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Effective population sizes of eastern oyster *Crassostrea virginica* (Gmelin) populations in Delaware Bay, USA

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ABSTRACT

Effective population size (N_e) is an important concept in population genetics as it dictates the rate of genetic change caused by drift. N_e estimates for many marine populations are small relative to the census population size. Small N_e in a large population may indicate high reproductive variance or sweepstakes reproductive success (SRS). The eastern oyster (*Crassostrea virginica*) may be prone to SRS due to its high fecundity and high larval mortality. To examine if SRS occurs in the eastern oyster, we studied N_e and genetic variation of oyster populations in Delaware Bay. Adult and spat oysters were collected from five locations in different years and genotyped with seven microsatellite markers. Slight genetic differences were revealed by *Fst* statistics between the adult populations and spat recruits, while the adult populations are spatially homogeneous and temporally stable. Comparisons of genetic diversity and relatedness among adult and spat samples failed to provide convincing evidence for strong SRS. N_e estimates obtained with five different methods were variable, small and often without upper confidence limits. For single sample collections, N_e estimates for spat (140–440) were consistently smaller than that for adults (589–2,779). Analysis of pooled adult samples across all sites suggests that N_e for the whole bay may be very large, as indicated by the large point estimates and the lack of upper confidence limits. These results suggest that N_e may be small for a given spat fall, but the entire adult population may have large N_e and is temporally stable as it is the accumulation of many spat falls per year over many years.

1. Introduction

Effective population size (N_e) or the number of breeding individuals in an idealized Wright-Fisher population (Wright, 1931) is an important concept in population genetics. It determines the rate of genetic change caused by random drift in a finite population. As genetic drift is a major evolutionary force, N_e is critical to our understanding of the evolutionary history, genetic variability and population structure of a species (Charlesworth,

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2009). N_e is also important to conservation biology and resource management as it predicts the rate of inbreeding in small populations (Berthier *et al.*, 2002; Kalinowski and Waples, 2002).

Many marine organisms have large and weakly differentiated populations. Interestingly, N_e estimates in most marine organisms studied so far are much smaller than the census population size (N). In a survey of 15 marine organisms, the N_e/N ratio was mostly below 0.0001 (Hauser and Carvalho, 2008), suggesting that only a small fraction of individuals may function as breeders. It has been suggested that the small N_e/N ratio may be a reflection of sweepstake reproductive success (SRS) resulting from high fecundity and type III survivorship (heavy larval mortality) that are characteristic of many marine organisms (Hedgecock, 1994; Hedgecock and Pudovkin, 2011). While SRS is supported by small N_e estimates in some studies, the prevalence and evolutionary significance of SRS are poorly understood. Most N_e estimates were obtained for a single cohort and at one time, and it is not clear if it has any meaningful impact on the genetic variation of a whole population over time (Buston *et al.*, 2009). Studies on temporal and spatial variations in N_e and its effects on population genetic structure should improve our understanding of the significance of SRS.

While the definition of N_e is simple, its estimation is notoriously difficult. As it is not possible to directly count the number of breeding individuals in a natural population over a lifetime, N_e must be inferred from genetic variation observed from genetic markers. The increasing availability of polymorphic genetic markers has made estimating N_e possible, and several estimation methods have been developed (Luikart *et al.*, 2010). N_e estimation methods can be divided into two main categories: one using a single sample and the other using two temporal samples. Single-sample estimators include the linkage disequilibrium (LD) method (Hill, 1981; Waples and Do, 2008), heterozygote excess method (Pudovkin *et al.*, 1996), sibship method (Wang and Santure, 2009), Bayesian partial likelihood method implemented in ONeSAMP (Tallmon *et al.*, 2008), and the rarefaction of alleles method (Hedgecock *et al.*, 2007). The LD method determines N_e based on linkage disequilibrium, which may produce biased results when the sample size is smaller than the estimate N_e (England *et al.*, 2006), but protocols have been developed to correct such bias (Waples, 2006). The heterozygote excess method exploits the excess of heterozygotes arising in a cohort of progeny produced by a limited number of parents, but it is not widely useful because it is not accurate unless the N_e is less than 30 (Zhdanova and Pudovkin, 2008). ONeSAMP has the greatest potential to provide improved precision because it calculates eight summary statistics that have relationship with N_e and thus uses more information from the data. The two-sample methods rely on temporal changes in allele frequency to estimate N_e based on the principal that the degree of allele frequency change from genetic drift is proportional to effective population size. The standard moment-based method follows the classical theory of the increase over time of the F-statistic due to genetic drift (Krimbas and Tsakas, 1971; Waples, 1989). Later, the maximum likelihood-based method (Tallmon *et al.*, 2004) and pseudo-likelihood method (Wang, 2001; Wang and Whitlock, 2003) were developed based on hidden Markov-chain model to measure temporal changes

in allele frequencies (Palstra and Ruzzante, 2008). Most methods assume one isolated population when estimating N_e. It is possible to accommodate several connected populations and estimate both N_e and temporal gene flow or migration rate simultaneously (Beerli and Felsenstein, 2001; Wang and Whitlock, 2003; Wilson and Rannala, 2003; Leberg, 2005).

The eastern oyster, *Crassostrea virginica* (Gmelin), is a marine bivalve widely distributed along the Atlantic Coast of North America, the Gulf of Mexico and Caribbean Sea. It is a keystone estuarine species that plays important roles in the ecology of estuaries such as Delaware Bay. Because of its abundance, high fecundity and typical type III survivorship, the eastern oyster provides a good model species to study N_e variation and SRS. Small N_es have been reported for the eastern oyster. Using 4–6 allozyme loci, Hedgecock *et al.* (1992) estimated N_e in four populations of the eastern oyster. For the three populations that produced confident estimates, N_e, ranged from 14.9 in upper Chesapeake Bay, 30 in James River to 33.8 in Delaware Bay. These surprisingly low estimates were cited as supporting evidence for SRS. In another study using eight microsatellites, Rose *et al.* (2006) obtained a likely N_e of 1,517 for the James River, which is about 500 times that estimated for the same population by Hedgecock *et al.* (1992). Further, contrary to SRS predictions, no differences in allelic richness or gene diversity were observed between different age classes by Rose *et al.* (2006). These rather conflicting results suggest that N_e may vary depending on sampling time and study methods, and further studies are needed in determining whether SRS exists as a major phenomenon in oysters.

Both previous studies in the eastern oyster assumed that samples were from a single isolated population, namely no migration. The eastern oyster, like most marine invertebrates, has a lengthy pelagic larval stage that can disperse over long distances. If genetic heterogeneity is detected in a near-by population, it may be necessary to incorporate larval migration from connected populations when estimating N_e.

To improve our understanding of temporal and spatial variation in N_e and possible SRS in oysters, we conducted a genetic analysis of eastern oyster populations in Delaware Bay, a well-flushed estuary system and a major oyster habitat, with microsatellite markers. We collected adults and spat from five locations in Delaware Bay in 2006 and 2009, genotyped them with seven putatively neutral microsatellite markers and estimated N_e with five different methods. Our objective was to test the hypothesis that significant SRS exists in eastern oyster populations in Delaware Bay causing small N_es and significant temporal and spatial genetic changes.

2. Materials and methods

a. Samples

Adult eastern oysters were collected from five locations in Delaware Bay (from upper to lower bay): Hope Creek (HC), Round Island (RI); Shell Rock (SR), Beadons (BD); Cape Shore (CS) in 2006 and again in 2009, except for Hope Creek where adult samples were

Table 1. Sampling site, sample size and date for eastern oyster collections used in this study.

Sample	Description	Sample size	Date collected	Latitude, longitude
HC07a	Hope Creek, adult	48	Sep 25, 2007	39°26.7′, 75°31.1′
HC09a	Hope Creek, adult	48	July 20, 2009	39°26.7′, 75°31.1′
HC09s	Hope Creek, spat	48	Oct 30, 2009	39°26.7′, 75°31.1′
RI06a	Round Island, adult	48	Nov 29, 2006	39°24.0′, 75°28.0′
RI06s	Round Island, spat	48	Nov 29, 2006	39°24.0′, 75°28.0′
RI09a	Round Island, adult	48	July 20, 2009	39°24.0′, 75°28.0′
RI09s	Round Island, spat	48	Oct 30, 2009	39°24.0′, 75°28.0′
SR06a	Shell Rock, adult	48	Nov 29, 2006	39°17.5′, 75°20.7′
SR09a	Shell Rock, adult	48	July 20, 2009	39°17.5′, 75°20.7′
BD06a	Beadons, adult	48	Nov 21, 2006	39°17.5′, 75°20.7′
BD09a	Beadons, adult	48	July 20, 2009	39°17.5′, 75°20.7′
BD09s	Beadons, spat	48	Oct 30, 2009	39°17.5′, 75°20.7′
CS06a	Cape Shore, adult	48	Dec12, 2006	39°04.4′, 74°55.0′
CS06s	Cape Shore, spat	48	Dec 8, 2006	39°04.4′, 74°55.0′
CS09a	Cape Shore, adult	48	July 20, 2009	39°04.4′, 74°55.0′
CS09s	Cape Shore, spat	48	Oct 30, 2009	39°04.4′, 74°55.0′

collected in 2007 instead of 2006 (Table 1; Fig. 1). Spat were collected from two locations (RI and CS) in 2006 (18.5±5.5 mm in size) and four locations (HC, RI, BD and CS) in 2009 (15.6±4.3 mm in size) (Table 1). Each sampling site contained 48 randomly selected oysters or spat. The total number of the samples analyzed was 768. All oysters were refrigerated until the adductor muscles or the whole spat were preserved in 95% ethanol.

b. DNA extraction, PCR amplification and genotyping

Genomic DNA was extracted with the Omega Bio-Tek Inc. E.Z.N.A.™ Mollusk DNA extraction kit according to supplied protocols. Oysters were genotyped at seven microsatellite markers, RUCV046, RUCV063 and RUCV091 from Wang and Guo (2007), RUCV176 and RUCV227 from Wang *et al.* (2009), Cvi1248 from Carlsson and Reece (2007), Cvi9 from Brown *et al.* (2000). The seven markers did not show significant changes in genotype frequency after disease-caused mortalities and were considered as putatively neutral (Guo *et al.* unpublished data). The forward primers of RUCV063, Cvi1248, and Cvi9 were directly labeled with fluorescence dyes, FAM, VIC and FAM, respectively. For these three markers, multiplex PCR (Polymerase Chain Reaction) was carried out in 10 μl with 1× PCR buffer, 1.5 mM $MgCl_2$, 1.0 mg/ml BSA, 0.2 mM dNTP, 0.2 μM of every primer, 0.08 U of *Taq* DNA polymerase (Promega GoTaq® DNA polymerase), and 20–50 ng of oyster genomic DNA. RUCV046, RUCV091, RUCV227 and RUCV176 were indirectly labeled by adding a M13 tail (Schuelke, 2000) to the forward primer and separately amplified with the inclusion of 0.2 μM of FAM, VIC, PET and NED-labeled M13 primers, respectively, in the same reagent mixture described above. For multiplex PCR of directly labeled primers,

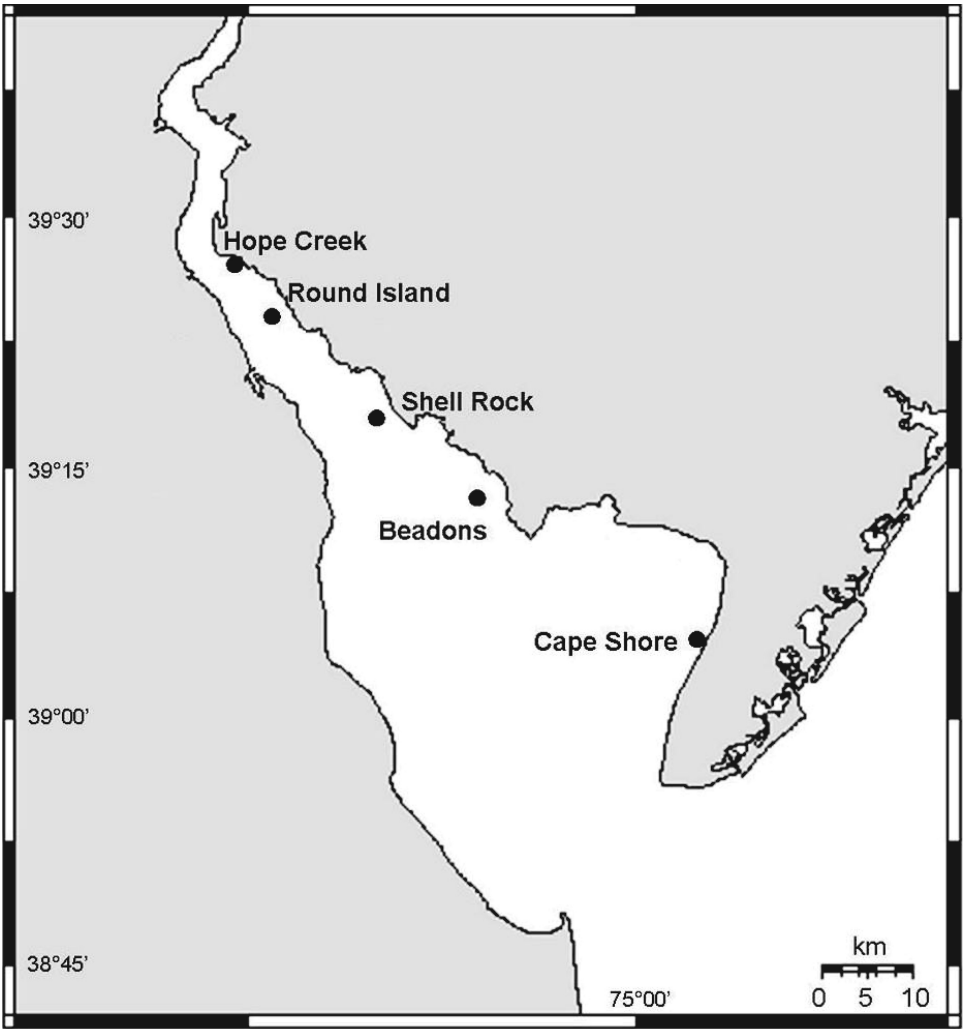

Figure 1. A map of Delaware Bay showing sampling sites.

the program was set as: initial denaturing at 95°C for 5 min; 35 cycles of 95°C for 45 s, 57°C for 45 s, 72°C for 45 s and ending with 72°C for 5 min. The M13-tailed markers were amplified using the following PCR profile (Schuelke, 2000): an initial denature for 5 min at 95°C, followed by 35 cycles of 95°C for 30 s, 55°C (RUCV091 and RUCV176) or 60°C (RUCV046 and RUCV227) for 30 s, and 72°C for 30 s; 19 cycles of: 95°C for 30 s, 53°C for 30 s, and 72°C for 30 s; ending with a final extension at 72°C for 10 min. PCR amplification was conducted on either a GeneAmp 9700 thermocycler (Perkin Elmer, Weiterstadt, CA) or an iCycler thermocycler (Bio-Rad, Hercules, CA).

Following amplification, PCR products from M13-tailed markers were mixed together. The mixed or multiplex PCR products were diluted three fold, and 0.5 μl of the diluted products were mixed with 12 μl of deionized formamide (Sigma) and 0.5 μl of GS-600LIZ size standard (Applied Biosystems). The mixture was electrophoresed with an ABI 3130xl Prism Genetic Analyzer. Allele scoring was performed with GeneMapper v4.0 (Applied Biosystems).

c. Statistical analyses

MICRO-CHECKER 2.2.3 was used to examine evidence of scoring error, large allele drop out (Wattier *et al.*, 1998), stuttering (Shinde *et al.*, 2003), and frequency of null allele assuming a single null allele based on Brookfield's approach (1996). Standard genetic indices, including the number of alleles (N), the observed heterozygosity (H_o) and the expected heterozygosity (H_e) were calculated using GENEPOP 4.0 (Raymond and Rousset, 1995) online version (http://genepop.curtin.edu.au/). Allelic richness (A_r) was estimated using FSTAT version 2.9.3.2 (Goudet, 1995). Deviation from Hardy-Weinberg equilibrium (HWE) was tested using the online version of the program GENEPOP employing a Markov chain method (Guo and Thompson, 1992). Significance criteria were adjusted for the number of simultaneous tests using sequential Bonferroni corrections (Rice, 1989). To assess genetic similarities among individuals in a population, mean pairwise relatedness (r) was calculated for each population using a maximum-likelihood relatedness estimator (Konovalov and Heg, 2008) implemented in software Kingroup version 2 (Konovalov *et al.*, 2004).

d. Temporal N_e estimators

Three temporal methods were used to estimate N_e for each population. The first method is the moment-based temporal estimator (Waples, 1989) implemented in NeEstimator version 1.3 (Peel *et al.*, 2004). The second method is MLNE 2.0, which implements the pseudo-likelihood method by assuming isolated populations (Wang, 2001). These two methods both assume that populations are closed, ignoring the role of migration on changing population allelic frequencies. To account for possible population heterogeneity and gene flow, we employed another method that relaxes the assumption of no migration by estimating N_e (N_{eopen}) and migration rate (m) jointly (Wang and Whitlock, 2003). This method is also implemented in MLNE 2.0. The maximum N_e was preset at 10,000 for the latter two methods as dictated by the software.

For temporal analysis, two temporally separated samples are needed. Eastern oysters may produce some gametes at one-year old, but most reach full maturation at two years of age and continue to spawn every year (Galtsoff, 1964). For the purpose of this study, we set generation time at two years. For the samples collected, we designated three different temporal sets as follows. Adult spawned in summer and spat were collected in fall, so adult-spat of the same year was one generation. The 2009 adults could be the F1 generation spawned by 2006 adults and the 2009 adults produced 2009 spat, so 2006 adult-2009 spat

Table 2. A Summary of N_e estimators used in this study.

Program	Description	Key assumptions	Comments	Reference
LDNe	One sample, based on linkage disequilibrium	LD signal only arises from genetic drift	Strongly biased by age structure and small samples	Waples and Do (2008)
ONeSAMP	One sample, uses approximate Bayesian computation	LD signal is only from genetic drift	User defined the prior N_e, after 50,000 simulated populations based on user data, summary statistics close to observed data delineates accepted range of N_e	Tallmon *et al.* (2008)
NeEstimator	Two samples, moment-based method	Allele frequency change is only from drift, no selection or immigration	Variance effective size estimator based on the allele frequency changes over temporal samples	Pell *et al.* (2004)
MLNE	Two samples, pseudo-likelihood temporal method	Allele frequency change is from drift or immigration	Allows to estimate N_e alone or estimate N_e and m jointly	Wang and Whitlock (2003)

Table 3. Allele number (N), allelic richness (A_r), observed and expected heterozygosities (H_o and H_e) for each microsatellite marker and populations. H_o in bold represents significant departures from Hardy-Weinberg equilibrium after Bonferroni correction.

	population															
	HC07a	HC09a	HC09s	RI06a	RI06s	RI09a	RI09s	SR06a	SR09a	BD06a	BD09a	BD09s	CS06a	CS06s	CS09a	CS09s
RUCV046																
N	23	18	20	21	20	23	17	19	19	20	21	19	23	18	19	25
A_r	22.833	17.938	19.916	20.937	19.916	22.854	16.916	19.000	18.895	19.958	21.000	18.917	22.895	17.916	18.937	25.000
H_o	**0.792**	**0.646**	**0.438**	**0.667**	**0.708**	**0.688**	**0.708**	**0.681**	**0.667**	**0.792**	**0.681**	**0.500**	**0.667**	**0.646**	**0.750**	0.809
H_e	0.940	0.937	0.932	0.949	0.926	0.935	0.910	0.921	0.929	0.942	0.933	0.933	0.946	0.920	0.938	0.946
RUCV063																
N	22	24	23	24	24	28	23	26	25	25	26	22	26	21	28	25
A_r	21.957	23.916	22.998	23.957	23.916	27.832	22.916	26.000	24.916	24.833	26.000	21.916	25.853	20.958	27.832	25.000
H_o	**0.708**	**0.688**	**0.646**	**0.813**	**0.583**	0.875	**0.646**	**0.766**	**0.813**	**0.771**	**0.723**	**0.667**	**0.667**	**0.563**	**0.813**	**0.596**
H_e	0.942	0.953	0.958	0.958	0.947	0.953	0.940	0.952	0.952	0.945	0.957	0.948	0.957	0.948	0.959	0.945
RUCV091																
N	13	13	13	12	14	11	16	12	10	15	11	10	12	13	13	14
A_r	12.937	12.916	12.937	11.937	13.875	10.937	15.833	12.000	9.938	14.937	11.000	9.937	11.916	12.958	12.916	14.000
H_o	**0.604**	0.708	0.708	0.750	0.688	0.792	0.688	0.830	0.792	0.813	0.766	0.771	**0.667**	0.708	0.813	0.809
H_e	0.834	0.823	0.824	0.832	0.824	0.809	0.782	0.804	0.809	0.857	0.787	0.790	0.793	0.808	0.822	0.848
RUCV227																
N	13	13	8	13	13	9	13	10	11	13	10	13	12	14	11	12
A_r	12.937	12.958	8.000	12.958	12.937	9.000	12.875	10.000	10.979	12.958	10.000	12.958	11.979	13.916	11.000	12.000
H_o	**0.563**	**0.708**	**0.458**	**0.583**	**0.563**	**0.333**	**0.521**	**0.468**	**0.500**	**0.521**	**0.489**	**0.625**	**0.521**	**0.500**	**0.417**	**0.511**
H_e	0.836	0.832	0.817	0.789	0.860	0.764	0.811	0.748	0.774	0.846	0.755	0.833	0.798	0.854	0.698	0.798

(Continued)

Table 3. (Continued)

	population															
	HC07a	HC09a	HC09s	RI06a	RI06s	RI09a	RI09s	SR06a	SR09a	BD06a	BD09a	BD09s	CS06a	CS06s	CS09a	CS09s
RUCV176																
N	5	4	4	3	4	5	5	6	4	3	6	5	5	5	3	4
A_r	4.979	4.000	4.000	3.000	4.000	4.958	4.979	6.000	3.979	3.000	6.000	5.000	4.938	4.979	3.000	4.000
H_o	**0.667**	0.292	**0.208**	0.146	0.229	0.146	**0.313**	0.255	0.208	0.229	0.277	0.292	0.146	0.250	0.146	**0.277**
H_e	0.939	0.297	0.431	0.176	0.375	0.196	0.532	0.323	0.263	0.261	0.372	0.405	0.194	0.363	0.192	0.523
Cvi1248																
N	26	32	27	29	28	26	22	25	28	30	25	26	24	29	27	27
A_r	25.894	31.831	26.874	28.749	27.811	25.853	21.915	25.000	27.811	29.769	25.000	25.853	23.873	28.729	26.832	27.000
H_o	**0.854**	**0.563**	**0.646**	**0.479**	**0.708**	**0.542**	**0.625**	**0.596**	**0.500**	**0.646**	**0.532**	**0.646**	**0.479**	**0.646**	**0.625**	**0.638**
H_e	0.890	0.932	0.918	0.935	0.929	0.917	0.885	0.872	0.918	0.901	0.845	0.923	0.845	0.887	0.916	0.909
CVi9																
N	14	17	15	16	13	14	17	15	14	18	16	15	15	16	14	16
A_r	13.937	16.916	14.917	15.958	12.958	13.958	16.875	15.000	13.937	17.896	16.000	14.979	14.916	15.916	13.979	16.000
H_o	0.854	0.813	**0.750**	0.750	0.833	0.729	0.792	0.851	0.875	0.854	0.83	0.813	0.792	0.854	**0.667**	0.915
H_e	0.911	0.907	0.903	0.916	0.896	0.888	0.91	0.897	0.906	0.922	0.913	0.915	0.905	0.911	0.892	0.901
Average																
N	16.571	17.286	15.714	16.857	16.571	16.571	16.143	16.143	15.857	17.714	16.429	15.714	16.714	16.571	16.429	17.571
A_r	16.496	17.211	15.663	16.785	16.488	16.485	16.044	16.143	15.779	17.622	16.429	15.651	16.624	16.482	16.357	17.571
H_o	0.720	0.631	0.551	0.598	0.616	0.586	0.613	0.635	0.622	0.661	0.614	0.616	0.563	0.595	0.604	0.651
H_e	0.899	0.812	0.826	0.794	0.822	0.780	0.824	0.788	0.793	0.811	0.795	0.821	0.777	0.813	0.774	0.839

were considered as two generations apart. To estimate N_e with temporal methods, it is necessary to define the source (first sample) and derived (second) populations. With little knowledge of population structure and actual larval movement, we estimated N_e using three types of sample pairings: (1) Adults from each population (or location) as the first sample, and spat collected from the same location as the second sample; (2) Pooling all the adult populations in the same year as the first sample, and the spat from each location as the second sample; (3) Pooling all the adult populations in the same year as the first sample and all the spat populations in the same year as the second sample. Additionally, the temporal method with migration requires the allele frequency data from the source population. As we do not know where the immigrants to each of the five populations are from, we pooled allele frequencies from all other four populations collected in the same year to represent the source population for the targeted focal population.

e. Single-sample N_e estimators

Two single-sample estimators, LDNe (Waples and Do, 2008) and ONeSAMP (Tallmon *et al.*, 2008) were used in this study. LDNe uses information on linkage disequilibrium and corrects biases due to small sample sizes (England *et al.*, 2006; Waples, 2006). Low frequency microsatellite alleles can also bias results, so we estimated N_e after removing alleles with frequencies lower than 0.02, as suggested by Waples and Do (2010). ONeSAMP implements multiple summary statistical methods using approximate Bayesian computation. This method calls for user-defined N_e priors (Tallmon *et al.*, 2008). We set 20–10,000 as the lower and upper bounds of N_e priors to get N_e estimation along with 95% confidence intervals (CIs). All of the 16 adult and spat samples collected in different locations and different years, were used for N_e estimation with the two single-sample estimators. A summary of N_e estimation programs used in this study is given in Table 2.

3. Results

a. Genetic diversity within populations

A total of 768 oysters, 48 from each of the 16 collections, were genotyped at seven microsatellite loci. No evidence of scoring error due to artifact peaks or large-allele drop out was detected at any loci by MICRO-CHECKER 2.2.3. Null alleles were suggested at RUCV046, RUCV063, RUCV227 and Cvi1248. Null allele frequencies did not vary significantly among samples or populations (paired two-sample t-test, $p > 0.05$ after Bonferroni's correction). Loci exhibited moderate or high gene diversity in populations. Numbers of alleles per locus ranged from 3 to 30, and allelic richness (A_r) ranged from 3.0 to 29.8 (Table 3). Averaged over all loci, allelic richness ranged from 15.7 to 17.6 without noticeable differences among populations. As a group, the adult populations had an allelic richness of 16.6, which is not different from the 16.3 observed for spat ($p = 0.3863$, two-sample t-test). Observed heterozygosity (H_o) didn't differ markedly among adult populations (mean $H_o = 0.62$) and spat recruits (mean $H_o = 0.61$) ($p = 0.4416$, two-sample t-test) either.

Per locus test for HWE within individual populations showed that 70 out of the 112 cases had significant deviations after sequential Bonferroni corrections. Further, loci RUCV046, RUCV063, RUCV227 and Cvi1248 had a particularly high number of locations showing HWE deviation (Table 3). Most of the deviated cases showed a significant heterozygote deficiency (Table 3), suggesting the possible presence of null alleles, which were detected by MICRO-CHECKER.

To determine if population structure exists in Delaware Bay, we obtained *Fst* statistics (a measurement of population differentiation) for all population pairs. *Fst* estimates were small, ranging from −0.0047 to 0.0133 (Table A1), and none was significant after Bonferroni correction, suggesting that there is no significant genetic differentiation among any of the population or sample collections. Before Bonferroni corrections, only one of 45 adult population pairs had a significant *Fst* value (0.0045, $p = 0.0208$), suggesting that the adult populations in Delaware Bay is genetically homogenous and temporally stable. Two of the 15 spat-spat sample pairs had significant *Fst* values, and they were between 2006 and 2009 spat collections only. No significant *Fst* was observed among spat samples collected during the same year. However, 24 of the 60 adult-spat pairs had significant ($p < 0.05$) *Fst* values (before Bonferroni corrections) (Table A1), which suggest that minor genetic differences exist between adult populations and spat collections.

Mean pairwise relatedness value (r), a measure of genetic similarity among individuals relative to the population mean, ranged from -0.81 ± 0.36 (HC09s) to -0.55 ± 0.31 (SR06a) across all populations (Fig. A1). These negative r values suggest that individuals within populations are unrelated.

b. N_e Estimates from temporal methods

N_e estimates based on three temporal methods are presented in Table 4. For all temporal sample pairs and estimated by all three methods, N_e estimates were surprisingly small, although many had no upper confidence limits. The lack of upper confidence limits put the N_e point estimates into question and may suggest the $N_e$is very large or cannot be resolved with available data. For temporal sample pairs within each site, N_e estimates ranged from 37 to 611. NeEstimator yielded slightly but consistently lower N_e point estimates than MLNE without migration except for the 2006–2009 CS adult sample pair. Considering gene flow in the pseudo-likelihood method (Wang and Whitlock, 2003), N_e estimates became lower in all cases, and migration rate (m, ranging from 0 to 1) ranged from 0.31 to 0.78. Migration rate was lower at the middle bay sites (SR and BD) than that of the upper (HC and RI) and lower bay (CS) sites (Table 4).

Within each site, N_e for a given sample estimated with different base populations and different methods varied considerably. In most cases, the 2009 adults had higher N_e estimates than 2009 spat. To estimate N_e for all oysters in Delaware Bay assuming they are from a homogenous population (which is confirmed by *Fst* statistics), we pooled all samples

Table 4. Effective population size (N_e) and 95% confidence intervals of eastern oyster populations in Delaware Bay estimated using temporal methods and different source population.

	Generation	NeEstimator	MLNE (Wang, 2001)	MLNE (Wang and Whitlock, 2003)	
				N_e	m
Hope Creek					
07a/09s	2	190 (76-∞)	229 (101-10000)	NA*	NA
09a/09s	1	86 (31-293)	112 (58-771)	91 (69-128)	0.72 (0.40-1)
09a all/09s	1	151 (68-1180)	273 (151-1186)	NA	NA
07a/09a	1	232 (57-∞)	99 (87-10000)	NA	NA
Round Island					
06a/09s	2	111 (56-348)	135 (80-344)	87 (63-136)	0.72 (0.46-1)
06a all/09s	2	126 (74-253)	181 (121-325)	NA	NA
06a/06s	1	74 (33-457)	194 (78-10000)	81 (62-111)	0.76 (0.42-1)
06a all/06s	1	111 (55-429)	381 (189-306)	NA	NA
09a/09s	1	37 (21-80)	101 (58-309)	73 (55-109)	0.49 (0.27-0.80)
09a all/09s	1	62 (37-117)	174 (117-332)	NA	NA
06a/09a	1	127 (44-∞)	254 (86-10000)	100 (74-152)	0.77 (0.37-1)
06a all/09a	1	232 (82-∞)	375 (156-10000)	NA	NA
Shell Rock					
06a/09a	1	84 (35-1169)	250 (89-10000)	119 (68-653)	0.31 (0.47-0.68)
06a all/09a	1	125 (58-686)	287 (135-10000)	NA	NA
Beadons					
06a/09s	2	102 (53-289)	109 (66-241)	121 (83-221)	0.49 (0.22-1)
06a all/09s	2	263 (120-1075)	228 (135-581)	NA	NA
09a/09s	1	67 (31-333)	161 (70-10000)	84 (60-150)	0.44 (0.20-0.76)
09a all/09s	1	86 (47-210)	210 (128-528)	NA	NA
06a/09a	1	142 (46-∞)	195 (77-10000)	128 (89-299)	0.52 (0.17-1)
06a all/09a	1	273 (86-∞)	611 (188-10000)	NA	NA
Cape Shore					
06a/09s	2	107 (55-313)	231 (112-306)	92 (68-136)	0.78 (0.37-1)
06a all/09s	2	236 (112-1107)	270 (155-771)	NA	NA
06a/06s	1	56 (28-176)	196 (79- 10000)	89 (66-128)	0.77 (0.40-1)
06a all/06s	1	110 (55-407)	289 (143-848)	52 (40-72)	0.55 (0.37-0.80)
09a/09s	1	65 (31-275)	277 (97-10000)	NA	NA
09a all/09s	1	126 (60-587)	184 (0-10000)	NA	NA
06a/09a	1	131 (42-∞)	87 (53-208)	99 (71-118)	0.61 (0.23-1)
06a all/09a	1	150 (64-2535)	310 (143-10000)	NA	NA
All sites					
09a/09s	1	437 (192-5916)	893 (369-10000)	NA	NA
09a all/09s	1	155 (83-412)	370 (189-306)	NA	NA
06a/09a	1	251 (160-427)	331 (230-534)	NA	NA
06a all/09a	1	81 (62-138)	184 (100-10000)	NA	NA

*NA, no source population was available while pooling all the adult populations as the first sample.

collected from different locations at a given time, for adults and spat separately. For the pooled bay-wide samples, the N_e estimates were only slightly higher than those obtained for individual sites, ranging from 81–893 (Table 4).

c. N_e estimates from single-sample methods

Two single-sample methods were used to estimate N_e for all 16 samples collected. The LDNe method yielded mostly negative N_e estimates, except for five samples (Table 5). Negative estimates can be explained by sampling error without invoking any genetic drift. Thus, the best biological interpretation for the negative estimates is N_e = infinity (Waples and Do, 2010). None of the N_e estimates had finite upper limits, except for 2009 spat from BD, which had a N_e of 270. On the other hand, N_e estimates from ONeSAMP were considerably higher than those from the LDNe method, ranging from 140 in 2006 CS spat to 2,779 in 2006 SR adults. At all sites, N_e estimates for adults were higher than that for spat. On average, N_e for adult populations was 1,601, ranging from 589 to 2,779. N_e for spat samples averaged 252, ranging from 140 to 440. The difference was significant ($p = 0.0002$, two-sample t-test). All 16 N_e estimates from ONeSAMP had finite 95% confidence intervals.

Analysis of the pooled samples suggests that the N_e for the whole bay may be very high. The N_e estimate for all spat collected in 2006 was 67,107 and that for spat collected in 2009 was 3,086. N_e estimates for adult populations were much higher than those for spat. The N_e for all adults collected in 2006 was 7.2×10^{10} and that for all 2009 adults was 3.0×10^{7}. These high point estimates suggest that the N_e could be very high.

4. Discussion

a. Interpreting N_e with different methods

In this study, we estimated N_e and examined temporal and spatial genetic variation in eastern oyster populations from Delaware Bay using adult and spat samples collected at five sites and over three years. Three temporal methods and two single-sample methods were used for N_e estimation. Overall, our results show that N_e estimates for individual sample collections were small and variable. Variation in N_e was evident not only among different sites and age-classes, but also among different methods. The latter variation suggests that some of the N_e estimates are not reliable. This is also indicated by the fact that many N_e estimates have no upper confidence limits. Caution is needed for interpreting the N_e results.

All N_e estimation methods make assumptions that, when violated, lead to biases in N_e estimates. Some of the assumptions may not hold for our study. The assumption that populations are in HWE was not true in 63% of cases tested in our study. Most of the departure from HWE might be caused by the presence of null alleles. Temporal methods should not be seriously affected if the null alleles are equally distributed across samples

(Jehle *et al.*, 2001; Zeller *et al.*, 2008). This is the case in our study as we did not see differences in null allele frequencies among samples.

Discrete generation is an important assumption for the temporal methods that is most easily violated. In our study, the adult populations almost certainly consisted of different year-classes, spanning 2–3 generations. Waples and Yokota (2007) showed that the bias is reduced if the generation interval is greater than 5. However, our samples are only one or two generations apart. This may be one reason why N_e estimates from temporal methods are relatively small. Estimates over two generations were generally larger than those over a single generation (Table 4).

If rare alleles observed in the first sample are absent in the second sample, the moment-based *Fst* method could produce biased estimates. The likelihood-based methods should provide more precise estimates than the moment-based method since they use more information from the data (Wang, 2001; Berthier *et al.*, 2002). In this study, some microsatellite markers used were highly polymorphic and may have many rare alleles. We tested the effects of rare alleles on N_e estimation by estimating N_e using markers with different allele numbers: 3 highly polymorphic markers with 20 to 27 alleles versus 3 moderately polymorphic markers with 12–15 alleles. Markers with high allele numbers did not significantly change N_e estimates from two temporal methods (data not shown). We also compared N_e estimates using the 3 most heterozygote deficient and the 3 least heterozygote deficient loci, but no significant difference in N_e estimates was found. This was expected as the null allele, which causes the heterozygote deficiency, is evenly distributed in samples.

When migration was permitted, the temporal MLNE method produced smaller N_e estimates, credible confidence intervals, and high migration rates (0.31–0.78). In some other studies where N_{eopen} of Wang and Whitlock (2003) and at least one temporal $N_{eclosed}$ method were used, N_{eopen} were all smaller than $N_{eclosed}$ (Ford *et al.*, 2004; Hoffman *et al.*, 2004; Johnson *et al.*, 2004; Consuegra *et al.*, 2005; Jensen *et al.*, 2005; Saillant and Gold, 2006; Fraser *et al.*, 2007a.b; Zeller *et al.*, 2008). This is not surprising assuming migration reduces genetic changes attributable to genetic drift.

The finding of high migration rates suggests that there is tremendous mixing of oysters in Delaware Bay (Narvaez *et al.*, this issue). This is reasonable as the bay is a well-flushed and mixed system, and the eastern oyster has a veliger larva that can disperse over large distances. It is interesting that migration rates are higher in upper and lower bay regions than the mid-bay region. This result suggests that middle bay populations may be the center of recruitment and contribute more to the next generation than the upper and lower bay populations, an idea that is supported by more than 50 years of population survey data (Powell *et al.*, 2008). Most recruits in upper and lower bay regions may come from the middle bay, represented by SR and BD in this study, while the middle bay population are mostly self-recruiting, or more so than the other regions of the bay. This is the first time that migration rates have been estimated for the eastern oyster. As uncertainty exists for N_e

estimates, the migration rates should also be considered as preliminary and viewed with caution.

N_e estimates from the two single-sample methods are larger than those from temporal methods. The LDNe method did not produce valid point estimates, but the negative estimates and the lack of upper confidence bounds may suggest that the population is very large (Waples and Do, 2010). As Fraser *et al.* (2007b) suggests, it is important to consider the confidence intervals rather than point estimates generated by different methods. The lower confidence bounds provide estimates of minimum N_e. The lack of upper confidence limits may mean that the N_e is very large. It could mean that the N_e cannot be estimated with available data, which was limited by the relatively small number of samples and markers. As a guideline for sampling requirements, Palstra and Ruzzante (2008) suggested that at least 10% of a population's effective size need to be sampled. The sample size (n = 48) in this study is not large, however, we see no correlation between sample size and N_e for the pooled spat samples. Spat from 2006 (n = 96) had a larger N_e than spat from 2009 (n = 192, Table 5). Eastern oysters in Delaware Bay spawn mostly from June to August. The 2006 spat were collected in late November and early December, sized at 18.54 ± 5.50 mm (length) while the 2009 spat were collected in Oct 30, sized at 15.64 ± 4.29 mm (length). It is possible that the 2006 collection covered more recruits, from more different parents than the 2009 collection. Environmental differences leading to differences in bay-wide reproduction between the two years may also explain the difference in N_e.

The ONeSAMP method based on Bayesian approximation produced valid N_e estimates for all 16 sample collections. All estimates had finite 95% confidence intervals, making them more reliable than those with infinity as the upper confidence limit. Among the five methods, N_e estimates from ONeSAMP were also among the highest. Beebee (2009) compared four single-sample estimators (heterozygote excess, linkage disequilibrium, Bayesian partial likelihood and sibship analysis) using microsatellite data from multiple natterjack toad (*Bufo calamita*) populations, and concluded that the Bayesian method was the most precise. Assuming the N_e estimates from ONeSAMP are reliable, we may conclude that N_e is temporally and spatially variable in Delaware Bay, and the adult populations have larger N_es (589 to 2,779) than spat (140–440). These estimates are in the same range of what has been reported for eastern oyster populations in James River (535–1,516) by Rose *et al.* (2006), but considerably higher than that reported for Delaware Bay (33.8) by Hedgecock *et al.* (1992).

b. Sweepstake reproduction success

It has been suggested that marine organisms with high fecundity and type III survivorship may be prone to SRS (Hedgecock and Pudovkin, 2011). One prediction of the SRS hypothesis is a small effective population size to census population size ratio (N_e/N), which indicates only a small proportion of adult oysters are successful in producing offspring that survive. Extremely low N_e/N ratios ($<10^{-2}-10^{-5}$) have been reported in many marine

Table 5. Effective population size of eastern oyster populations in Delaware Bay estimated using LD-based single-sample estimators.

	LDNe	ONeSAMP
Hope Creek		
07a	4366 (279-∞)	1127 (492-5689)
09a	∞ (1380-∞)	2285 (1075-19137)
09s	∞ (490-∞)	205 (123-638)
Round Island		
06a	∞ (1439-∞)	1160 (460-5517)
09a	507 (178-∞)	1579 (778-11703)
06s	∞ (804-∞)	440 (231-1964)
09s	∞ (339-∞)	190 (118-581)
Shell Rock		
06a	∞ (216-∞)	2779 (981-21354)
09a	∞ (303-∞)	1333 (675-9073)
Beadons		
06a	785 (217-∞)	2438 (982-17713)
09a	∞ (331-∞)	1606 (778-13519)
09s	270 (136-2742)	299 (165-1191)
Cape Shore		
06a	∞ (391-∞)	1113 (483-5150)
09a	∞ (552-∞)	589 (271-2293)
06s	∞ (798-∞)	140 (76-365)
09s	309 (140-∞)	236 (134-689)
All sites		
06 adult all	∞ (1304-∞)	7.2×10^{10} (∞-∞)
09 adult all	∞ (1209-∞)	3.0×10^{7} (∞-9.54×10^{13})
06 spat all	∞ (9301-∞)	67107 (13138-1.6×10^{8})
09 spat all	∞ (1502-∞)	3086 (1149-16342)

invertebrates and fishes (Hedgecock, 1994; Hauser *et al.*, 2002; Arnason, 2004; Hedrick, 2005; Hoarau *et al.*, 2005; Zeller *et al.*, 2008), which are in agreement with SRS predictions. In this study, despite the difficulties of estimating N_e and some uncertainties, all N_e estimates for individual sample collections were much smaller than the expected census size. The census size of adult eastern oyster populations from the natural beds on the New Jersey side of Delaware Bay was estimated at 1.6×10^9 as of October 2009 (Hofmann *et al.*, 2009). Even with our highest N_e estimate for a given population, 2,779 for SR adults of 2006, the N_e/N ratio is as small as 10^{-6}. Assuming these small N_e estimates are accurate, the extremely small N_e/N ratio supports the SRS hypothesis.

It should be cautioned that the small N_e estimates may not be reliable, as they are often without upper confidence limits. The pooled adult samples across the bay gave very large N_e estimates: 3.0×10^7 for 2009 adults and 7.2×10^{10} for 2006 adults, which do suggest that the

N_e for the bay-wide population could be very large. Given the difficulties in N_e estimation and uncertainties, we should view both the extremely low estimates from individual samples and the very high estimates for the pooled samples with caution. The small estimates may be equally unreliable as the infinite estimates since many of the former are without upper confidence limits.

There are two main characteristic signatures left by SRS: reduction of genetic diversity and increased relatedness among recruits (Hedgecock *et al.*, 2007). The slight genetic differences between adult populations and spat collections as indicated by moderate *Fst* values (only significant before Bonferroni corrections) support some variation in reproduction success. However, some of the results do not support SRS as a major phenomenon in the eastern oyster. There was no detectable reduction in genetic diversity (in terms of allelic richness or observed heterozygosity) between spat recruits and adult populations. This finding is in agreement with the results of Rose *et al.* (2006) and in conflict with SRS predictions. In addition, the negative relatedness estimation both in adult populations and spat recruits indicate that individual oysters are unrelated within the populations studied. This also argues against significant SRS. Further, the bay-wide population as a whole is homogenous and temporally stable (albeit only measured over a short time), which would not be expected under strong impact of SRS. Strong SRS would create rapid genetic changes due to drift and greatly diminish genetic variability over time. Empirical data show that the eastern oyster genome is highly polymorphic (Zhang and Guo, 2010) and eastern oyster populations are weakly differentiated over large geographic ranges (Karl and Avise, 1992; Gaffney, 1996). It is possible that weak SRS exists but cannot be detected by available statistics. SRS, if any, after major epizootics may help the development of disease resistance in Delaware Bay (Ford and Bushek, this issue), although SRS may work against the development of resistance in the long run as the population can sway back to a susceptible state.

In conclusion, N_e estimates for eastern oyster populations in Delaware Bay are highly variable and uncertain. Each spat fall may have a small N_e but the N_e for the entire bay could be very large. The relatively small N_e for a given spat collection and the slight genetic differences between spat and adult populations support some variation in reproductive success. The lack of significant changes in genetic diversity and temporal genetic differentiation along with negative relatedness argues against any lasting impact by SRS on the adult population in Delaware Bay. These results suggest that, while each spat fall may involve a small set of parents and carry some genetic drift, such variance in reproductive success does not have a strong effect on the genetic variation of the entire bay-wide population, as the adult population is an accumulation of many spat falls per year over many years.

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APPENDIX

Table A1. Pairwise *Fst* values (below diagonal) and associated p-values (above diagonal) among 16 populations/samples of eastern oyster from Delaware Bay. None of the *Fst* values is significant at $p < 0.05$ after Bonferroni's correction.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 HC07a	–	0.7267	0.5113	0.4050	0.8450	0.6413	0.7992	0.5467	0.8704	0.4704	0.4221	0.0413	0.0179	0.0742	0.4971	0.3800
2 HC09a	−0.0036	–	0.9267	0.5421	0.7713	0.5425	0.9213	0.7667	0.4746	0.4325	0.1017	0.0754	0.0004	0.1329	0.1954	0.0833
3 RI06a	−0.0011	−0.0047	–	0.5008	0.2842	0.3417	0.8558	0.7342	0.9233	0.6221	0.1729	0.1238	0.0171	0.6688	0.1125	0.2658
4 RI09a	−0.0007	−0.0014	−0.0027	–	0.2450	0.1508	0.3675	0.2988	0.6904	0.3313	0.0571	0.0017	0.0013	0.0013	0.0050	0.0833
5 SR06a	−0.0023	−0.0032	0.0006	0.0018	–	0.1613	0.0208	0.6300	0.3429	0.0842	0.1133	0.1400	0.0429	0.2038	0.1042	0.5250
6 SR09a	−0.0025	−0.0022	−0.0014	−0.0009	−0.0003	–	0.2096	0.2188	0.0754	0.3008	0.0675	0.0042	0.0004	0.0008	0.0050	0.0179
7 BD06a	−0.0031	−0.0019	−0.0009	0.0025	0.0045	0.0003	–	0.5817	0.9058	0.1888	0.2683	0.1433	0.0004	0.0221	0.0196	0.0933
8 BD09a	−0.0018	−0.0013	−0.0004	0.0011	−0.0015	0.0004	0.0009	–	0.4183	0.6517	0.2067	0.0275	0.0042	0.1254	0.0250	0.1967
9 CS06a	−0.0016	0.0002	−0.0020	0.0005	0.0016	0.0017	−0.0016	−0.0006	–	0.5971	0.0233	0.1321	0.0017	0.1308	0.1033	0.0458
10 CS09a	0.0014	0.0007	−0.0030	0.0003	0.0026	−0.0001	0.0035	−0.0005	−0.0004	–	0.0754	0.0029	0.0004	0.0763	0.0083	0.0571
11 HC09s	0.0020	0.0041	0.0071	0.0072	0.0075	0.0045	0.0031	0.0049	0.0105	0.0116	–	0.2813	0.1408	0.8633	0.1779	0.6592
12 RI06s	0.0024	0.0013	0.0028	0.0071	0.0054	0.0043	0.0006	0.0049	0.0034	0.0086	0.0002	–	0.0354	0.0683	0.1208	0.5788
13 RI09s	0.0070	0.0098	0.0114	0.0133	0.0086	0.0128	0.0122	0.0076	0.0112	0.0132	0.0029	0.0042	–	0.2521	0.0558	0.1500
14 BD09s	0.0028	0.0013	0.0011	0.0071	0.0035	0.0061	0.0045	0.0040	0.0052	0.0061	−0.0026	0.0016	0.0021	–	0.0204	0.3871
15 CS06s	0.0011	0.0024	0.0050	0.0063	0.0073	0.0067	0.0047	0.0052	0.0040	0.0091	0.0016	−0.0007	0.0032	0.0026	–	0.2063
16 CS09s	0.0044	0.0064	0.0088	0.0096	0.0050	0.0057	0.0078	0.0065	0.0110	0.0105	0.0013	0.0026	0.0008	0.0012	0.0059	–

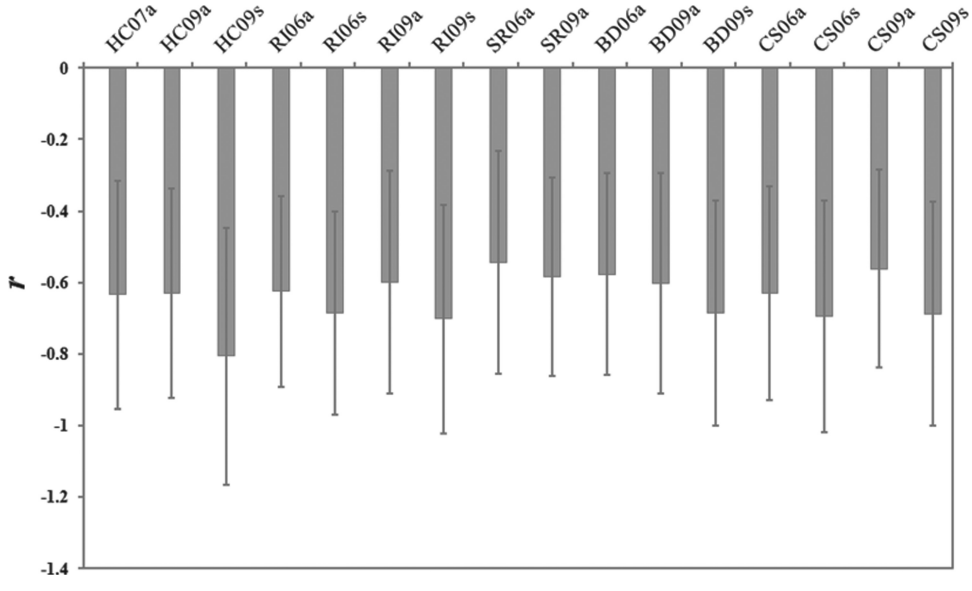

Figure A1. Mean relatedness values (r) for eastern oyster populations in Delaware Bay. Bars show means (±SE) following the methods of Konovalov and Heg (2008).

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