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Genetic diversity and phenotypic variation within hatchery-produced oyster cohorts predict size and success in the field

A. Randall Hughes [1,5] Torrance C. Hanley [1] James E. Byers,[2] Jonathan H. Grabowski,[1] Tom McCrudden,[3] Michael F. Piehler,[4] and David L. Kimbro[1]

[1]*Marine Science Center, Northeastern University, Nahant, Massachusetts 01908 USA*
[2]*Odum School of Ecology, University of Georgia, Athens, Georgia 30602 USA*
[3]*Research Aquaculture, Inc., Tequesta, Florida 33469 USA*
[4]*Institute of Marine Sciences, University of North Carolina at Chapel Hill, Morehead City, North Carolina 28557 USA*

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Abstract. The rapid growth of the aquaculture industry to meet global seafood demand offers both risks and opportunities for resource management and conservation. In particular, hatcheries hold promise for stock enhancement and restoration, yet cultivation practices may lead to enhanced variation between populations at the expense of variation within populations, with uncertain implications for performance and resilience. To date, few studies have assessed how production techniques impact genetic diversity and population structure, as well as resultant trait variation in and performance of cultivated offspring. We collaborated with a commercial hatchery to produce multiple cohorts of the eastern oyster (*Crassostrea virginica*) from field-collected broodstock using standard practices. We recorded key characteristics of the broodstock (male : female ratio, effective population size), quantified the genetic diversity of the resulting cohorts, and tested their trait variation and performance across multiple field sites and experimental conditions. Oyster cohorts produced under the same conditions in a single hatchery varied almost twofold in genetic diversity. In addition, cohort genetic diversity was a significant positive predictor of oyster performance traits, including initial size and survival in the field. Oyster cohorts produced in the hatchery had lower within-cohort genetic variation and higher among-cohort genetic structure than adults surveyed from the same source sites. These findings are consistent with "sweepstakes reproduction" in oysters, even when manually spawned. A readily measured characteristic of broodstock, the ratio of males to females, was positively correlated with within-cohort genetic diversity of the resulting offspring. Thus, this metric may offer a tractable way both to meet short-term production goals for seafood demand and to ensure the capacity of hatchery-produced stock to achieve conservation objectives, such as the recovery of self-sustaining wild populations.

Key words: conservation aquaculture; diversity; genetic variation; hatchery; intraspecific variation; oyster; sex ratio.

Introduction

Appreciation for intraspecific variation, whether the result of artificial selection, genetic by environmental interactions, or phenotypic plasticity, has a long history in applied science fields such as aquaculture, agronomy, and silviculture. In fact, the role of selective breeding in generating lines of animal and plant species that varied in key traits was influential in Charles Darwin's development of the theory of natural selection (Darwin 1859). Today, the selection of favorable traits of terrestrial and marine organisms is still critical in agriculture and aquaculture systems. For example, the Sydney rock oyster (*Saccostrea glomerata*) is the focus of an aquaculture industry in New South Wales, Australia, that is supported by a highly controlled breeding program of more than 160 single pairwise mated family lines (Dove and O'Connor 2009). Although variation among populations (i.e., varieties, lines, cohorts, or stocks) is a key feature of such breeding programs, the selection process often leads to reduced genetic diversity within these populations, in part because such variation may counteract performance of the trait(s) of interest, such as disease resistance or rapid growth. As a result, the effective population size and/or genetic diversity of cultivated stocks is often lower than natural stocks (Williams and Davis 1996, Notter 1999, van de Wouw et al. 2010, Araki and Schmid 2010, Morvezen et al. 2016, Zhang et al. 2017). Even when overall genetic variation remains high in

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[5] E-mail: rhughes@northeastern.edu

cultivated stocks, as in the grain crop maize, artificial selection can lead to low genetic diversity at key genes, such as those involved in starch production and yield, compromising future breeding efforts (Whitt et al. 2002). Understanding relationships among breeding practices, genetic variation, and key traits is critical to meet current production needs and to allow for changing production objectives (Notter 1999, Whitt et al. 2002).

Aquaculture, or the controlled cultivation and production of aquatic organisms, is now on par with wild seafood harvest (Bostock et al. 2010) and is the fastest growing food industry in the world (Froehlich et al. 2018). This rapid growth offers both risks and opportunities for resource management and conservation (Froehlich et al. 2017). For instance, aquaculture can have negative impacts on adjacent ecosystems (Silva 2012), as well as on the target species themselves through reduced performance and viability due to inbreeding, genetic drift, and hybridization (Bostock et al. 2010, Christie et al. 2016); these negative impacts have resulted in aquaculture at times being viewed as at odds with conservation (Froehlich et al. 2017). However, aquaculture can reduce fishing pressure on wild fish stocks, which is especially important for fishery species that are ecosystem engineers, such as oysters and mussels, that provide several ecosystem services.

Aquaculture efforts focus not only on food production, but also on stock restorations and stock enhancements to enable recovery of self-sustaining wild populations (Grant et al. 2017). Such "conservation aquaculture" efforts, implemented primarily through the use of hatcheries, aim to support the sustainable use and/or recovery of natural resources through mitigation, prevention, or restoration (Froehlich et al. 2017). Despite their potential, key research gaps regarding the impacts of hatchery practices remain, particularly regarding the genetic diversity, trait variation, and fitness of hatchery-reared populations in natural environments (Araki and Schmid 2010, Froehlich et al. 2017, Grant et al. 2017). Such intraspecific variation can be important not only for population performance, but also for community interactions and ecosystem function (Hughes et al. 2008, Bolnick et al. 2011), with important implications for conservation and management outcomes.

The bulk of aquaculture production for food still comes from wild or recently domesticated stocks (Bostock et al. 2010). Reduced effective population size and decreased genetic diversity can occur during cultivation from these stocks if they have high fecundities and associated high variance in reproductive success (i.e., sweepstakes reproductive success; Hedgecock and Pudovkin 2011), as appears to be the norm in shellfish aquaculture (Hedgecock et al. 1996, Boudry et al. 2002, Lind et al. 2009, 2010), even if they are not being actively selected for a particular trait (Morvezen et al. 2016). Thus, hatchery cultivation practices are expected to generate enhanced variation among populations, but reduced variation within populations. Experiments with natural populations of aquaculture species suggest that these changes in intraspecific variation within and across populations could have large effects on both production and resilience to environmental change. For example, genetic identity, diversity, and relatedness within populations of seagrasses and marsh plants have clear benefits for plant production, resistance to disturbance, and community composition, abundance, and diversity (Hughes and Stachowicz 2004, 2011, Hughes et al. 2014). In addition, genetic diversity and relatedness within and between populations of marine invertebrates have strong effects on settlement and colonization success of early life-history stages (Gamfeldt et al. 2005, Aguirre et al. 2013, Smee et al. 2013, Plough et al. 2016). These examples suggest that understanding the links between hatchery cultivation practices, intraspecific genetic and trait variation, and population performance will be critical to the success of conservation aquaculture efforts.

Filter-feeding shellfish like oysters and mussels comprise ~30% of worldwide aquaculture production (Froehlich et al. 2018), and hatcheries play a critical role in supplying juveniles ("seed") for these efforts, particularly in areas where natural recruitment is limiting (Proestou et al. 2016). Along the Atlantic and Gulf coasts of the United States, the Eastern oyster (*Crassostrea virginica*) once supported a widespread wild fishery, and it is now the focus of extensive and increasing aquaculture, stock enhancement, and stock restoration efforts (Kirby 2004, Beck et al. 2011, Kennedy et al. 2011, Grabowski et al. 2012). Across this region, some hatcheries maintain selective breeding programs to produce *C. virginica* lines with particular traits (e.g., disease resistance; Degremont et al. 2015), whereas others use field-collected broodstock for each spawn (R. Hughes, *personal observation*). In *C. virginica*, prior efforts have focused on performance across selected oyster lines (Proestou et al. 2016), but we have little knowledge of how hatchery practices may influence genetic and trait variation within and across lines produced from field-collected broodstock. In this study, we collaborated with a commercial hatchery to produce multiple cohorts of *C. virginica* from field-collected broodstock using standard practices. These cohorts were used in prior studies to examine the effects of cohort diversity (the number of cohorts) on oyster performance (Hanley et al. 2016) and the potential for local adaptation across cohorts (Hughes et al. 2017; Appendix S1). In this study, we examined key traits of the broodstock, quantified the genetic diversity of each resulting cohort, compared adult genetic structure to observed juvenile structure, and evaluated links between genetic diversity and performance in the field to inform key knowledge gaps regarding the role of hatcheries in conservation aquaculture.

Materials and Methods

Study species

The oyster *C. virginica* is an ecologically important and economically valuable bivalve species found throughout the Gulf of Mexico and the Atlantic coast of the United States (Dame et al. 1984). Oysters create structured habitat in an otherwise soft-sediment environment; oyster reefs in turn serve as key habitat for a range of recreationally and commercially important fishes and invertebrates (Wells 1961, Bahr and Lanier 1981, Coen et al. 1999, Peterson et al. 2003). In addition, oysters filter large volumes of water and promote denitrification, removing excess nitrogen and filtering down the abundance of harmful algae and microbes (Dame et al., 1984 Newell 1988, Piehler and Smyth 2011, Smyth et al. 2015). Intraspecific variation in oysters has been shown to affect oyster population performance, highlighting the potential importance of hatchery practices for aquaculture success. For instance, experimental assemblages of adult oysters from multiple bays had greater settlement of oyster larvae and produced more and larger offspring compared to assemblages of adult oysters from a single bay (Smee et al. 2013). Similarly, in a prior study using the same oyster cohorts analyzed here, we found higher recruitment of juvenile oysters to experimental assemblages created from four juvenile oyster cohorts (i.e., lines produced in a hatchery from wild stocks) compared to those from only one or two cohorts (Hanley et al. 2016). Survivorship of juvenile oysters was also associated with decreasing genetic relatedness (Hanley et al. 2016), suggesting that intraspecific variation in oysters may enhance production via effects on multiple vital rates.

Creating oyster cohorts

In April 2012, we collected 100 adult oysters (80–100 mm shell length) from three to five separate reefs at each of six sites: St. Augustine, Florida (FL-1; 30.0224, −81.3287), Jacksonville, Florida (FL-2; 30.4446, −81.4199), Sapelo Island, Georgia (GA/SC-1; 31.4777, −81.2726), Ace Basin, South Carolina (GA/SC-2; 32.4846, −80.6001), Masonboro, North Carolina (NC-1; 34.1510, −77.8551), and Middle Marsh, North Carolina (NC-2; 34.6951, −76.6183). They were held in flowing seawater tanks or suspended in cages from docks in their home region for 2–3 weeks until 30 oysters from each site could be tested and certified as disease free. The remaining 70 oysters were then shipped on ice to a single hatchery facility in Florida (Research Aquaculture, Tequesta, Florida, USA; 26.9607, −80.0931) at the end of April.

The adult oysters from each site were used as the broodstock to produce six separate site-specific "cohorts" (one cohort per site). From their arrival at the hatchery, the adult oysters were held for 2 weeks until they were ready to spawn under the same conditions in separate flow-through seawater systems to prevent cross-contamination. All families were manually spawned (i.e., strip spawned) on 7 May. Because the original FL-1 family did not produce many offspring, the remaining broodstock oysters from this site were spawned on 1 June using the same process. Due to variation in ripeness and sex, the number of oysters spawned and the ratio of males to females varied across broodstock (Table 1), though our broodstock numbers for each cohort are comparable to those commonly used in hatchery settings (30–60 individuals; Morvezen et al. 2016).

The broodstock oysters from each source site were strip spawned, sexed, and fertilized on the same day by a team of seven people, who each had a specific job to perform: shucking the animals, sampling and preparing tissue for microscopic analysis of sex, identifying the sex, stripping the male sperm, stripping the female eggs, mixing the sperm and eggs after all of the animals from a particular source were stripped, overseeing the process and keeping track of broodstock source. We sanitized equipment between individuals and again between broodstock sources. Stripping was done by broodstock source independently and quickly so that the sperm and eggs would remain viable, and all viable sperm and eggs were used. During the gamete mixing process, the eggs from all females and the sperm from all males were first pre-mixed and then combined to ensure equal access of gametes to one another. We allowed 30–60 min for fertilization; once 75–90% of the eggs were fertilized, they were moved to larval tanks. All larvae were retained except for minimal numbers of individuals in each cohort that did not grow or had improper development. Larval culture occurred in 60-gallon (1 gallon = 3.79 L) conical tanks utilizing a flow-through seawater system with Banjo screens that is commercially used in multiple bivalve hatcheries (e.g., Taylor Shellfish in Washington; Cherrystones in Virginia).

Over a period of 3 d during the week of 28 May, oysters were sieved on a 250-μm sieve and settled on crushed oyster cultch in a recirculating flow-through system. The week of 11 June, once they reached 800 μm in size, they were moved into a nursery facility compliant with state regulations, again under flow-through seawater conditions (salinity 32 ppt, temperature 30°C). In the hatchery and nursery stages, the oysters were fed a mixed diet of *T. isochrysis*, *Chaetocerous gracilis*, and *Tetraselmis* via a constantly running peristaltic pump. Although growth was similar during the larval culture phase, some cohorts produced more juvenile oysters ("spat") than others during settlement, despite following the same procedures for all. To maintain consistency in their growing conditions, we selected a random sample of each cohort to yield similar total abundances across cohorts on 18 June. At the end of June (27 June), at ~4 mm in size, the six cohorts were transferred to a common flow-through facility at the Whitney Marine Biological Laboratory in St. Augustine, Florida, USA. To

TABLE 1. Characteristics of the juvenile cohorts and adult oysters analyzed in our study.

Site	No. oysters analyzed	Effective allelic diversity	Allele number	H_O	H_E	G_{IS}	Broodstock					
							No. males	No. females	Total	Male : female ratio	Effective population size (N_E)	Effective allelic diversity
Original 6 juvenile cohorts (2012)												
FL-1	44	4.952 (0.876)	11.583 (2.028)	0.636 (0.070)	0.732 (0.042)	**0.131 (0.068)**	6	34	40	0.18	20.40	no data
FL-2	46	3.781 (0.458)	8.333 (1.170)	0.609 (0.066)	0.689 (0.047)	**0.116 (0.069)**	7	18	25	0.39	20.16	no data
GA/SC-1	49	4.725 (0.756)	8.583 (1.164)	0.634 (0.078)	0.717 (0.050)	**0.115 (0.073)**	8	20	28	0.40	22.86	no data
GA/SC-2	54	5.042 (0.873)	9.000 (1.308)	0.608 (0.082)	0.725 (0.050)	**0.162 (0.082)**	19	22	41	0.86	40.78	no data
NC-1	46	6.025 (1.059)	11.583 (1.885)	0.611 (0.055)	0.745 (0.061)	**0.181 (0039)**	12	13	25	0.92	24.96	no data
NC-2	50	5.786 (0.960)	12.917 (2.130)	0.605 (0.073)	0.749 (0.063)	**0.192 (0.075)**	10	17	27	0.59	25.19	no data
Additional three juvenile cohorts (2016)												
FL-3	48	3.526 (0.541)	7.583 (1.041)	0.491 (0.095)	0.628 (0.065)	**0.218 (0.113)**	2	12	14	0.17	6.86	6.791 (1.413)
FL-4	48	4.844 (0.704)	8.583 (1.090)	0.575 (0.078)	0.729 (0.058)	**0.211 (0.087)**	2	13	15	0.15	6.93	7.365 (1.594)
FL-5	47	3.256 (0.299)	6.833 (0.588)	0.525 (0.089)	0.678 (0.029)	**0.225 (0.131)**	4	10	14	0.40	11.43	8.538 (1.836)
Adults (2014)												
FL-1	51	9.123 (2.225)	18.833 (3.772)	0.569 (0.059)	0.793 (0.059)	**0.283 (0.057)**	NA	NA	NA	NA	NA	NA
FL-2	47	10.343 (2.560)	18.750 (3.620)	0.624 (0.053)	0.818 (0.051)	**0.237 (0.054)**	NA	NA	NA	NA	NA	NA
GA/SC-1	46	9.746 (2.824)	18.000 (3.834)	0.587 (0.062)	0.791 (0.051)	**0.258 (0.053)**	NA	NA	NA	NA	NA	NA
GA/SC-2	47	10.074 (2.687)	18.167 (3.605)	0.606 (0.055)	0.815 (0.045)	**0.256 (0.061)**	NA	NA	NA	NA	NA	NA
NC-1	50	10.316 (2.727)	19.000 (4.004)	0.618 (0.047)	0.803 (0.051)	**0.230 (0.028)**	NA	NA	NA	NA	NA	NA
NC-2	45	9.813 (2.552)	17.833 (3.680)	0.602 (0.057)	0.792 (0.060)	**0.240 (0.043)**	NA	NA	NA	NA	NA	NA

Notes: The original six cohorts were spawned in a single hatchery in 2012, and the additional three cohorts were spawned at the same hatchery in 2016. The adults were collected from the same source reefs in 2014. Indices of genetic diversity include effective allelic diversity, allele number, observed heterozygosity H_O, expected heterozygosity H_E, and inbreeding coefficient G_{IS}. See *Methods* for a description of the loci used. Numbers in parentheses indicate standard error estimated from a jackknifing procedure in GenoDive. Boldface type indicates significant G_{IS} values using a bootstrap procedure in GenoDive with 1,000 permutations (Bonferroni correction applied, $\alpha = 0.05$). NA indicates not applicable.

assess genetic diversity within and between oyster cohorts produced in the hatchery, 50 individuals were haphazardly collected from each juvenile cohort prior to the start of the field experiments and preserved at −80°C for genetic analysis. This sample size is sufficient to estimate allele frequencies accurately (Hale et al. 2012).

To extract DNA, we ground each tissue sample with a pestle and used the tissue centrifugation protocol from the Omega Bio-Tek E-Z 96 Tissue DNA Kit (Norcross, Georgia, USA). We determined genetic diversity and population structure using 12 highly variable microsatellite loci developed for *C. virginica*: Cvi9, Cvi11, and Cvi13 from Brown et al. (2000); Cvi1i24b, Cvi2i23, Cvi2j24, and Cvi2k14 from Reece et al. (2004); Cvi4313E-VIMS from Carlsson and Reece (2007); and RUCV1, RUCV66, RUCV73, and RUCV74 from Wang and Guo (2007). We amplified four loci in each multiplexed polymerase chain reaction (PCR) using the Qiagen Type-It Microsatellite PCR Kit (Hilden, Germany). Each 10 μL reaction consisted of 1 μL DNA template, 5 μL 2X type-it multiplex master mix (Qiagen), 2.4 μL water, and 0.2 μL each 10 μmol/L primer. Using a T100 thermal cycler (Bio-Rad, Hercules, California, USA), PCR cycling conditions included initial activation/denaturation at 95°C for 5 min, followed by 28 cycles of 95°C for 30 s, 60°C for 90 s, and 72°C for 30 s, and final extension at 60°C for 30 min. PCR products were separated on a 3730xl Genetic Analyzer (Applied Biosystems) with the internal size standard GeneScan 500 LIZ (Applied Biosystems, Foster City, California, USA), and fragment analysis was performed using GeneMarker version 2.6 (SoftGenetics, State College, Pennsylvania, USA).

We created panels for each multiplexed reaction in GeneMarker, which included bins that were assigned manually for all alleles; the same panels were used to score all samples, and the alignment of the panels was checked prior to each analysis to account for any run-to-run variation and to identify any new alleles. We used these panels to do a preliminary first assignment of alleles based on peak position and bin position, but every sample was then scored manually for all loci to examine signal intensity, to confirm the presence/absence of alleles, and to identify any reruns. A subset of samples was then rerun (at least 15% per multiplex PCR reaction) and manually scored again to confirm any uncertain allele calls and account for any genotyping error.

Performance of juvenile oyster cohorts in the field

We experimentally evaluated the performance (size, growth, survivorship) of each 2012 juvenile oyster cohort in the field as a function of within-cohort effective allelic diversity. These same oysters were analyzed for different response variables as part of two other studies (Hanley et al. 2016, Hughes et al. 2017; see Appendix S1 for additional information). These studies used the same experimental design. Namely, in each experiment, 12 spat from a single cohort were affixed to 10 × 10 cm experimental tiles using the marine adhesive Z-spar (Kop-Coat Marine Group, Rockaway, New Jersey, USA). Tiles were held in flow-through seawater tables for less than 48 hours until being deployed to the field. Prior to deployment, we measured shell height of each spat and photographed all tiles. At the end of each experiment, live oysters were counted and measured.

Oysters at three of the five sites included here have previously been analyzed in a test of genetic by environmental variation across oyster cohorts (Hughes et al. 2017): spat from each cohort were deployed on 12–14 July 2012 across three field sites in the South Atlantic Bight that spanned the geographic range of the source populations: FL-EXP (29.6714, −81.2162); GA-EXP (31.9213, −80.9880), or NC-EXP (34.7069, −76.7631). At each field site, we deployed 18 tiles (six cohorts × three tiles per cohort) to each of nine natural intertidal oyster reefs. Low spat abundance in the FL-1 cohort limited replication of this cohort to four reefs per experimental site (N = 147 tiles total). The three tiles from each cohort were haphazardly assigned to one of three predation treatments (full cage, with mesh with 6 × 6 mm openings; partial cage to control for caging artifacts; no cage) and deployed on the reef in a completely randomized design; only the full cage and no cage treatments are addressed further here. This experiment lasted 6 weeks.

Data collected on oysters deployed at the other two field sites used in the present study come from a concurrent longer-term experiment focused on the effects of oyster cohort diversity that included additional treatments not analyzed here (Hanley et al. 2016). In this study, 36 tiles were deployed (six cohorts × six tiles per cohort) at each of two sites in the Matanzas River estuary, Florida (FL-North: 29.75177, −81.25578; FL-South: 29.65838, −81.22193) on 24–25 July 2012. The six tiles from each cohort were split across the same three predation treatments as above and deployed in a completely randomized design. This experiment lasted 6 months.

Adult oyster genetic diversity and sex ratios within and across source reefs

Because we found significant structure within the juvenile oyster cohorts produced in this study (see *Results*), we conducted a survey of adult oyster genetic diversity on our source reefs in 2014 to assess the extent to which adult structure corresponded to the observed juvenile structure. We also tested whether site-specific differences in the ratio of males to females observed in the 2012 broodstock oysters were consistent in 2014. *Crassostrea virginica* is a sequential protandrous hermaphrodite, first maturing as male and then changing to female. Thus, the proportion of females generally increases with size and age, yet increases in the

proportion of males have been observed at higher densities and under stressful environmental conditions (Kennedy 1983, Harding et al. 2013). In May 2014, we collected 100 adult oysters from the same oyster reefs at each of our 2012 source sites. The oysters were shipped live to the Northeastern University Marine Science Center. The shell height of each oyster was measured prior to shucking and a small sample of gill and mantle tissue was collected and stored at −80°C for genetic analysis using the same protocols for DNA extraction, PCR, and genotyping as for the hatchery-produced juvenile oysters used in the field experiment. We then scored oysters as male, female, or indeterminate based on the presence or absence of eggs or sperm in the gonadal fluid using the methods of Harding et al. (2013) and a Leica DMLB microscope (Leica Camera, Wetzlar, Germany). Because of differences between the primary goals of the 2012 and 2014 collections (i.e., produce cohorts vs. characterize sex ratio, respectively), we had a larger sample size of sexed oysters in 2014 (Fig. 4).

Analyses

We used rarefaction and extrapolation in EstimateS (Colwell 2013) to confirm that our sample size was sufficient to characterize genetic diversity accurately (see Appendix S2 for details). We then examined measures of genetic diversity per locus and per source site and assessed cohort structure and differentiation for both the juvenile oyster cohorts produced in 2012 and the adult oysters sampled in 2014 using GenoDive version 2.0b27 (Meirmans and van Tienderen 2004). We calculated allele number (a), observed (H_O) and expected (H_E) heterozygosity, and inbreeding coefficient (G_{IS}, which is analogous to F_{IS}; Nei 1987, Meirmans and van Tienderen 2004). We also calculated genetic relatedness (R) using STORM (Frasier 2008), a metric that accounts for the frequency of alleles to determine the relatedness, or genetic (dis)similarity, of a population. In addition, we calculated pairwise F_{ST} (Weir and Cockerham 1984) and analysis of molecular variance (AMOVA; Michalakis and Excoffier 1996) using GenoDive to assess cohort structure and differentiation. Finally, we used the M-ratio test (Garza and Williamson 2001) to determine whether any of the 2012 or 2016 juvenile cohorts showed evidence of a population bottleneck.

Our juvenile oyster cohorts differed from each other in several measures of genetic diversity (see *Results*), so we tested whether genetic variation predicted ecological trait variation across cohorts in our experiments. We focused on effective allelic diversity, a metric similar to allelic richness (i.e., number of alleles) that weights the number of alleles by their frequencies to determine the effective number of alleles in the population (Meirmans and van Tienderen 2004). Thus, effective allelic diversity integrates information about the number of alleles and their distribution (Meirmans and van Tienderen 2004). This metric is sensitive to variation in sample size (Meirmans 2013), so we held sample sizes consistent across groups of juvenile and adult oysters (Table 1). Effective allelic diversity was highly correlated with both allelic richness (positive; $R^2 = 0.96$; $y = 1.71x + 1.49$) and genetic relatedness (negative; $R^2 = 0.98$; $y = -0.08x + 0.52$), so we focused only on effective allelic diversity here. Results of analyses including allelic richness rather than effective allelic diversity were similar (Appendix S3). Our response variables included: initial size (average shell height per tile before being deployed in the field); survival in the absence of predation (number of live oysters in cages at the end of the experiment, modeled with a binomial generalized linear model [GLM] with logit link); survival in the presence of predation (number of live oysters on open tiles at the end of the experiment, modeled with a binomial GLM with logit link); final size (average shell height per tile at the end of the experiment); and oyster growth (standardized as (final oyster shell height − initial oyster shell height)/initial oyster shell height for each individual per tile). Because we hypothesized that differences in initial oyster size may affect oyster performance, we included initial size as a covariate in our analyses. In all analyses, we tested linear models including a fixed effect of effective allelic diversity with experimental site as a random factor to account for differences in experimental duration or other unmeasured variables. Analyses were run in R software (version 3.0.2; R Core Team 2014) using the packages lme4 and lmerTest (which calculates F and P values using the Satterthwaite approximation for degrees of freedom).

Several characteristics of the broodstock may have contributed to the observed variation in juvenile oyster genetic diversity, including variation in broodstock genetic diversity, effective population size (N_E, calculated as ([4 × number of females × number of males]/[number of females + number of males]), and/or sex ratio. We did not sample genetic diversity of the 2012 broodstock used to produce our original six cohorts at the time of spawning, precluding a direct analysis of the relationship between broodstock genetic diversity and cohort genetic diversity. To address this gap, we first examined the relationship between effective allelic diversity of the adult oysters from each site (sampled in 2014) and the juvenile oyster cohorts (spawned and sampled in 2012). We conducted our analyses on a subset of the adult oysters sampled in 2014 to maintain consistent sample sizes with juveniles (Table 1). In addition, we produced three additional oyster cohorts in 2016 in the same hatchery using broodstock from three field sites in Florida (FL-3: 30.0224, −81.3287; FL-4: 30.4446, −81.4199; FL-5: 29.7181, −84.9739) and the same methods as described above. We assessed effective allelic diversity of the 2016 juvenile cohorts and also measured the following variables on the broodstock: genetic diversity (effective allelic diversity), effective population size (N_E), and sex ratio (number of males to females). We then tested whether there were significant correlations

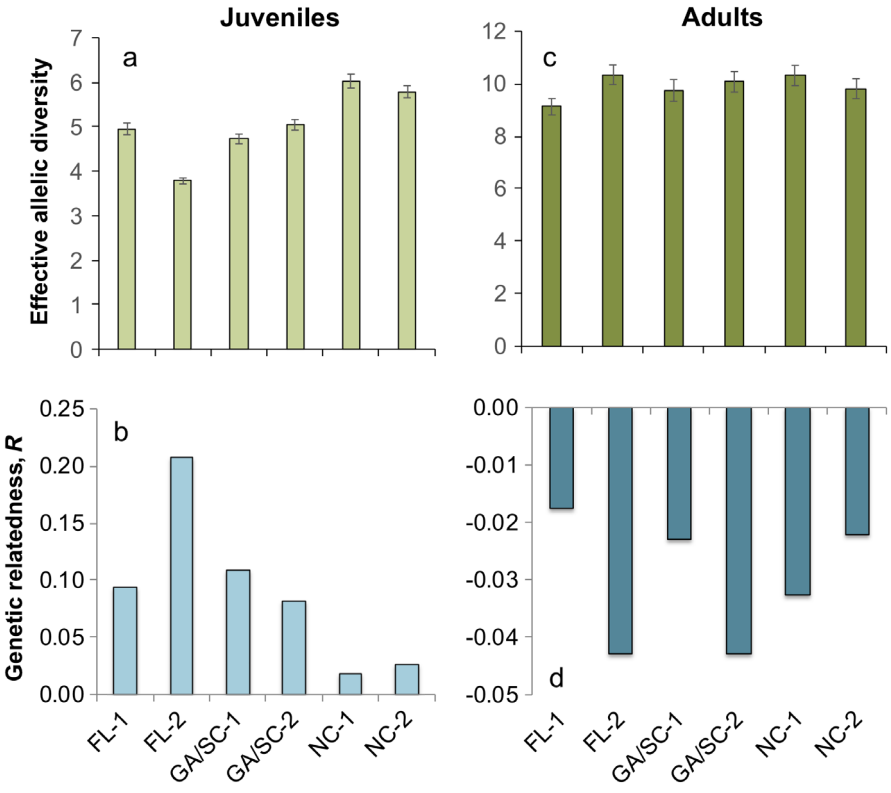

FIG. 1. (a, c) Effective allelic diversity and (b, d) genetic relatedness in (a, b) juvenile and (c, d) adult oysters. Error bars in panels a and c represent ±SE as estimated by a jackknifing procedure in GenoDive. Juvenile oysters were produced in 2012 in a single hatchery from adult broodstock collected from six field sites. Adult oysters for these analyses were collected from the same field sites in 2014. Note the different *y*-axis for panels a and c.

between these predictors and effective allelic diversity of the 2016 juvenile cohorts. Thus, the analyses of broodstock effective population size and sex ratio include nine cohorts (the original six plus the additional three), whereas the analysis of broodstock genetic diversity only includes the later three cohorts. Analyses were conducted with R statistical software (version 3.0.2; R Core Team 2014) using the lm and glm functions in the lme4 package.

To evaluate the potential for reproductive skew, we ran a parentage analysis of the 2016 broodstock and juveniles using Cervus (version 3.0.7; Kalinowski et al. 2007), assigning each juvenile to the most likely male and female parents from the known pool of broodstock.

Broodstock sex ratio was positively correlated with effective allelic diversity across cohorts (see *Results*). To examine the consistency in sex ratios through time, we also compared sex ratios of adult oysters collected from source reefs in 2012 and 2014 using a paired *t* test.

RESULTS

Genetic diversity within and across juvenile oyster cohorts

The rarefaction analysis indicated that a sample size of ≥44 oysters per cohort was sufficient to capture the genetic variation present in the original six juvenile oyster cohorts (Appendix S2). These cohorts varied almost twofold in genetic diversity, with effective allelic diversity (number of alleles weighted by their relative frequencies) ranging from 3.8 in FL_2 to 6.0 in NC_1 (Fig. 1a), and genetic relatedness ranging from 0.018 in NC_1 to 0.208 in FL_2 (Fig. 1b), though observed heterozygosity (H_O) did not clearly differ between cohorts (Table 1; Appendix S4: Table S1). In addition to genetic variation within cohorts, we identified significant genetic differentiation between cohorts for all pairwise comparisons except GA/SC_2 and GA/SC_1 (Table 2a). Similarly, hierarchical AMOVA identified significant effects of individuals within cohorts and cohorts within regions (i.e., Florida, Georgia/South Carolina, and North Carolina) on juvenile oyster genetic structure (Appendix S4: Table S2). We also found evidence of a recent population bottleneck for all hatchery-produced juvenile cohorts based on the *M*-ratio test ($0.34 < M < 0.43$ for all cohorts, with any value of $M < 0.68$ indicating that a population likely experienced a bottleneck; Garza and Williamson 2001).

Table 2. Pairwise comparison of genetic differentiation among source sites calculated using the infinite alleles model (F_{ST}) for (a) juvenile oysters produced in the hatchery in 2012 and (b) adult oysters collected in the field in 2014.

Source	FL-2	FL-1	GA/SC-2	GA/SC-1	NC-1	NC-2
a) Juveniles						
FL-2						
FL-1	**0.114**					
GA/SC-2	**0.099**	**0.070**				
GA/SC-1	**0.099**	**0.064**	0.017			
NC-1	**0.072**	**0.081**	**0.086**	**0.098**		
NC-2	**0.088**	**0.079**	**0.090**	**0.093**	**0.067**	
b) Adults						
FL-2						
FL-1	−0.002					
GA/SC-2	0.001	0.006				
GA/SC-1	0.001	0.005	0.002			
NC-1	−0.001	0.004	0.001	0.001		
NCx002D;2	0.001	0.002	0.001	−0.001	−0.001	

Notes: Boldface type indicates significant F_{ST} values using a bootstrap procedure in GenoDive with 1,000 permutations (Bonferroni correction applied, $\alpha = 0.05$.

Performance of juvenile oyster cohorts in the field

Effective allelic diversity was a significant positive predictor of initial average oyster size ($F_{1,163} = 176.41$, $P < 0.001$, $R^2 = 0.63$; Fig. 2a). Both effective allelic diversity ($P < 0.05$; Fig. 2b) and initial average oyster size ($P < 0.001$; Appendix S5: Fig. S1a) influenced survival in the absence of predation (i.e., cage treatment): juvenile cohorts with greater allelic diversity and larger initial size had generally higher survivorship. In contrast, initial average size ($P < 0.001$; Appendix S5: Fig. S1b), but not effective allelic diversity, was a significant positive predictor of survival in the presence of predation (no cage treatment). Similarly, only initial average size was significantly positively correlated with final average oyster size (initial size: $F_{1,163} = 29.41$, $P < 0.001$; Appendix S5: Fig. S1c). Growth across experimental sites did not differ significantly by effective allelic diversity ($F_{1,163} = 0.65$, $P = 0.42$) or initial average size ($F_{1,163} = 0.01$, $P = 0.92$).

Genetic diversity within and across adult oysters on source reefs

In our analysis of genetic diversity of adult oysters sampled from the field in 2014, there were minimal differences among sites in effective allelic diversity (ranging from 9.12 in FL_1 to 10.34 in FL_2; Fig. 1c) and genetic relatedness (ranging from -0.043 in FL_2 to −0.017 in FL_1; Fig. 1d). For each source site, effective allelic diversity was generally higher and genetic relatedness was substantially lower in the adult oysters collected from the field than in the juvenile oysters produced in the hatchery (Fig. 1), though observed heterozygosity (H_O) did not consistently differ between the 2012 juvenile and the 2014 adult oyster samples (Table 1). Further, our analysis of the 2014 adult oyster samples identified no significant genetic differentiation between populations, with F_{ST} values < 0.01 for all pairwise comparisons (Table 2b). Based on the results of the hierarchical AMOVA, most of the genetic variance was distributed within individuals (75%), but differences among individuals within sites accounted for 25% of the variation ($P = 0.001$; Appendix S4: Table S2).

Predictors of juvenile oyster genetic diversity

There was no relationship between effective allelic diversity of the 2014 adult oysters from each source site and effective allelic diversity of the 2012 juvenile oyster cohorts from those same sites ($R^2 = 0.002$, $P = 0.92$). However, there was a positive correlation between broodstock and cohort effective allelic diversity for the three 2016 cohorts for which data were both available ($R^2 = 0.99$). In general, broodstock diversity was approximately two times higher than cohort diversity.

Broodstock male-to-female ratio was positively correlated with effective allelic diversity across the nine cohorts ($R^2 = 0.33$, $P = 0.004$; $y = 1.92x + 3.78$; Fig. 3a), whereas effective population size showed no relationship ($R^2 = 0.21$, $P = 0.12$; Fig. 3b). These results were consistent when we analyzed only the original 6 oyster cohorts. The ratio of males to females in the 2012 broodstock varied across source site (range = 0.18–0.92; Fig. 4). Our 2014 survey from these same sites revealed that adult oyster sex ratios did not vary significantly across the two time points (*t* test, $P = 0.59$; Fig. 4).

The parentage analysis of the three 2016 cohorts demonstrated considerable reproductive skew (Appendix S4: Table S3). For example, in the FL-3 cohort, one male and female parental combination (M2 and F9) generated almost one-half of the sampled cohort, whereas some broodstock (e.g., females F4, F5, F6, and F8) were not represented in the sampled cohort (Appendix S4: Table S3).

Discussion

Our hatchery-produced oyster cohorts exhibited significant intraspecific trait variation in initial size, final size, and survival across a range of field experimental conditions, consistent with other findings of ecologically relevant intraspecific trait variation across cohorts of oysters (Smee et al. 2013) and other bivalves (Yund and McCartney 2016). This variation occurred despite the cohorts being produced in a single hatchery at the same time using the same methods, highlighting the need to identify measurable proxies for post-hatchery performance to promote the success of aquaculture efforts. To that end, we found that juvenile cohort genetic diversity explained ~60% of variation in juvenile oyster size when leaving the hatchery. Juvenile oyster size is a key variable influencing oyster performance in the wild, because of

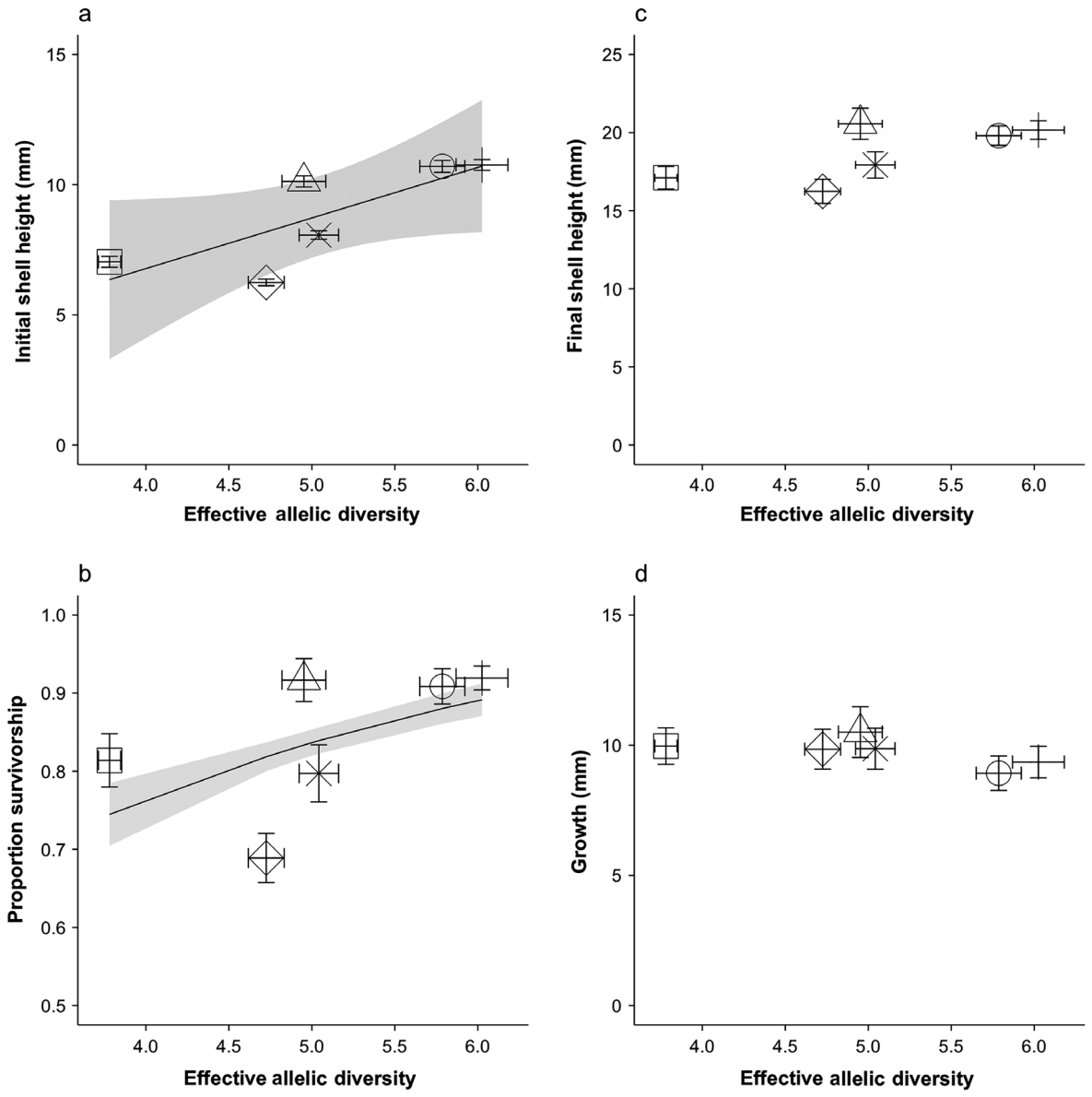

FIG. 2. Relationships among juvenile cohort effective allelic diversity and (a) average shell height when leaving the hatchery, (b) average shell height at the end of the experiments, (c) average survivorship of oysters in cages in the field (out of 12 oysters total), and (d) average growth over the course of the experiments. Different symbols represent individual cohorts: triangle, FL-1; square, FL-2; diamond, GA/SC-1; x, GA/SC-2; +, NC-1; circle, NC-2. Error bars represent ±SE.

the high mortality during the settlement stage and the inverse allometric relationship between mortality and size (David 1998, Lorenzen 2000, Grant et al. 2017, Plough 2018). Consistent with this relationship, juvenile outplant size alone had a positive influence on oyster survival in the presence of predation, and both size and cohort genetic diversity were positively correlated with cohort survival in the absence of predation outside the hatchery. Similar positive relationships between population genetic diversity and individual size and/or performance have been observed in other marine invertebrates (Aguirre and Marshall 2012*a*, *b*), suggesting it may be a general pattern of relevance to multiple aquaculture applications, from food production to conservation.

Although we do not know the underlying genetic or physiological mechanisms, the relationships between cohort effective allelic diversity and performance are consistent with the large literature on positive heterozygosity-fitness correlations in marine bivalves (Allendorf and Leary 1986, Zouros et al. 1988, Hedgecock et al. 1996, David 1998). Both single- and multi-locus allozyme heterozygosity have been linked with viability, growth, and reproductive effort in natural populations of bivalves (Allendorf and Leary 1986, Hedgecock et al.

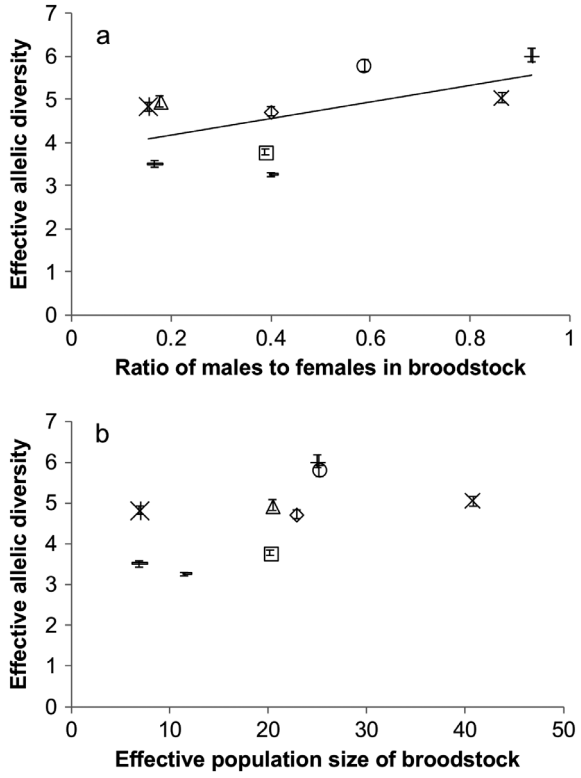

FIG. 3. Relationship between juvenile cohort effective allelic diversity and (a) the ratio of males to females in the broodstock and (b) the effective population size of the broodstock. Different symbols represent individual cohorts: triangle, FL-1; square, FL-2; diamond, GA/SC-1; x, GA/SC-2; +, NC-1; circle, NC-2; long line, FL-3; star, FL-4; short line, FL-5. Error bars represent ±SE as estimated by a jackknifing procedure in GenoDive.

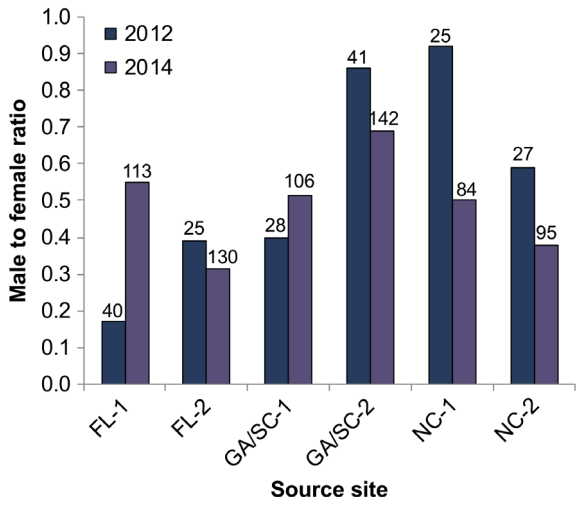

FIG. 4. Ratio of male to female oysters for adults collected from our six source sites in 2012 and again in 2014. The 2012 adults were used as the broodstock for our six juvenile oyster cohorts. The 2014 adults were sampled for genetic diversity and relatedness. Numbers above each bar indicate the total number of oysters sexed for each site.

1996). For example, shell length was positively correlated with the degree of heterozygosity in a natural population of mussels (Zouros et al. 1988). Individual heterozygosity may be linked to increased bivalve viability and growth due to a reduction in protein turnover and/or routine metabolic costs (Hedgecock et al. 1996). Although bivalves provide some of the best evidence for positive heterozygosity-fitness correlations, the strength of these relationships is highly variable even within the same species (Allendorf and Leary 1986, David 1998). Whether there is similar variation in the relationships documented here between effective allelic diversity and oyster fitness components warrants further investigation.

Because we were interested in understanding the effects of standard hatchery practices on the genetic diversity and performance of cultured populations, we did not manipulate genetic diversity directly. However, our results are consistent with observed ecological benefits of intraspecific variation from experimental manipulations and surveys across a range of systems (Hughes et al. 2008, Bolnick et al. 2011). For example, life-history diversity across salmon (*Oncorhynchus nerka*) populations reduces variation in salmon returns, resulting in a substantial reduction in the frequency of fishery closures (Schindler et al. 2010). Importantly, these ecological benefits have been demonstrated even when diversity is quantified using putatively neutral genetic markers (e.g., Hughes and Stachowiz 2009, Stachowicz et al. 2013), as in this study, despite the typically weak correlation between these markers and variation in particular functional traits (Reed and Frankham 2001). Neutral markers such as microsatellites are also useful for determining the contribution of hatchery-produced populations to future generations in the wild (Araki and Schmid 2010, Morvezen et al. 2016), helping to evaluate the success of conservation aquaculture efforts.

The juvenile oyster cohorts produced in 2012 exhibited greater genetic structure and lower genetic variation than the adults collected from the same source sites in 2014. Both of these findings are consistent with "sweepstakes reproduction" in oysters, whereby high variance in individual reproductive success causes reduced allelic diversity, increased genetic relatedness, and increased among-cohort diversity compared to the adult population, even when manually spawned, primarily due to differential mortality during early life-history stages (Boudry et al. 2002, Hedgecock and Pudovkin 2011, Plough and Hedgecock 2011, Morvezen et al. 2016, Plough et al. 2016, Plough 2018). Although we do not have data to assess the genetic variation and structure of the adult oysters used as broodstock in 2012, our limited comparison of broodstock and cohort effective allelic diversity for three later oyster cohorts confirms the lower effective allelic diversity of the juvenile oysters relative to the adult broodstock (Table 1), as well as considerable reproductive skew in the broodstock contributing to these cohorts (Appendix S4: Table S3). Further, we

found evidence of a population bottleneck in all hatchery-produced juvenile cohorts, including those produced in 2012 and 2016; low *M*-ratio values reflected the relatively low number of alleles compared to the relatively large range in alleles for each locus. Combined with the commonly observed loss of genetic diversity in hatchery-produced cohorts relative to wild populations of fish and invertebrates (Araki and Schmid 2010, Grant et al. 2017), our results highlight that hatchery production of highly fecund marine fishes and invertebrates is likely quite vulnerable to the effects of sweepstakes reproduction and population bottlenecks. Further, our results are consistent with previous studies demonstrating the potential for significant kin structure among oysters at small spatial scales despite little population structure at regional scales (Diaz-Ferguson et al. 2010, Adrian et al. 2017).

Among the three cohorts for which we have genetic data on both the adult broodstock and the resulting juveniles, there was a positive correlation between adult broodstock and juvenile cohort diversity. This pattern suggests that genetic variation of broodstock may be a useful predictor of genetic variation in the resulting juveniles. However, the lack of any relationship between genetic variation in non-broodstock adults sampled in 2014 from the same sites as the six juvenile cohorts produced in 2012 highlights that a snapshot of oyster reef genetic diversity at one point in time may not be a good indicator of diversity in cohorts produced from a subset of adults from those reefs at different times. Rather, oyster sex structure may be a less expensive, more readily measured metric for predicting juvenile oyster diversity. Large asymmetries in the ratio of adult males to females are expected to contribute to sweepstakes reproduction, resulting in a less diverse cohort of offspring (Yund and McCartney 2016). Consistent with this prediction, we found that as the ratio of males to females in the broodstock approached one, effective allelic diversity of the resulting offspring increased across nine juvenile cohorts produced from the northern Gulf of Mexico and the South Atlantic Bight. While we do not have data for ratios greater than one, we expect that effective allelic diversity would again decline as the ratio continues to increase. Thus, aiming for an ~1:1 broodstock male-to-female ratio is a potentially useful method for hatcheries to ensure genetic diversity in their product (Grant et al. 2017), thereby enhancing initial size and survival.

We found substantial spatial variation in broodstock sex ratios across the natural reefs we sampled. We also observed temporal variation in sex ratios on the oyster reefs (Fig. 4), consistent with past studies (Haley 1977, Kim and Powell 1998), though in some cases (e.g., FL-1, NC-1) this variation was greater than that predicted by modeling sex–size relationships (Harding et al. 2013). Because we targeted larger oysters for broodstock collection, our expectation was that broodstock sex ratios would be skewed female; oysters can transition from males to females at ~30 mm shell length (Thompson et al. 1996), and the sex ratio of oysters in a prior study was <0.25 male : female once oysters were 60 mm (Mroch et al. 2012). However, in both 2012 and 2014, at least half of our survey sites had male : female ratios greater than or equal to 0.5, with some approaching 1.0, despite the fact that greater than 90% of the oysters were larger than 60 mm. Even replicate reefs separated by as little as 100 m within a single site exhibited substantial variation in male-to-female ratio (e.g., 0.3–1.1 at FL-1), suggesting that site-level differences were not simply driven by seasonal variation associated with latitude. Given that these spatial differences were fairly consistent across multiple years (2012 and 2014), the sex structure of natural reefs may be an important determinant of local reproductive success.

Hatchery production efforts in marine fishes and invertebrates can serve a wide range of purposes, including closed culture for food production, sea "ranching" for food production, stock restoration to replenish a depressed natural population, and stock enhancements to boost production of a self-sustaining natural population (Froehlich et al. 2017, Grant et al. 2017). We found that effective allelic diversity was correlated with survival in the absence of predation. In contrast, survival was generally low in the presence of predation, complicating our ability to detect effects of effective allelic diversity while also highlighting that some environmental factors are sufficient to overwhelm the effects of diversity. The positive effect of effective allelic diversity within a single cohort contrasts with the negative effects on survivorship of the number of these same cohorts (Hanley et al. 2016) but, in both cases, these relationships were only detected in the absence of predation. These results suggest that methods to increase genetic diversity may be more relevant to closed culture and sea ranching (where individuals are generally protected from predators) than to stock restoration and stock enhancement (where individuals are exposed to predators when they are present). However, the positive relationship between effective allelic diversity and juvenile oyster size when leaving the hatchery, which was in turn positively correlated with oyster size and survival across all environments, highlights the potential importance of genetic diversity across a range of aquaculture applications.

When hatchery production is used for conservation purposes (stock restoration or enhancement), or when sea ranching operations do not harvest hatchery-produced individuals before they reproduce, there is the potential for the hatchery individuals to reduce the effective population size of natural populations (Morvezen et al. 2016). This potential problem increases as the ratio of hatchery individuals to wild individuals increases (Morvezen et al. 2016). To counter these problems, we join the calls of others in recommending that hatcheries use the largest possible broodstock, aim for an equal ratio of males to females in the broodstock, renew the broodstock regularly, release multiple cohorts into natural populations in equal quantities, and perform genetic

monitoring of recipient populations (Morvezen et al. 2016, Grant et al. 2017).

The considerable trait and performance variation across cohorts in this study, and populations more generally (Albert et al. 2010, Bolnick et al. 2011), argue not only for conservation aquaculture practices that maintain within-population genetic variation, but also for strategies that incorporate multiple populations. Including multiple populations increases the chances of capturing existing intraspecific variation (Yund and McCartney 2016) that can contribute to increased production (similar to selection effects in the species diversity–ecosystem function literature; Loreau and Hector 2001). In addition, increased population or cohort diversity can lead to greater consistency in production, particularly across different environmental conditions (i.e., the insurance effect; Yachi and Loreau 1999). Further, more populations will ensure a greater range of traits are represented, since a population with high growth may not also have high disease resistance, for example. Ultimately, hatchery methods that maintain genetic variation within and between populations are not just important to meet short-term production goals; they are critical to allow populations and species to respond to longer-term environmental change, and thus the use of aquaculture to meet conservation objectives.

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Supporting Information

Additional supporting information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/eap.1940/full

Data Availability

Data are available from the Biological and Chemical Oceanography Data Management Office: https://www.bco-dmo.org/project/709942