurricane Sandy “culling” process, may explain why Oyster Creek appears to have reduced inbreeding close to HWE and increased diversity (Table 2).

All the populations appear to manifest evidence of historical bottlenecks (Fig. 4), but we can find no evidence of bottlenecks within the last 2–3 years (Table 4), suggesting that the populations have been bolstered by active sexual reproduction during the recovery from the storm. The 2008 Barnegat populations all showed evidence of recent bottlenecks using the Wilcoxon sign-rank tests for heterozygosity excess (Campanella et al., 2010b). During the decade preceding the evaluation of genetic diversity in 2008, several key environmental stressors occurred including a massive macroalgal bloom in 1988 which resulted in mass die-offs of *Z. marina* near Ham Island (Bologna et al., 2001,

2007) and repeated brown-tide events severely restricting light in Barnegat Bay (Gastrich and Wazniak, 2002). These repeated stress events could have led to those bottlenecks among populations. This present trend of improved *Z. marina* genetic health may be evidence that these previous environmental stresses that caused historical genetic bottlenecks and loss of diversity have been relieved by Hurricane Sandy, supporting the "Storm Stimulus" hypothesis.

4.2. Implications for restoration

During the last 20 years, we have been involved with several mitigation and restoration efforts to replace and enhance the spatial extent of *Z. marina* in Barnegat Bay (Bologna and Sinnema, 2006, 2012; Bologna unpubl.). Our early attempts relied upon transplanting existing shoots to document survival success as a metric for management and permit compliance (Bologna and Sinnema, 2006). However, after initial analyses identified low genetic diversity and significant population bottlenecks (Campanella et al., 2010a, 2010b), we combined transplant and seeding in mitigation and restoration efforts (Sinnema and Bologna, 2009) to promote greater genetic diversity and increase spatial coverage (*sensu* Orth et al., 2020).

Consequently, it appears that the use of seeds may be required to counteract flowering and seed destruction due to higher sea surface temperatures in systems where inbreeding, bottlenecks, and haplotype diversity are under changing climate stress (Qin et al., 2020). Hopefully, through active restoration efforts, we may limit severe bottlenecks, as somatic, clonal reproduction can result in fixation and limited diversity (Yu et al., 2020). However, the underlying factor that impacts population diversity in organisms which exhibit both sexual and asexual reproduction is recruitment of new individuals.

Under the Storm Stimulus hypothesis, the intermittent destruction of existing habitats opens sediments for seeds to germinate and support sexual recombination leading to greater genetic diversity, which is supported by the findings of our research. Ultimately, the long-term recovery of seagrasses on a global stage will provide necessary ecosystem services and support Sustainable Development Goals (Unsworth et al., 2022).

4.3. Conclusions

The recovery of the Barnegat Bay *Z. marina* populations may be accounted for by alternative explanations than the Storm Stimulus effect. The OCNGS has had a long-term and lasting effect on the whole bay since its opening in 1969. Even without the high temperatures in the immediate vicinity of the power plant, its operation induced substantial circulation changes for decades. It is possible that removing the power plant heat source and inflow/outflow circulation effects in 2018 had an ameliorative effect on the population genetics of the bay by helping revert the system closer to a process of natural selection. It is unlikely that the processes of migration or outside gene flow occurred, as seen in animals (Dobzhansky, 1955), especially in the sessile eelgrass beds of Barnegat Bay with limited outside tidal access due to barrier islands (Fig. 1).

Future stressors in Barnegat Bay are not just a possibility, but a likelihood (Scalpone et al., 2020). Even though there is no more nuclear power plant altering water temperatures in the bay, global climate change seems likely to increase the temperature of the New Jersey coastal region (Wilson and Lotze, 2019). Already populations of *Z. marina* in North Carolina, the southernmost Western Atlantic eelgrass beds, are beginning to lose ground to the more tropical *Halodule wrightii*, which is moving north (Shields et al., 2019; Nguyen et al., 2021). Combs et al. (2020) found that *Z. marina* in North Carolina has shifted to flowering earlier in the season due to temperature cues being reached sooner. Farther north, Hensel et al. (2023) reported the more temperature tolerant *Ruppia maritima* rapidly recolonizing Chesapeake Bay ahead of *Z. marina* after a heat wave dieback; this also occurred in Barnegat Bay following the algal induced mass die off of *Z. marina* near Ham Island (Bologna et al., 2007).

Given that Wilson and Lotze (2019) predicted that eelgrass could be extirpated from its current southern range limit in North Carolina by the end of this century, we are naturally concerned for the long-term survival of the species along the entire Western Atlantic Coast. Because of this concern, we suggest that studying thermal tolerant strains may be critical to the long-term survival of this species under elevated temperature regimes. The genetics of the Oyster Creek *Z. marina* population, which may have become more temperature resistant through selection at the outflow of the OCNGS, could be critical to understanding thermal tolerance evolution. Future experiments with these heat-resistant ecotypes should involve Next Generation DNA Sequencing, RNA expression analysis, and genetic comparisons to wild-type heat-sensitive ecotypes of the species. The information generated from this ecotype may become quite valuable in future advances in restoration against marine global warming and loss of submerged aquatic vegetation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

Abdel-Latif, A., Osman, G., 2017. Comparison of three genomic DNA extraction methods to obtain high DNA quality from maize. Plant Methods 13, 1. https://doi.org/10.1186/s13007-016-0152-4.

Ackerman, J., 2006. Sexual reproduction of seagrasses: pollination in the marine context. In: Larkum, A.W.D., Orth, R.J., Duarte, C. (Eds.), Seagrasses: Biology, Ecology And Conservation. Springer, Dordrecht, pp. 89–109.

Arnaud-Haond, S., Stoeckel, S., Bailleul, D., 2020. New insights into the population genetics of partially clonal organisms: when seagrass data meet theoretical expectations. Mol. Ecol. 29 (17), 3248–3260.

Barbier, E.B., Barbier, J.C.B., Folke, C., 2019. Paradise Lost?: The Ecological Economics of Biodiversity, 2, Routledge, New York.

Beaudet, M., Brisson, J., Messier, C., Gravel, D., 2007. Effect of a major ice storm on understory light conditions in an old-growth Acer–Fagus forest: pattern of recovery over seven years. . Ecol. Manag. 242 (2–3), 553–557.

Bell, S.S., 2006. Seagrasses and the metapopulation concept. In: Kritzer, J.P., Sale, P.F. (Eds.), Marine Metapopulations. Academic Press, London, pp. 387–411.

Blake, E.S., Kimberlain, T.B., Berg, R.J., Cangialosi, J.P., Beven Ii, J.L., 2013. Tropical cyclone report: Hurricane Sandy. Natl. Hurric. Cent. 12, 1–10.

Blok, S.E., Olesen, B., Krause-Jensen, D., 2018. Life history events of eelgrass *Zostera marina* L. populations across gradients of latitude and temperature. Mar. Ecol. Prog. Ser. 590, 79–93.

Bologna, P., Sinnema, M., 2006. Assessing the success of eelgrass restoration in New Jersey. In: S. Treat, S., Lewis, R. (Eds.), Seagrass Restoration: Success, Failure and the Costs of Both. Lewis Environmental Services. Inc, Valrico, FL, pp. 79–90.

Bologna, P., Sinnema, M., 2012. Restoration of seagrass habitat in New Jersey, USA. J. Coast. Res. 28, 99–104.

Bologna, P., Balzano, S., Suleski, A., 2014. Impacts of Hurricane Sandy on submerged aquatic vegetation in Barnegat Bay, New Jersey. In : Proceedings of the Conference Forty Third Benthic Ecology Meeting Presentation, Jacksonville, Florida, USA,. March 19–22.

Bologna, P., Gibbons-Ohr, S., Downes-Gastrich, M., 2007. Recovery of eelgrass (*Zostera marina*) after a major disturbance event in Barnegat Bay, New Jersey, USA. Bull. N.J. Acad. Sci. 52, 1–7.

Bologna, P., Wilbur, A., Able, K., 2001. Reproduction, population structure, and recruitment failure in a bay scallop (*Argopecten irradians*) population from coastal New Jersey. Usa. J. Shellfish Res 20, 89–96.

Booy, G., Hendriks, R.J.J., Smulders, M.J.M., Van Groenendael, J.M., Vosman, B., 2000. Genetic diversity and the survival of populations. Plant Biol. 2 (04), 379–395.

Campanella, J.J., Bologna, P.A.X., Smalley, J.V., Rosenzweig, E.B., Smith, S.M., 2010b. Population structure of *Zostera marina* (eelgrass) on the Western Atlantic Coast is characterized by poor connectivity and inbreeding. J. Hered. 101 (1), 61–70.

Campanella, J.J., Bologna, P.A.X., Smith, S.M., Rosenzweig, E.B., Smalley, J.V., 2010a. *Zostera marina* population genetics in Barnegat Bay, New Jersey, and implications for grass bed restoration. Pop. Ecol. 52 (1), 181–190.

Cavalli-Sforza, L.L., Edwards, A.W., 1967. Phylogenetic analysis. Models and estimation procedures. Am. J. Hum. Genet 19 (3 Pt 1), 233.

Chapuis, M.P., Estoup, A., 2007. Microsatellite null alleles and estimation of population differentiation. Mol. Biol. Evol. 24 (3), 621–631.

Combs, A., Jarvis, J., Kenworthy, W., 2020. Quantifying Variation in *Zostera marina* seed size and composition at the species' southern limit in the Western Atlantic: Implications for Eelgrass population resilience. Estuaries Coasts 44, 367–382.

Cornuet, J.M., Luikart, G., 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144, 2001–2014.

Cristescu, R., Sherwin, W.B., Handasyde, K., Cahill, V., Cooper, D.W., 2010. Detecting bottlenecks using BOTTLENECK 1.2.02 in wild populations: the importance of the microsatellite structure. Conserv. Genet. 11, 1043–1049.

Defne, Z., Ganju, N.K., 2015. Quantifying the residence time and flushing characteristics of a shallow, back-barrier estuary: application of hydrodynamic and particle tracking models. Estuaries Coasts 38, 1719–1734.

DiRienzo, A., Peterson, A.C., Garza, J.C., 1994. Mutational processes of simple-sequence repeat loci in human populations. Proc. Natl. Acad. Sci. USA 91 (8), 3166–3170.

Dobzhansky, T., 1955. A review of some fundamental concepts and problems of population genetics. In: Warren, K.B. (Ed.), Cold Spring Harbor Symposia on Quantitative Biology (Vol. 20). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 1–15.

Dunic, J.C., Brown, C.J., Connolly, R.M., Turschwell, M.P.C.ôté, M, I., 2021. Long-term declines and recovery of meadow area across the world's seagrass bioregions. Glob. Chang. Biol. 27 (17), 4096–4109.

Ehrlich, P.R., 1988. The loss of diversity. In: Wilson, E.O., Peter, F.M. (Eds.), Biodiversity. National Academy Press, Washington, DC, pp. 21–27.

Felsenstein, J., 1989. PHYLIP-phylogeny interference package (version 3.2). Cladistics 5, 164–166.

Fertig, B., Kennish, M.J., Sakowicz, G.P., 2013. Changing eelgrass (*Zostera marina* L.) characteristics in a highly eutrophic temperate coastal lagoon. Aquat. Bot. 104, 70–79.

Frankham, R., Ballou, S.E.J.D., Briscoe, D.A., Ballou, J.D., 2002. Introduction to Conservation Genetics. Cambridge University Press, Cambridge.

Garza, J.C., Williamson, E.G., 2001. Detection of reduction in population size using data from microsatellite loci. Mol. Ecol. 10 (2), 305–318.

Gastrich, M.D., Wazniak, C., 2002. A brown tide bloom index based on the potential harmful effects of the brown tide alga, *Aureococcus anophagefferens*. Aquat. Ecosyst. Health Manag. 33, 175–190.

Glaz, B., Gilbert, R.A., 2006. Sugarcane response to water table, periodic flood, and foliar nitrogen on organic soil. Agron. J. 98 (3), 616–621.

Gower, J.C., 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53 (3–4), 325–338.

Harwell, M.C., Orth, R.J., 2002. Seed bank patterns in Chesapeake Bay eelgrass (*Zostera marina* L.): a bay-wide perspective. Estuaries 25, 1196–1204.

Hensel, M.J., Patrick, C.J., Orth, R.J., Wilcox, D.J., Dennison, W.C., Gurbisz, C., Lefcheck, J.S., 2023. Rise of *Ruppia* in Chesapeake Bay: climate change–driven turnover of foundation species creates new threats and management opportunities. Proc. Natl. Acad. Sci. USA 120 (23), e2220678120.

Hernández-Delgado, E.A., Toledo-Hernández, C., Ruíz-Díaz, C.P., Gómez-Andújar, N., Medina-Muñiz, J.L., Canals-Silander, M.F., Suleimán-Ramos, S.E., 2020. Hurricane impacts and the resilience of the invasive sea vine, *Halophila stipulacea*: a case study from Puerto Rico. Estuaries Coasts 43, 1263–1283.

Jarvis, J.C., Moore, K.A., Kenworthy, W.J., 2012. Characterization and ecological implication of eelgrass life history strategies near the species' southern limit in the western North Atlantic. Mar. Ecol. Prog. Ser. 444, 43–56.

Johnson, A.J., Orth, R.J., Moore, K.A., 2020. The role of sexual reproduction in the maintenance of established *Zostera marina* meadows. J. Ecol. 108 (3), 945–957.

Johnson, A.J., Shields, E.C., Kendrick, G.A., Orth, R.J., 2021. Recovery dynamics of the seagrass *Zostera marina* following mass mortalities from two extreme climatic events. Estuar. Coasts 44, 535–544.

Kendall, M.S., Battista, T., Hillis-Starr, Z., 2004. Long term expansion of a deep *Syringodium filiforme* meadow in St. Croix, US Virgin Islands: the potential role of hurricanes in the dispersal of seeds. Aquat. Bot. 78 (1), 15–25.

Kennish, M.J., Bricker, S.B., Dennison, W.C., Glibert, P.M., Livingston, R.J., Moore, K.A., Noble, R.T., Paerl, H.W., Ramstack, J.M., Seitzinger, S., Tomasko, D.A., 2007. Barnegat Bay–Little Egg Harbor Estuary: case study of a highly eutrophic coastal bay system. Ecol. Appl. 17 (sp5). S3-S16.

Kennish, M.J., Haag, S.M., Sakowicz, G.P., 2010. Seagrass decline in New Jersey coastal lagoons: a response to increasing eutrophication. In: Kennish, M.J., Paerl, H.W. (Eds.), Coastal Lagoons: Critical Habitats of Environmental Change. CRC Press, Boca Raton, pp. 167–201.

Kritzer, J.P., Sale, P.F., 2006. Marine Metapopulations. Academic Press, London.

Lathrop, R.G., Styles, R.M., Seitzinger, S.P., Bognar, J.A., 2001. Use of GIS mapping and modeling approaches to examine the spatial distribution of seagrasses in Barnegat Bay, New Jersey. Estuaries 24, 904–916.

Lienert, J., 2004. Habitat fragmentation effects on fitness of plant populations– a review. J. Nat. Conserv. 12 (1), 53–72.

Luikart, G., Cornuet, J.M., 1998. Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. Conserv. Biol. 12 (1), 228–237.

Minch, E., Ruiz-Linares, A., Goldstein, D., Feldman, M., Cavalli-Sforza, L.L., 1995. Microsat (version 1.4 d): a Computer Program for Calculating Various Statistics on Microsatellite Allele Data. Stanford University, Stanford, California.

Nei, M., 1972. Genetic distance between populations. Am. Nat. 106 (949), 283–292.

Nei, M., 1977. F-statistics and analysis of gene diversity in subdivided populations. Ann. Hum. Genet. 41, 225–233.

Nei, M., Feldman, M.W., 1972. Identity of genes by descent within and between populations under mutation and migration pressures. Theor. Pop. Biol. 3 (4), 460–465.

Nelson, D.C., Flematti, G.R., Ghisalberti, E.L., Dixon, K.W., Smith, S.M., 2012. Regulation of seed germination and seedling growth by chemical signals from burning vegetation. Ann. Rev. Plant Biol.. 63, 107–130.

Nguyen, H.M., Ralph, P.J., Marín-Guirao, L., Pernice, M., Procaccini, G., 2021. Seagrasses in an era of ocean warming: a review. Biol. Rev. 96 (5), 2009–2030.

Nordlund, L.M., Jackson, E.L., Nakaoka, M., Samper-Villarreal, J., Beca-Carretero, P., Creed, J.C., 2018. Seagrass ecosystem services–What's next? Mar. Pollut. Bull. 134, 145–151.

O'Brien, K.R., Waycott, M., Maxwell, P., Kendrick, G.A., Udy, J.W., Ferguson, A.J., Dennison, W.C., 2018. Seagrass ecosystem trajectory depends on the relative timescales of resistance, recovery and disturbance. Mar. Pollut. Bull. 134, 166–176.

Oetjen, K., Ferber, S., Dankert, I., Reusch, T., 2010. New evidence for habitat-specific selection in Wadden Sea *Zostera marina* populations revealed by genome scanning using SNP and microsatellite markers. Mar. Biol. 157, 81–89.

Olesen, B., Sand-Jensen, K., 1994. Patch dynamics of eelgrass Zostera marina. Mar. Ecol. Prog. Ser. 106, 147-147.

Olivieri, I., 2000. Preface— Special issue on plant metapopulations. Evol. Ecol. 14 (iii-vii).

Olsen, J.L., Stam, W.T., Coyer, J.A., Reusch, T.B., Billingham, M., Boström, C., Wyllie-Echeverria, S., 2004. North Atlantic phylogeography and large-scale population differentiation of the seagrass *Zostera marina* L. Mol. Ecol. 13 (7), 1923–1941.

Olson, E., Vasslides, J.M., 2022. Multi-decadal declines and species assemblage shifts in the fish community of a Northeast US Temperate Estuary. Estuar. Coast 45 (7), 2219–2240.

Orth, R.J., Harwell, M.C., Inglis, G.J., 2007. Ecology of seagrass seeds and seagrass dispersal processes. In: Larkum, A.W.D., Orth, R.J., Duarte, C.M. (Eds.), Seagrasses: Biology, Ecology, and Conservation. Springer, Dordrecht, pp. 111–133.

Orth, R.J., Lefcheck, J.S., McGlathery, K.S., Aoki, L., Luckenbach, M.W., Moore, K.A., Oreska, M.P., Snyder, R., Wilcox, D.J., Lusk, B., 2020. Restoration of seagrass habitat leads to rapid recovery of coastal ecosystem services. Sci. Adv. 6 (41), eabc6434.

Orth, R.J., Luckenbach, M., Moore, K.A., 1994. Seed dispersal in a marine macrophyte: implications for colonization and restoration. Ecology 75 (7), 1927–1939.

Page, R.D., 2003. Visualizing phylogenetic trees using TreeView. Curr. Protoc. Bioinforma. (1) 6-2.

Park, S.D.E., 2001. The Excel microsatellite toolkit (version 3.1). Animal Genomics Laboratory, University College Dublin, Ireland.

Parsons Brinckerhoff, Inc., 2013. Route 72 Manahawkin Bay Bridges Improvement Project: Submerged Aquatic Vegetation Supplemental Survey Report 21p. Prepared for the New Jersey Department of Transportation.

Peakall, R., Smouse, P.E., 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes 6 (1), 288–295.

Peduzzi, P., 2019. The disaster risk, global change, and sustainability nexus. Sustainability 11 (4), 957–978.

Piry, S., Luikart, G., Cornuet, J.M., 1999. BOTTLENECK: a program for detecting recent effective population size reductions from allele data frequencies. J. Hered. 90 (4), 502–503.

Qin, L.Z., Kim, S.H., Song, H.J., Kim, H.G., Suonan, Z., Kwon, O., Kim, Y.K., Park, S.R., Park, J.I., Lee, K.S., 2020. Long-term variability in the flowering phenology and intensity of the temperate seagrass *Zostera marina* in response to regional sea warming. Ecol. Indic. 119, 106821.

Ray, J.D., Sinclair, T.R., Glaz, B., 2009. Sugarcane response to high water tables and intermittent flooding. J. Crop Improv. 24 (1), 12–27.

Reusch, T., 1999. Five microsatellite loci in eelgrass *Zostera marina* and a test of cross-species amplification in *Z. noltii* and *Z. japonica*. Mol. Ecol. 9, 365–378.

Reusch, T., 2000. Pollination in the marine realm: microsatellites reveal high outcrossing rates and multiple paternity in eelgrass *Zostera marina*. Heredity 85 (5), 459–464.

Reusch, T.B., Boström, C., Stam, W.T., Olsen, J.L., 1999a. An ancient eelgrass clone in the Baltic. Mar. Ecol. Prog. Ser. 183, 301–304.

Reusch, T., Stam, W.T., Olsen, J.L., 1999b. Size and estimated age of genets in eelgrass, *Zostera marina*, assessed with microsatellite markers. Mar. Biol. 133, 519–525.

Scalpone, C.R., Jarvis, J.C., Vasslides, J.M., Testa, J.M., Ganju, N.K., 2020. Simulated estuary-wide response of seagrass (*Zostera marina*) to future scenarios of temperature and sea level. Front. Mar. Sci. 7, 539946.

Setchell, W.A., 1929. Morphological and phenological notes on *Zostera marina L*. Univ. Calif. Publ. Bot. 14, 389–452.

Shields, E.C., Parrish, D., Moore, K., 2019. Short-term temperature stress results in seagrass community shift in a temperate estuary. Estuaries Coasts 42, 755–764.

Sinnema, M.S., Bologna, P., 2009. Channel Islands eelgrass mitigation year 1 monitoring report. Atlantic City Electric Project Report, Atlantic City, NJ.

Takezaki, N., Nei, M., 1996. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. Genetics 144 (1), 389–399.

Turschwell, M.P., Connolly, R.M., Dunic, J.C., Sievers, M., Buelow, C.A., Pearson, R.M., Brown, C.J., 2021. Anthropogenic pressures and life history predict trajectories of seagrass meadow extent at a global scale. Proc. Natl. Acad. Sci. USA 118 (45), e2110802118.

Tweitmann, A., Dietl, G.P., 2018. Live-dead mismatch of molluscan assemblages indicates disturbance from anthropogenic eutrophication in the Barnegat Bay-Little Egg Harbor estuary. J. Shellfish Res. 37 (3), 615–624.

Unsworth, R.K., Cullen-Unsworth, L.C., Jones, B.L., Lilley, R.J., 2022. The planetary role of seagrass conservation. Science 377 (6606), 609–613.

van Lent, F., Verschuure, J.M., van Veghel, M.L., 1995. Comparative study on populations of *Zostera marina* L. (eelgrass): in situ nitrogen enrichment and light manipulation. J. Exp. Mar. Biol. Ecol. 185 (1), 55–76.

Walz, Y., Janzen, S., Narvaez, L., Ortiz-Vargas, A., Woelki, J., Doswald, N., Sebesvari, Z., 2021. Disaster-related losses of ecosystems and their services. Why and how do losses matter for disaster risk reduction? Int. J. Disaster Risk Reduct. 63, 102425–102441.

Weir, B.S., 1996. Genetic Data Analysis II. Sinauer, Sunderland, Massachusetts.

Wilkinson, M., 1996. Majority-rule reduced consensus trees and their use in bootstrapping. Mol. Biol. Evol. 13 (3), 437–444.

Wilson, K.L., Lotze, H.K., 2019. Climate change projections reveal range shifts of eelgrass *Zostera marina* in the Northwest Atlantic. Mar. Ecol. Prog. Ser. 620, 47–62.

Wright, S., 1950. Genetical structure of populations. Nature 166, 247–249.

Yeh, F.C., Yang, R.-C., Boyle, T.B.J., Ye, Z.-H., Mao, J.X., 1997. POPGENE Ver. 1.32. The User-Friendly Software for Population Genetic Analysis; University of Alberta, Molecular Biology and Biotechnology Center: Edmonton, AB, Canada.

Yu, L., Boström, C., Franzenburg, S., Bayer, T., Dagan, T., Reusch, T.B., 2020. Somatic genetic drift and multilevel selection in a clonal seagrass. Nat. Ecol. Evol. 4 (7), 952–962.