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ASSESSMENT OF GENETIC DIVERSITY IN WILD AND AQUACULTURE STOCKS OF *MERCENARIA MERCENARIA* IN FLORIDA

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ABSTRACT The northern hard clam *Mercenaria mercenaria* is one of the most commercially important bivalve species produced via aquaculture in the eastern United States. Breeding practices associated with large-scale hatchery production of hard clam seed may result in unintended, potentially negative consequences including inbreeding and reduced levels of genetic diversity. Seven microsatellite markers were used to compare levels of genetic diversity in six hatchery stocks and four wild stocks from the state of Florida. Wild stocks of *M. mercenaria* had slightly higher levels of allelic richness and similar observed heterozygosity (wild = $A_r = 3.61$, $H_o = 0.448$; hatchery = $A_r = 3.46$, $H_o = 0.452$); however, these differences were not statistically significant (A_r: $P = 0.145$; H_o: $P = 0.931$). Differentiation was highest between hatchery stocks ($G_{ST} = 0.03$, SD = 0.007; $P < 0.001$) but minimal among hatchery-by-wild comparisons. In contrast, wild stocks were not differentiated ($G_{ST} = -0.002$, SD = 0.004; $P \approx 0.58$) and Bayesian comparisons of competing gene flow models (panmixia, stepping stone, and full-migration) suggested that wild stocks were effectively panmictic at this scale. Together, these results indicate that some genetic drift has occurred within hatchery populations, possibly as a result of broodstock selection and spawning practices. The genetic divergence between wild and hatchery stocks observed in this study are not as pronounced as seen in other aquaculture bivalve species. Given the large scale of hard clam production in Florida we argue that hatchery practices should incorporate long-term genetic goals (i.e., avoidance of inbreeding) to maintain the integrity of hatchery stocks. A secondary objective of this study was to amplify a congeneric species, *Mercenaria campechiensis*, using the same suite of microsatellite markers to assess their utility for detecting interspecific hybridization. Amplification success was poor and failed for four of the seven loci. The identification of microsatellite loci for different species of *Mercenaria* should be identified *de novo* in the future.

KEY WORDS: *Mercenaria mercenaria*, aquaculture, population genetics, microsatellites, panmixia, clam, northern quahog

INTRODUCTION

The hard clam *Mercenaria mercenaria* (Linnaeus, 1758), is a marine bivalve native to the eastern seaboard of the United States in coastal waters from the Gulf of St. Lawrence to Florida (Harte 2001). Populations of *M. mercenaria* have been commercially exploited for over 100 y (MacKenzie et al. 2001); declines in harvests and fluctuations in abundance, however, prompted the development of aquaculture technology to procure seed for large-scale clam production (Arnold et al. 2009). Propagation of *M. mercenaria* has increased substantially in the last 35 y (Castagna 2001) in part due to advances in culture techniques made during the 1950s (Manzi & Castagna 1989). Most notably, the state of Florida has seen dramatic increases in *M. mercenaria* production, which has largely been attributed to job retraining programs for underemployed fishermen, increased availability of aquaculture leases, and refined production techniques (Colson & Sturmer 2000).

The commercial hard clam industry in Florida is comprised of more than 300 shellfish growers who farm submerged land leases totaling over 2,100 acres (Adams & Sturmer 2012). Hatchery production of *Mercenaria mercenaria* is typically accomplished via a five-stage process including broodstock selection and maintenance, spawning, larval culture, postset culture, and nursery culture in raceways or upwellers (Hadley & Whetstone 2007). Because the production of wild seed varies annually as a function of environmental conditions, clam aquaculture is almost entirely dependent upon hatcheries for seed (FAO 2013). Given the reliance on hatcheries for the mass production of offspring, resource managers would benefit from considering the genetic consequences of current breeding practices. For example, large-scale seed production accomplished using small numbers of broodstock may result in a significant reduction in genetic diversity and an increased likelihood of inbreeding relative to wild populations (Lutz 2001). Such outcomes may have potential negative consequences for survival and growth. The application of genetic techniques to inform aquaculture production has resulted in increased yields (Langdon et al. 2003), disease resistance (Ford & Haskin 1987), and growth rates in a variety of shellfish species through selective breeding practices (see Guo 2009 for review). Baseline information on hatchery stock diversity remains an important goal in aquaculture given the potential consequences, both positive and negative, associated with broodstock selection and management techniques.

Culture of *Mercenaria mercenaria* in Florida occurs primarily in the Gulf of Mexico, where the natural occurrence of this species is controversial. Palmer (1927) listed the species distribution as occurring in waters along the Texas coast; Harte (2001), however, contends that these were mostly likely mistaken identifications. Sources of *M. mercenaria* introductions

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into the Gulf of Mexico may be derived from either experimental plantings to assess the suitability for potential aquaculture circa 1960 (Woodburn 1961) or as a result of contemporary aquaculture escape (Arnold et al. 2004). It is difficult to distinguish *M. mercenaria* morphologically from the closely related *Mercenaria campechiensis* (Gmelin, 1791), which does occur naturally in the Gulf of Mexico, and the two species hybridize naturally in Florida (Bert & Arnold 1995, Arnold et al. 1996, Arnold et al. 2004). Given the magnitude of the hard clam industry (upward of 184 million hard clams were produced in 2007; Adams et al. 2009), examination of genetic consequences (reduced genetic diversity, inbreeding) associated with present culture techniques warrant consideration.

The objective of this study was to compare levels of neutral genetic variation within and among hatchery stocks relative to wild populations. We hypothesized that hatchery-produced hard clams would show evidence of reduced allelic diversity and lower levels of heterozygosity relative to wild conspecifics as a result of breeding techniques that use limited numbers of broodstock. Genetic differentiation of wild and hatchery stocks was quantified to understand the extent to which isolation has impacted allele frequencies in hatchery populations. We evaluated competing models of gene flow among wild stocks to test if differences between hatchery and wild stocks were the product of gene flow, genetic drift, or a combination of the two. Lastly, we amplified the same suite of microsatellite markers on a closely related species, *Mercenaria campechiensis*, to assess their efficacy for the detection of interspecific hybridization.

MATERIALS AND METHODS

Individuals of *Mercenaria mercenaria* were collected from four Atlantic coastal Florida locations (north to south): St. Augustine, St. Johns County, $n = 53$ (N 29.728275, W 81.251642); Mosquito Lagoon, Volusia County, $n = 13$ (N 28.922448, W 80.842134); Mosquito Lagoon, Brevard County, $n = 22$ (N 28.723907, W 80.713320); and Indian River Lagoon, St. Lucie County, $n = 8$ (N 27.543921, W 80.338136) (Fig. 1). Samples of hatchery stocks were collected from four hatcheries operating along the Atlantic coast and two operating in the Gulf of Mexico. Sample sizes ranged from 15 to 36 individuals per hatchery stock. Samples ($n = 48$) of *Mercenaria campechiensis* were collected from one population sampled at Bradenton Beach, Manatee County, FL (N 27.44832, W 82.68879, Fig. 1).

DNA was extracted from adductor muscles (~3 mm^3 tissue per sample) using the plate extraction protocol described by Ivanova et al. (2006). DNA was quantified using a Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE) and standardized at 20 ng/μL prior to PCR amplification. Seven microsatellite loci (Wang et al. 2010) were amplified in 15 μL simplex reactions containing 20 ng template DNA, 7.5 μL Qiagen Multiplex PCR Master Mix (Qiagen, Valencia, CA), 5.9 μL ddH_20, 0.02 μM forward primer, 0.2 μM reverse primer, and 0.2 μM dye primer. A CAC-sequence tag was added to the 5′ end of all forward primers (5′-CACGACGTTGTAAAACGAC-3′). A third dye-primer was used in each PCR reaction that contained the same CAC-sequence and one of two 5′-fluorescent dyes [6-carboxyfluorescein (FAM) or hexachlorofluorescein (HEX)]. Thermal cycling conditions were: 95°C for 15 min, followed by 35 cycles of 94°C for 30 sec, 58°C for 1 min 30 sec, 72°C for 1 min 30 sec, followed by a 10 min extension at 72°C. Electrophoresis of PCR products was performed using an ABI 3130xl (Applied Biosystems, Foster City, CA) with a ROX 500 size standard (Applied Biosystems, Foster City, CA). Multiple PCR products from the same individual were combined before electrophoresis for select primer pairs (combo one: RUMM 34, RUMM 32; combo two: RUMM 21, RUMM 47, and RUMM 18). For the remaining primer pairs (RUMM 17, RUMM 20) PCR products were electrophoresed individually. PCR products for marker RUMM 17 were diluted (1 μL PCR product: 30 μL double-distilled water) to facilitate microsatellite scoring (i.e., reducing excess stutter). For combo one, PCR product generated for loci RUMM 34 and RUMM 32 were mixed at a ratio of 2:1 to generate peaks of equal intensity. Microsatellites used in combos were identified based on the known characteristics of individual microsatellite regions (i.e., size range, repeat motif) (Wang et al. 2010).

Allele calls were automatically scored using GeneMarker software (SoftGenetics, State College, PA) and manually confirmed. The number of alleles per locus, observed (H_o) and expected heterozygosities (H_e), and allelic richness (A_r) were calculated in Genalex 6.5 (Peakall & Smouse 2012). Deviation from Hardy–Weinberg Equilibrium (HWE) and linkage equilibrium were tested using GENEPOP (Rousset 2008) with the following parameters: dememorization = 5,000, batches = 5,000, iterations per batch = 1,000. The frequency of null alleles was estimated using the individual breeding model (10,000 iterations) implemented in INEst v 1.0 (Chybicki & Burczyk 2009) that accounts for background levels of inbreeding. Sequential Bonferroni corrections were performed for multiple comparison tests (Rice 1989).

Global genetic differentiation among wild and hatchery stocks was estimated by computing the fixation index G_{ST} (Nei 1987), the standardized fixation index G'_{ST} (Hedrick 2005), and Jost's (2008) differentiation (D) that is independent of the amount of within-population diversity. Multiple metrics of genetic differentiation were included to account for the potential influences of mutation rates and initial heterozygosity that may bias individual measures (Leng & Zhang 2011). A lack of discrepancy among metrics was interpreted as meaning potential biases were minimal. We used 10,000 permutations to estimate the proportion of simulated data sets that would have lower differentiation under a truly random mating scenario. We estimated pairwise F_{ST} (Weir & Cockerham 1993, Slatkin 1995) and 10,000 permutations were used to test for significant differences. We applied hierarchical analysis of molecular variance (AMOVA) to examine the apportionment of genetic variance between sources (wild versus hatchery), among stocks (hatcheries 1, 2, etc.) within sources, and among individuals within individual stocks. We assumed an infinite allele model and used 10,000 permutations to test for significance of the variance partitions. All differentiation and AMOVA calculations were performed using GENODIVE v2.0 (Meirmans & Van Tienderen 2004). Inbreeding (F_{IS}) was estimated using SPAGEDI (Hardy & Vekemans 2002) after Weir and Cockerham (1984). Group average estimates of allelic richness, heterozygosity, and inbreeding were compared between wild and hatchery groups using FSTAT (Goudet 2001). Tests for significance were based on two-sided tests estimated using 1,000 permutations.

The program STRUCTURE v2.3 (Pritchard et al. 2000, Falush et al. 2003) was used to examine K, the number of genetically differentiated clusters. We included sample identification in

Figure 1. Collection sites for wild hard clam samples used to assess genetic diversity in wild and aquaculture stocks of *Mercenaria mercenaria* from the state of Florida, United States. Sampling sites for *M. mercenaria* were: (1) St. Augustine, St. Johns County, (2) Mosquito Lagoon, Volusia County, (3) Mosquito Lagoon, Brevard County, and (4) Indian River Lagoon, St. Lucie County. Point 5 was the sample site for *M. campechiensis* collections.

advance to help inform the search for the "true" K in case the data were weakly informative (Hubisz et al. 2009). We used the admixture model with correlated allele frequencies between populations. Likelihoods [LnP (K)] were examined for a range of potential population numbers ($K = 1$–15) and each population scenario was performed 10 times. MCMC consisted of 10×10^5 burn-in iterations followed by 2.0×10^6 iterations. Results from STRUCTURE runs were evaluated using STRUCTUREHARVESTER version 0.6.8 (http://taylor0.biology.ucla.edu/struct_harvest/) to facilitate the interpretation and detection of genetic clusters. Specifically, results were examined for: (1) an asymptote point at which the increase of likelihood values begins to plateau, or decrease; (2) K values at or near the asymptote having low variance across replicate runs; and (3) corresponding large K values (e.g., >100) (Earl & von Holdt 2012).

To examine alternative models of genetic population structure among wild stocks, we used the Bayesian coalescent framework implemented in MIGRATE 3.6.4 (Beerli & Felsenstein 2001, Beerli 2006). MIGRATE uses thermodynamic integration to calculate the marginal likelihood of a specified population model, a technique shown to outperform Markov chain Monte Carlo and harmonic mean estimators of model fit (Beerli & Palczewski 2010). Three competing models were examined: (1) a full migration model where each wild population represents a distinct stock having potentially different sizes (θ) that exchange migrants at unequal rates; (2) a stepping stone model where migration occurs between adjacent stocks (potentially at unequal rates); and (3) a model of panmixia assuming the sampled stocks represent a single effectively random mating population. For the MIGRATE runs, the Indian River Lagoon samples were excluded due to low sample size.

Within the program MIGRATE, a preliminary model was run to determine the appropriate run length and suitable priors for subsequent analyses (i.e., full, stepping stone, and panmixia models). Three identical, independent runs were generated for each model and the results from each model were combined. Final individual run parameters were under default conditions with the following exceptions: Brownian motion mutation model for microsatellite data; uniform theta (scaled population size) priors (min. = 0, max. = 10,000, delta = 2,000); increment between sampled genealogies (100); recorded samples per run (150,000); initial discarded samples per replicate (burn-in) (100,000). We ran six static heated chains (temperatures: 1, 4.19, 10.58, 23.35, 48.90, and 100). Natural log Bayes factors (LBF) were calculated as $\ln[mL(\text{model}_1)] - \ln[mL(\text{model}_2)]$. Model probabilities were generated by subtracting the highest marginal likelihood from each of the alternative models, taking the exponent of each pairwise difference, summing these values, and using them as the denominator. Each exponentiated value was divided by the sum to estimate the probability of an individual model (Beerli personal communication).

RESULTS

Overall, hatchery stocks exhibited a smaller range of observed heterozygosities (H_o range = 0.392–0.536) relative to the wild stocks (H_o range = 0.379–0.527; Table 1). The number of alleles across all loci was on average higher in wild stocks (mean $N_a = 7.04$) relative to hatchery stocks (mean $N_a = 6.38$). Allelic richness (A_r) averaged across loci was 3.61 for wild stocks and 3.46 for hatchery stocks. The number of alleles per locus ranged from a minimum of two (loci: RUMM 20) to a maximum of 20 (RUMM 18). We identified eight private alleles distributed across the four wild populations, and five private alleles across the six hatchery stocks (Table 1). Overall, locus-specific and multilocus F_{IS} estimates reflected high levels of inbreeding (Table 1). Differences in A_r, H_o, H_e, and F_{IS} between wild and hatchery groups were not significant (Table 2).

Null alleles were detected in both wild and hatchery *Mercenaria mercenaria*. Four loci in particular (RUMM 20, RUMM 21, RUMM 32, and RUMM 34) displayed elevated levels across multiple populations (Table 1). Of the wild populations, St. Johns County population displayed evidence of null alleles at the greatest number of loci (3) and St. Lucie County samples had the fewest (1). Hatchery groups 5 and 6 had null alleles at multiple loci (4) whereas all other hatchery populations displayed evidence of null alleles at either one or two loci. Null alleles in *Mercenaria campechiensis* were estimated for the three loci with sufficient data (RUMM 32, RUMM 34, and RUMM 17), one of which (RUMM 34) showed evidence for null alleles.

Across all stocks, 18 of 70 tests for Hardy–Weinberg equilibrium deviated from expectation after sequential Bonferroni correction (Table 1). Four loci had significant deviation from HWE in more than one population. Five of two hundred and ten tests for linkage disequilibrium were significant after Bonferroni correction (Hatchery group 2, RUMM 18–RUMM 34; group 4, RUMM 18–RUMM 34, RUMM 32–RUMM 34, RUMM 32–RUMM 17; group 6; RUMM 18–RUMM 17).

Global genetic differentiation among wild and hatchery stocks was low ($G_{ST} = 0.022$, SD = 0.003) even after correction for highly variable markers ($G'_{ST} = 0.060$, SD = 0.019; $D = 0.039$, SD = 0.018). The permuted data sets were all smaller than observed test statistics (all $P < 0.001$), suggesting that although we detected low allelic differentiation, the observed patterns across all population samples were still greater than expected under random mating. When considered separately, wild samples had little differentiation ($G_{ST} = -0.008$, SD = 0.007; $G'_{ST} = 0.019$, SD = 0.019; $D = 0.013$, SD = 0.014) and permuted data sets were nonsignificant (for all results; $0.15 < P < 0.17$). Hatchery stocks displayed overall differentiation similar to the combined wild + hatchery data set ($G_{ST} = 0.030$, SD = 0.007; $G'_{ST} = 0.070$, SD = 0.021; $D = 0.046$, SD = 0.019) and values were significantly larger than permuted data sets (all $P = 0.001$). Pairwise F_{ST} estimates were all nonsignificant between wild stocks, whereas 23 pairwise F_{ST} values were significantly greater than zero between hatchery and wild stocks and between hatchery stocks (Table 3). Differences in F_{IS} between wild and hatchery groups were not significant (Table 2).

Levels of genetic differentiation tested by AMOVA were primarily explained by high levels of variation among individuals nested within stocks (Table 4). The variances among stocks (e.g., hatcheries 1, 2, etc.) and the variances among sources (wild or hatchery) were low, with the highest variance at 4.5%. Overall, the variance partitions among individual and stock were significant although variance partitioned among stocks was low. Population source (hatchery versus wild) predicted less than 1% of the observed variation.

Bayesian estimates of genetic clustering based on likelihood and delta K scores revealed limited genetic distinction. For $K = 1$ to $K = 3$, low variance across replicate analyses was observed, making the choice of best model ambiguous. Variance increased considerably, and likelihood scores decreased beginning at $K = 4$ (Fig. 2). Delta K values were greatest at $K = 2$ and $K = 11$; these values were, however, low (both <10), limiting their utility as an estimator of ad hoc support. Given the low likelihood and large variance, it is unlikely that $K = 11$ is a good estimate of the number of genetic groups. Graphs showing individuals at $K = 2$ and $K = 3$ are shown in Figure 2. In both cases there is little structure distinguishing wild from hatchery groups, with the exception of hatchery group 4, which seems to consist of two distinct genetic groups.

Results from thermodynamic integration of the competing gene flow models (full, stepping stone, and panmixia) applied to the three largest wild stocks showed that panmixia had the highest marginal likelihood (–2,928) (Table 5). The likelihood associated with the panmixia model far exceeded the full migration (–25,746) and stepping stone models (–35,091). An LBF of 22,548 was observed based on the likelihoods of panmixia and full migration, which is very strong preference for the panmixia model (Kass and Raftery 1995). The corresponding model probability for panmixia was ~1.0. A characteristic of the full model was the similar rates per generation of mutation-scaled allelic migration (M) among locations (mean = 168.27, SD = 7.50). Additionally, a very large theta was observed (mutation-scaled effective population size) for the intermediate location (Brevard; $\theta = 2083.5$, 95% CI = 0–3393.3) relative to the northern ($\theta = 24.7$, 95% CI = 0–166.7) and southern populations ($\theta = 22.4$, 95% CI = 0–160.0). The panmixia model had a $\theta = 100.93$ (95% CI = 33.4–266.7).

For *Mercenaria campechiensis*, three of seven loci amplified at greater than 50% of the available samples (Table 1). PCR success rates based on a minimum of two amplification attempts per sample were: RUMM 17%–88%, RUMM 32%–85%,

TABLE 1.

Summary statistics for seven microsatellite loci used to screen four wild and six hatchery populations of *Mercenaria mercenaria* from Florida, United States.

Source		Locus							Average
		RUMM 21	RUMM 18	RUMM 47	RUMM 32	RUMM 34	RUMM 20	RUMM 17	across loci
Wild									
St. Johns County ($n = 53$)									
	N_a	4	16	3	8	12	4	9	8.000
	H_o	0.200	0.813	0.208	0.415	0.460	0.170	0.804	0.439
	H_e	0.326	0.920	0.191	0.722	0.771	0.504	0.757	0.599
	P HWE	*0.002*	0.040	1.000	*0.000*	*0.000*	*0.000*	0.143	–
	Freq null	0.116	0.045	0.044	**0.152**	**0.152**	**0.217**	0.015	0.106
	F_{IS}	0.394	0.133	–0.079	0.408	0.412	0.668	–0.052	0.273
	N_p	1 (0.045)	1 (0.010)	–	–	–	–	1 (0.010)	3
Volusia County ($n = 13$)									
	N_a	4	10	3	8	7	3	6	5.900
	H_o	0.200	0.846	0.154	0.692	0.750	0.231	0.818	0.527
	H_e	0.410	0.843	0.145	0.769	0.719	0.411	0.719	0.574
	P HWE	0.029	0.684	1.000	0.032	0.040	0.076	0.603	–
	Freq null	**0.161**	0.039	0.112	0.066	0.057	**0.157**	0.047	0.092
	F_{IS}	0.556	0.175	–0.044	0.139	0.000	0.471	–0.091	0.146
	N_p	–	–	–	–	1 (0.042)	–	–	1
Brevard County ($n = 22$)									
	N_a	4	14	3	6	6	4	7	6.300
	H_o	0.250	0.667	0.182	0.450	0.150	0.273	0.682	0.379
	H_e	0.519	0.899	0.169	0.626	0.696	0.470	0.772	0.593
	P HWE	*0.000*	*0.006*	1.000	0.083	*0.000*	0.032	0.302	–
	Freq null	**0.146**	0.097	0.117	0.090	**0.222**	0.110	0.047	0.119
	F_{IS}	0.231	0.309	–0.073	0.305	0.583	0.495	0.023	0.293
	N_p	–	2 (0.044)	–	–	–	–	1 (0.015)	3
St. Lucie County ($n = 8$)									
	N_a	3	9	3	4	6	4	5	4.900
	H_o	0.000	0.750	0.500	0.500	0.429	0.375	0.625	0.454
	H_e	0.449	0.867	0.398	0.648	0.776	0.578	0.695	0.630
	P HWE	*0.007*	0.219	1.000	0.591	0.016	0.165	0.425	–
	Freq. null	**0.267**	0.066	0.090	0.110	0.140	0.136	0.085	0.128
	F_{IS}	1.000	0.207	–0.200	0.079	0.507	0.408	0.167	0.321
	N_p	–	1 (0.063)	–	–	–	–	–	1
Hatchery									
Group #1 ($n = 15$)									
	N_a	4	16	3	4	6	3	7	6.143
	H_o	0.214	0.733	0.214	0.400	0.286	0.133	0.667	0.393
	H_e	0.311	0.911	0.196	0.544	0.735	0.407	0.769	0.593
	P HWE	0.001	0.009	0.887	0.046	*0.000*	0.013	0.217	
	Freq null	0.123	0.041	0.082	0.099	**0.196**	**0.184**	0.058	0.112
	F_{IS}	0.334	0.228	0.297	0.634	0.691	0.167	0.442	0.348
	N_p	–	–	1 (0.036)	–	–	1 (0.033)	–	2
Group #2 ($n = 31$)									
	N_a	3	17	3	6	7	5	8	7
	H_o	0.143	0.862	0.333	0.233	0.517	0.500	0.742	0.466
	H_e	0.249	0.914	0.290	0.483	0.682	0.583	0.701	0.561
	P HWE	*0.000*	0.005	0.996	*0.000*	*0.000*	0.010	0.424	–
	Freq null	**0.168**	0.027	0.059	0.039	0.119	0.090	0.030	0.076
	F_{IS}	0.442	0.075	–0.130	0.529	0.258	0.159	–0.042	0.164
	N_p	–	–	–	–	–	1 (0.100)	–	1
Group #3 ($n = 32$)									
	N_a	4	20	3	6	9	5	8	7.857
	H_o	0.310	0.821	0.429	0.625	0.469	0.345	0.677	0.536
	H_e	0.547	0.925	0.357	0.521	0.725	0.579	0.769	0.642
	P HWE	*0.000*	0.009	0.930	0.234	0.001	*0.000*	0.015	–
	Freq null	0.130	0.055	0.115	**0.163**	0.045	0.102	0.036	0.093

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TABLE 1.

continued

Source		Locus							Average across loci
		RUMM 21	RUMM 18	RUMM 47	RUMM 32	RUMM 34	RUMM 20	RUMM 17	
	F_{IS}	0.447	0.130	–0.183	0.183	0.367	0.419	0.135	0.185
	N_p	–	–	–	–	–	1 (0.017)	–	1
Group #4 ($n = 36$)									
	N_a	3	13	3	4	7	2	8	5.714
	H_o	0.100	0.720	0.308	0.139	0.600	0.167	0.944	0.419
	H_e	0.471	0.894	0.266	0.422	0.740	0.239	0.834	0.582
	P HWE	*0.000*	*0.000*	1.000	*0.000*	0.001	0.010	0.057	–
	Freq null	0.072	0.079	0.066	**0.146**	0.214	**0.225**	0.061	0.124
	F_{IS}	0.797	0.214	–0.139	0.678	0.204	0.316	–0.119	0.247
	N_p	–	–	–	–	–	–	–	0
Group #5 ($n = 9$)									
	N_a	5	9	3	3	5	2	6	4.714
	H_o	0.625	0.667	0.750	0.333	0.250	0.111	0.778	0.486
	H_e	0.500	0.809	0.531	0.512	0.719	0.278	0.716	0.648
	P HWE	0.629	0.288	0.995	0.003	*0.000*	0.178	0.643	–
	Freq null	**0.230**	0.065	0.123	**0.246**	**0.231**	**0.248**	0.030	0.168
	F_{IS}	–0.186	0.232	–0.286	0.400	0.689	0.636	–0.028	0.201
	N_p	–	–	–	–	–	–	–	0
Group #6 ($n = 26$)									
	N_a	5	17	3	5	7	3	8	6.857
	H_o	0.087	0.833	0.217	0.227	0.308	0.192	0.885	0.392
	H_e	0.274	0.904	0.199	0.611	0.805	0.510	0.805	0.595
	P HWE	*0.000*	0.023	0.999	*0.000*	*0.000*	0.003	0.337	–
	Freq null	**0.250**	0.036	0.112	**0.274**	**0.291**	**0.276**	0.049	0.184
	F_{IS}	0.694	0.098	–0.068	0.642	0.630	0.634	–0.079	0.349
	N_p	–	–	–	–	–	–	1 (0.021)	1
M. campechiensis ($n = 48$)									
	N_a	–	–	–	8	8	–	8	8
	H_o	–	–	–	0.585	0.229	–	0.548	0.454
	H_e	–	–	–	0.735	0.725	–	0.650	0.703
	P HWE	–	–	–	0.042	0.000*	–	0.089	–
	Freq null	–	–	–	0.052	**0.205**	–	0.046	0.101
	F_{IS}	–	–	–	0.215	0.692	–	0.170	–

Data from *M. campechiensis* is included only for loci that amplified in greater than 50% of individuals.
n, number of individuals; N_a, number of alleles; H_o, observed heterozygosity; H_e, expected heterozygosity; *P* HWE, probability of allele frequency in Hardy-Weinberg equilibrium following Bonferroni correction Freq null = null allele frequency; F_{IS}, inbreeding; N_p, number of private alleles and their frequencies. Bold values indicate loci with null alleles present.

RUMM 34%–73%, RUMM 47%–48%, RUMM 21%–10%, RUMM 18%–4%, and RUMM 20%–0%. For markers with consistent amplification, the observed heterozygosity was 0.45, expected heterozygosity was 0.70 (SE = 0.03), the number of alleles was eight (SE = 0.00), and allelic richness was 2.25.

DISCUSSION

The aquaculture production of molluscs has expanded significantly in recent decades and now accounts for 24% of the total aquaculture production worldwide (FAO 2012). Genetic resources represent an invaluable asset in the aquaculture production of molluscs as molecular techniques have been successfully used to increase both growth and survival (e.g., Langdon et al. 2003, Guo 2009). In this study, we examined if current propagation techniques for *Mercenaria mercenaria* were effective at capturing levels of the genetic diversity similar to those observed in wild stocks. Overall, allelic richness and levels of heterozygosity were similar in wild stocks relative to hatchery-produced clams. Pairwise differentiation was significant between many hatchery-by-hatchery, and some hatchery-by-wild comparisons, likely reflecting genetic drift associated with broodstock selection and breeding practices.

The low levels of genetic differentiation between hatchery and wild hard clams observed in this study may be explained by the selection of genetically varied broodstock and the periodic supplementation of broodstock. Hatchery populations founded with few individuals would be expected to show signs of reduced genetic diversity and genetic drift after even a few generations of isolation (Gosling 1982, Hedgecock & Sly 1990). If hard clam hatchery seed were used as broodstock each generation, and each hatchery represented a closed population, we would expect a greater extent of differentiation between hatchery and wild stocks than observed here (e.g., Li et al. 2003). As reported by Whetstone et al. (2005), *Mercenaria mercenaria* can live up to 30 y of age, and hatcheries included in this study have been functional for approximately 15 y (Sturmer personal observation). Assuming an annual production cycle, inbreeding levels

TABLE 2.

Average values of allelic richness, observed and expected heterozygosities, and inbreeding grouped by source population (wild or hatchery) for *Mercenaria mercenaria* collected from Florida.

	Wild	Hatchery	*P*-value
A_r	3.610	3.460	0.145
H_o	0.448	0.452	0.931
H_e	0.613	0.591	0.318
F_{IS}	0.269	0.236	0.490

P values indicate significant differences in values as estimated using FSTAT.

A_r, allelic richness; H_o, observed heterozygosity; H_e, expected heterozygosity; F_{IS}, inbreeding coefficient.

following 15 y of propagation would be sufficient for hatchery populations to display evidence of genetic drift associated with decreased effective population size (Durand et al. 1993, Evans et al. 2004). The extent to which spawning practices vary between hatcheries in Florida is not known, though considerable variation in techniques is present. Some hatcheries select ripe adults for use in seed production from the harvests of cultured stocks. One hatchery maintains a large (~200) group of adults, but also supplements broodstock from harvests. The addition of novel genetic material may serve to limit the extent of genetic differentiation between stocks (Waples 1998). An alternative explanation is that periodic supplementation from wild stocks has occurred, although *M. mercenaria* wild stocks are rare, particularly along the Gulf of Mexico coast, and unlikely to be used by hatchery owners.

The loss of genetic diversity was not as pronounced as seen in other comparisons of wild and aquacultured bivalves. Genetic differences in microsatellite genotypes between cultured, native, and naturalized populations of Pacific oysters *Crassostrea gigas* revealed significantly lower levels of allelic richness (36.5% decrease) and expected heterozygosity (5.6% decrease) in hatchery relative to wild populations (Miller et al. 2012). Genetic variation in cultured populations of Asian Suminoe oysters *Crassostrea ariakensis* was indicative of a genetic bottleneck, with a 6-fold increased extent of genetic differentiation and a loss of allelic richness in excess of 60% among hatchery stocks (Xiao et al. 2011). Our results mirror that of Dillon and Manzi (1987), who found allele frequencies were similar between wild and hatchery stocks of *Mercenaria mercenaria* based on allozyme data, although evidence for the loss of rare alleles and genetic drift was inferred for hatchery stocks. Given the potential for negative consequences associated with losses of genetic diversity, hatchery stocks would benefit from maintaining large numbers of potential broodstock to increase effective population size and minimize genetic drift.

Regional panmixia among wild stocks of hard clams was inferred based upon several lines of genetic evidence. First, AMOVA tests of genetic differentiation among wild stocks were nonsignificant. Second, there was a distinct lack of population clustering associated with Bayesian analysis of individual genotypes. And lastly, statistical support was strongest for a panmictic model of gene flow. On the basis of its planktonic larval stage, *Mercenaria mercenaria* was predicted to have little or no genetic structuring at the scale examined here (Eversole 2001, Hellberg et al. 2002), and previous genetic work revealed limited organellar genetic structuring range-wide (Baker et al. 2008). As the southernmost population of wild hard clams was omitted from model tests, our results suggest that individuals from Indian River Lagoon and St. Augustine are genetically similar. This is not surprising given the close geographic proximity of the wild populations and a lack of major biogeographic barriers that would prevent gene flow. These results are conditional based on the limitations of sample sizes herein. The number of clams available from wild and hatchery-produced stocks was limited in some cases. For example, STRUCTURE may be biased when samples sizes are small (e.g., Fogelqvist et al. 2010) or unbalanced, as is the case here. Empirical data, however, suggest that STRUCTURE is biased toward over splitting when sample sizes are unbalanced (Onogi et al. 2011). Thus, our inference of a single genetic cluster is probably not overly conservative given the other lines of evidence (e.g., independent estimates of effective population size and migration using MIGRATE).

One important consideration for population genetic analysis of bivalves is the presence of null alleles and their potential to confound interpretation of differentiation metrics (Chapuis & Estoup 2006). Null alleles have been reported at a significant

TABLE 3.

Pairwise F_{ST} and *P* values based on microsatellite data collected for six hatchery and four wild populations of *Mercenaria mercenaria* collected in Florida, United States.

	St. Johns	Volusia	Brevard	St. Lucie	Hatchery 1	Hatchery 2	Hatchery 3	Hatchery 4	Hatchery 5	Hatchery 6
St. Johns	–	0.662	0.064	0.164	0.253	*0.004*	*0.005*	*0.001*	*0.047*	*0.001*
Volusia	–0.004	–	0.286	0.291	0.445	*0.020*	*0.008*	*0.015*	0.126	*0.009*
Brevard	0.009	0.005	–	0.171	0.101	*0.003*	*0.010*	*0.001*	0.152	*0.001*
St. Lucie	0.010	0.008	0.016	–	0.269	*0.048*	0.081	*0.018*	0.345	0.131
Hatchery 1	0.005	0	0.016	0.011	–	0.280	0.388	*0.005*	0.582	0.217
Hatchery 2	0.021	0.029	0.03	0.03	0.004	–	0.145	*0.001*	0.094	*0.001*
Hatchery 3	0.02	0.029	0.019	0.02	0.001	0.007	–	*0.001*	0.341	*0.002*
Hatchery 4	0.053	0.045	0.038	0.068	0.06	0.085	0.073	–	*0.028*	*0.001*
Hatchery 5	0.024	0.02	0.019	0.009	–0.007	0.02	0.003	0.048	–	*0.026*
Hatchery 6	0.038	0.038	0.054	0.022	0.009	0.042	0.032	0.104	0.039	–

Pairwise F_{ST} values are below diagonal, *P* values above diagonal (italicized *P* values are significant after sequential Bonferroni correction < 0.05).

TABLE 4.

Analysis of molecular variance (AMOVA) performed on *Mercenaria mercenaria* collected from wild and hatchery sources from Florida, United States.

Locus	Source of variation	Nested in	SSD	d.f.	Estimated variance	% Variance	*P*-value
Wild	Within individual	–	31.00	88	1.557	72.7	–
	Among individual	Stock	82.86	85	0.591	27.6	**<0.000**
	Among stock	–	5.98	2	–0.007	–0.3	0.690
Hatchery	Within individual	–	242.00	149	1.624	74.6	–
	Among individual	Stock	362.41	143	0.455	20.9	**<0.000**
	Among stock	–	36.18	5	0.098	4.5	**<0.000**
Combined	Within individual	–	390.50	245	1.594	73.1	–
	Among individual	Stock	620.79	235	0.524	24	**<0.000**
	Among stock	Source	43.11	8	0.061	2.8	**<0.000**
	Among source	–	7.06	1	0.001	0.1	0.428

Sources of variation are divided by individuals nested within stock and for combined analysis nested by source (wild versus hatchery).

portion of loci used in genetic research on bivalves (McGoldrick et al. 2000, Hedgecock et al. 2004, Reece et al. 2004). Potential mechanisms to explain the increased prevalence of null alleles in bivalves include high intraspecific polymorphism within the genome (Hedgecock et al. 2004, Reece et al. 2004) and recombination that acts in addition to neutral point mutations (McGoldrick et al. 2000). Various studies have dealt with null alleles in different ways. Some have elected to conduct population level analysis with and without affected markers (Carlsson et al. 2006), whereas others have included all loci under the assumption that all populations are likely impacted equally (Miller et al. 2012). We elected to include all loci in our analysis as null alleles were present in similar frequencies across wild and hatchery populations. As a result, differentiation measured in this study might represent a conservative estimate as levels of homozygosity may be artificially inflated due to a failure to amplify null alleles (Jones et al. 1998).

A secondary objective of this study was to assess the utility of these microsatellite markers for use in *Mercenaria* spp. hybridization. Amplification of *Mercenaria campechiensis* was largely unsuccessful, a result that was not surprising given the high degree of intraspecific genome diversity observed in bivalve

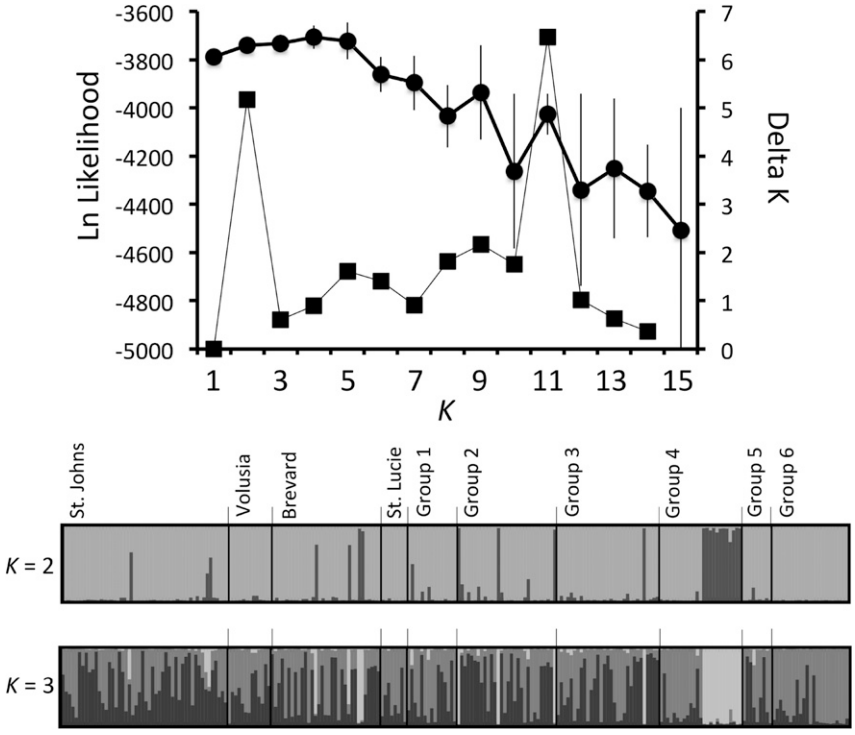

Figure 2. Results from STRUCTURE analyses of microsatellite genotypes collected for *Mercenaria mercenaria* specimens collected from wild and aquaculture stocks in Florida, United States. Top: Graph of the Ln likelihood scores (SD) (circles) and delta K scores (squares) across tested *K* values. Bottom: Bar plots of genetic clusters for $K = 2$ and $K = 3$. Each individual bar is one individual genotype, and different grey-scales represent the proportion of an individual genome assigned to one of *K* clusters.

TABLE 5.

Log marginal likelihoods (LML) based on thermodynamic integration of three competing gene flow models used to examine gene flow in wild populations of *Mercenaria mercenaria* collected from the Atlantic Coast of Florida.

Model	Model rank	LML	Difference from best model	Model probability
Panmixia	1	–2,928	–	>0.999
Full	2	–25,476	22,548	~0
Stepping stone	3	–35,091	32,163	~0

species (Reece et al. 2004). Hedgecock et al. (2004) found that useful cross-species amplification rates were highly variable across loci, where successful amplifications ranged from 12.8% to 96.5%. Evolutionary divergence among species and nucleotide substitutions in microsatellite flanking regions are suggested mechanisms for the failure to amplify DNA from closely related species (Hedgecock et al. 2004). In developing microsatellite markers for blacklip abalone *Haliotis rubra*, Li et al. (2003) reported high rates of null alleles in a single family and poor cross-species amplification. Increased mutation rates as a by-product of high fecundity was suggested as a potential explanatory mechanism. Based on our own findings, it appears that both null alleles and poor cross-species amplification are present in *Mercenaria* spp. Research involving known family lines would be useful for understanding the transmission and behavior of microsatellite markers in hard clams (Li et al. 2003). Development of microsatellites for hard clam species in the future should be performed *de novo*.

Moving forward, knowledge of the specific practices used by individual hatcheries, including numbers of broodstock and their retention times, would be helpful in elucidating the mechanisms responsible for the observed differences in levels of genetic diversity. Broodstock are thought to be supplemented using clams harvested from aquaculture farms that display favorable characteristics (e.g., physical appearance, ripeness; Sturmer personal observation). Additionally, the Florida clam aquaculture industry is largely decentralized and clam seed are produced by a number of different commercial operations. These factors may have the opposite effect than might be expected from breeding programs that use a mass selection approach (e.g., the Pacific oyster), where breeding for specific traits can reduce the standing levels of genetic variation (Appleyard & Ward 2006). Increasingly, genomic tools are being used to characterize putatively adaptive markers, markers under selection, and quantitative traits in aquaculture species (Cerda & Manchado 2013). Identifying genes and gene families associated with specific traits of interest (e.g., growth, heat stress response) would enable future studies to examine the difference in genetic diversity measured using neutral and nonneutral genetic markers (e.g., Monzón-Argüello et al. 2013). Such investigations would help to further our understanding of how the genes and genomes of species such as *Mercenaria mercenaria* are influenced by breeding practices associated with aquaculture production techniques.

In conclusion, the findings of this study suggest that differences in genetic diversity and differentiation between wild and aquaculture stocks of hard clams in Florida is limited relative to other, previously published aquaculture studies. Recommendations for maintaining genetic diversity in hard clams produced via aquaculture include: (1) keeping detailed records of the numbers of broodstock used in seed production and their sources; (2) making efforts to equalize contribution by individual families; and (3) incorporating large numbers of rotating broodstock to minimize the potential for genetic drift associated with closed populations. As aquaculture production of hard clams in the southeastern United States expands, the continued use of genetic techniques to inform and enhance broodstock selection techniques and clam production should remain an important goal.

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