

Effects of intraspecific diversity on survivorship, growth, and recruitment of the eastern oyster across sites

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Abstract. Intraspecific diversity, particularly of foundation species, can significantly affect population, community, and ecosystem processes. Examining how genetic diversity relates to demographic traits provides a key mechanistic link from genotypic and phenotypic variation of taxa with complex life histories to their population dynamics. We conducted a field experiment to assess how two metrics of intraspecific diversity (cohort diversity, the number of independent juvenile cohorts created from different adult source populations, and genetic relatedness, genetic similarity among individuals within and across cohorts) affect the survivorship, growth, and recruitment of the foundation species *Crassostrea virginica*. To assess the effects of both cohort diversity and genetic relatedness on oyster demographic traits under different environmental conditions, we manipulated juvenile oyster diversity and predator exposure (presence/absence of a cage) at two sites differing in resource availability and predation intensity. Differences in predation pressure between sites overwhelmingly determined post-settlement survivorship of oysters. However, in the absence of predation (i.e., cage treatment), one or both metrics of intraspecific diversity, in addition to site, influenced long-term survivorship, growth, and recruitment. While both cohort diversity and genetic relatedness were negatively associated with long-term survivorship, genetic relatedness alone showed a positive association with growth and cohort diversity alone showed a positive association with recruitment. Thus, our results demonstrate that in the absence of predation, intraspecific diversity can affect multiple demographic traits of a foundation species, but the relative importance of these effects depends on the environmental context. Moreover, the magnitude and direction of these effects vary depending on the diversity metric, cohort diversity or genetic relatedness, suggesting that although they are inversely related in this system, each captures sufficiently different components of intraspecific diversity. Given the global loss of oyster reef habitat and rapid decline in oyster population size, our results are particularly relevant to management and restoration. In addition, aquaculture, which commonly excludes predators during early life history stages, may benefit from incorporation of oyster cohort diversity into standard practice.

Key words: context dependent; demography; genetic diversity; genetic relatedness; growth; oyster; predation; recruitment; survivorship; trait.

INTRODUCTION

Intraspecific diversity, including genotypic richness and phenotypic variation, can significantly affect population dynamics, community structure, and ecosystem function (Bolnick et al. 2003, 2011, Whitham et al. 2003, 2006, Hughes et al. 2008), which in turn impact ecosystem services (Cardinale et al. 2012). Within-species variation of foundation and keystone species may be particularly important in shaping population, community, and ecosystem processes (Bruno and Bertness 2001, Hughes et al. 2008). However, understanding the fundamental mechanistic links between genotypic

richness and community and ecosystem processes requires consideration of genetically based phenotypic trait variation (Agashe 2009, Steiner and Masse 2013), particularly for demographic traits that determine the population dynamics of these key species (Gamfeldt et al. 2005, Crawford and Whitney 2010, Aguirre and Marshall 2012a,b, Hufbauer et al. 2013, Smee et al. 2013, Szucs et al. 2014). For species with complex life histories and a strong dependence on successful recruitment and colonization, in addition to growth and survivorship, understanding the direction and strength of the relationship between genetic diversity and demography across life history may also better inform conservation and restoration efforts.

The most common metric of intraspecific diversity is genotypic richness (i.e., the number of genotypes), but this measure of diversity is not as informative for species that reproduce sexually (Hughes et al. 2008, Agashe

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2009). In addition, genotypic richness does not incorporate information on genomic similarity or functional dissimilarity, which may be particularly important for studies seeking to link genotype-based measures of diversity to phenotypic trait variation, intra- and interspecific interactions, and ultimately ecological processes (Jousset et al. 2011, Avolio et al. 2012, Stachowicz et al. 2013). Genetic relatedness is an alternative metric of diversity that takes into account the genetic similarity (i.e., the number of shared alleles between individuals relative to the frequency of those alleles in the population; Frasier 2008), and corresponding phenotypic and functional differences, of individuals in a population, rather than treating each genotype as equally distinct (Stachowicz et al. 2013). Although genotypic richness and genetic relatedness are fundamentally linked (i.e., changing the number of genotypes affects genetic relatedness), their effects on ecosystem function may differ in direction and magnitude (Jousset et al. 2011, Stachowicz et al. 2013). Further exploration of the relationship between diversity metrics and their effects on demographic traits is needed, particularly for species of conservation concern.

The strength of intraspecific diversity effects, like the direction and magnitude of species interactions (Agrawal et al. 2007, Chamberlain et al. 2014), can be context dependent. Environmental conditions may interact with intraspecific diversity, such that the relative effects of genetic variation on demographic traits depend on the abiotic or biotic context. Often the effects of biodiversity on ecosystem function vary across environmental stress gradients, with the negative effects of high stress environments being greater in low diversity than in high diversity communities (Stuedel et al. 2012) and the benefits of genetic diversity being more apparent under stressful conditions (e.g., Hughes and Stachowicz 2009, Agashe and Bolnick 2010, Caesar et al. 2010). Despite the potential importance of diversity in response to stress, relatively few studies explicitly test the effects of diversity in multiple environments.

In this study, we examined how different metrics of intraspecific diversity affected key demographic traits of the eastern oyster (*Crassostrea virginica*). The global decline of oyster reefs (~85%) due to overharvesting, coastal development, and declining environmental quality has diminished the capacity for this habitat to provide key ecosystem functions and services (Beck et al. 2011). The mixed success of oyster reef restoration efforts thus far (Beck et al. 2011, Grabowski et al. 2012) suggests that additional factors, such as intraspecific diversity of this habitat-forming species, may play a critical role in population recovery. For example, Smee et al. (2013) found greater settlement of oyster larvae on experimental assemblages of adult oysters from three bays (high diversity) than on experimental assemblages of adult oysters from one bay (low diversity). Consequently, the combination of population decline and corresponding intraspecific diversity loss may further exacerbate the negative effects of overharvesting on the eastern oyster (Smee et al. 2013).

The population dynamics of this foundation species also depend on individual growth rates and survival, yet it remains unknown how different metrics of genetic diversity affect oyster growth and survivorship.

Environmental conditions, such as resource availability, predator identity/predation intensity, and habitat complexity, are known to structure oyster reef communities and determine the strength of trophic cascades (e.g., Grabowski et al. 2005, 2008, Kimbro et al. 2009, 2014). To examine whether the relationship between intraspecific diversity and demography of the eastern oyster varies across environmental conditions, we manipulated oyster diversity at two sites that differ in predation intensity, physical stress, and resource availability, and measured growth and survivorship of juvenile oyster transplants, and recruitment of new juvenile oysters. Specifically, we manipulated oyster cohort diversity, which refers to the number of source populations (see *Methods* for details), and is arguably the most relevant diversity metric for aquaculture practices and restoration efforts. We also determined genetic relatedness within and across cohorts using microsatellite loci to examine the relationship between different metrics of intraspecific diversity and demographic traits, and to assess which diversity measure was a better predictor of demography under different environmental conditions. Knowing how cohort diversity relates to genetic relatedness is particularly informative for species that have likely experienced substantial population restructuring and altered gene flow as a result of human recreational and interventional activities (Rose et al. 2006, Allendorf et al. 2008).

METHODS

Study system

In northeast Florida, the Matanzas River estuary (29.91386° N, 81.28368° W) extends 37 km north and 11 km south of the Matanzas Inlet. Previous research in this system identified a north-south spatial gradient in predation (by mud crabs, *Panopeus herbstii*; higher in north), physical stress (i.e., salinity, temperature, and reef submergence time; lower in north), and resource availability (phytoplankton abundance and chlorophyll *a*, higher in north; Garland and Kimbro 2015, *data available online*)⁴. We capitalized on these previously identified differences by selecting one site in the north (29.75177° N, 81.25578° W) and one site in the south (29.65838° N, 81.22193° W) to conduct our experiment on the relative importance of juvenile cohort diversity on oyster (*Crassostrea virginica*) survivorship, growth, and colonization enhancement.

Oyster cohort establishment

To experimentally manipulate juvenile cohort diversity, we first established juvenile oyster cohorts from six

⁴ <http://www.nerrsdata.org/>

different parental broodstocks at a hatchery in Jupiter, Florida, USA (Research Aquaculture). The broodstock oysters all came from the Southeastern Atlantic Bight, with two collection sites in North Carolina (NC), one collection site in South Carolina (SC), one collection site in Georgia (GA), and two collection sites in Florida (FL); collection sites within the same state were located in independent embayments (Appendix S1: Table S1). To establish each cohort, we collected 100 adult oysters (8.5–10 cm shell length) from three to five reefs at each site in April 2012, tested and certified 30 oysters as being free of disease using microscopy at the Aquatic Animal Health Laboratory at Florida Atlantic University, and then shipped the remaining 70 oysters to Research Aquaculture. At the hatchery, adult oysters and their offspring were held in separate flow-through seawater systems under identical conditions to prevent cross contamination. The broodstock from each site were manually spawned on the same day, though one FL cohort did not produce enough offspring so the remaining broodstock were spawned 3 weeks later. The larvae were held until they settled (~3 weeks) and then moved to a nursery facility at the hatchery under flow-through seawater conditions with standard food concentrations. In June 2012, the cohorts were transferred to a common flow-through facility at the Whitney Marine Biological Laboratory in St. Augustine, Florida, where 50 individuals were haphazardly collected for genetic analysis and stored at -80°C . Because these individuals were reared in a common environment, they represented juvenile oyster cohorts of the same age that had experienced identical conditions, thus making it easier to distinguish variation in traits due to geographic origin and genetic identity.

Field experiment

From July 2012 to December 2012, we conducted a field experiment to assess the relative influence of juvenile cohort diversity (one, two, or four cohorts) on three key aspects of oyster reef development – post-settlement survivorship, post-settlement growth, and the recruitment of successive oyster cohorts. At one site north and one site south of Matanzas Inlet, we identified one large reef (20×30 m) and established three 18.0-m transects at the reef's intertidal midpoint. Along each transect, we placed 36 experimental units at 0.5-m intervals. Each experimental unit consisted of 12 juvenile oysters (mean shell length 8 mm) that were attached to a ceramic tile (13×13 cm) using a marine epoxy, and these tiles were affixed to concrete pavers in a vertical position. The juvenile oysters were attached to tiles in the laboratory and immediately returned to a flow-through seawater system for 24 h prior to deployment. The experimental design included three juvenile cohort diversity treatments (one, two, or four cohorts per tile). The one-cohort treatment included two replicate “monocultures” per cohort and the two- and four-cohort treatments were

random combinations drawn from the pool of six cohorts. Experimental units were randomly assigned to receive one of three predator-exclosure treatments: cage, cage control, and no cage control (i.e., open). There were 12 replicates of each cohort diversity treatment \times predator-exclosure treatment combination at each site (total = 216 tiles). The cage ($0.3 \times 0.3 \times 0.5$ m) was constructed of vinyl-coated wire mesh (0.64 cm). To identify any cage artifact effects, the cage control lacked two walls, thereby mimicking the material effects of the exclosure without restricting predator access.

To compare short-term survivorship of outplanted oysters in the presence and absence of predation (i.e., across all exclosure treatments), we focused on the first 3 weeks of the experiment; sampling thereafter revealed significant oyster depletion in the cage control and open treatments that compromised further comparisons among these treatments. We then used the cage treatment to examine the effects of cohort diversity on survivorship, growth, and recruitment in the absence of predation over the course of the 6-month experiment. To determine the effect of cohort diversity on long-term founder cohort survivorship, we measured survivorship at the end of the experiment. To assess growth, we measured the shell length of each oyster monthly from the beginning (July 2012) to the end (December 2012) of the experiment. Finally, we assessed recruitment of juvenile oysters (2–5 mm) that settled on the experimental oysters and surrounding tile at once every other week intervals from the onset of the recruitment period (September 2012) to the end of the experiment.

Genetic diversity and relatedness

To assess genetic diversity within and between oyster cohorts and to examine the relationship between cohort diversity and genetic relatedness, we genotyped 44–54 individuals from each juvenile oyster cohort using 12 microsatellite loci developed for *Crassostrea 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)). After grinding each sample with a pestle, we used the Omega Bio-Tek E-Z 96 Tissue DNA Kit to extract DNA from individual spat (Omega Bio-Tek; Norcross, Georgia, USA). In each multiplexing polymerase chain reaction (PCR), we amplified four loci using the Qiagen Type-It Microsatellite PCR Kit (Qiagen; Valencia, California, USA). Each 10- μL reaction consisted of 1 μL DNA template, 5 μL $2\times$ type-it multiplex master mix (Qiagen), 2.4 μL water, and 0.2 μL each 10 $\mu\text{mol/L}$ 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; Foster City, California, USA) and fragment analysis was performed using GeneMarker version 2.6 (SoftGenetics; State College, Pennsylvania, USA).

To examine measures of genetic diversity per locus and per cohort, 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)) using GenoDive version 2.0b27 (Meirmans and van Tienderen 2004). In addition, we calculated pairwise F_{ST} (Weir and Cockerham 1984) using GenoDive to assess cohort structure and differentiation (see Appendix S2: Genotyping, Tables S1 and S2 for a summary of the genetic diversity statistics).

To determine genetic relatedness within each cohort diversity treatment, we calculated relatedness for every pairwise combination of individuals within and between each cohort using STORM version 2.0 (Frasier 2008). For the one-cohort treatment, this included only pairwise comparisons of individuals from the same cohort. For the two- and four-cohort treatments, this included all possible pairwise comparisons consisting of combinations of individuals from the same cohort and combinations of individuals from different cohorts.

Data analysis

To assess the relative influence of juvenile cohort diversity (i.e., number of cohorts), predator-exclosure treatment, and environment (i.e., site) on oyster survivorship, growth, and recruitment, we used a linear mixed-effects model selection approach in R (The R Foundation for Statistical Computing, Vienna, Austria; version 3.0.2, lme4 and bblme packages). In this study, predator-exclosure treatment, cohort diversity, and genetic relatedness were designated as fixed factors, with a random effect of transect (i.e., block). In addition, environment (site) was treated as a fixed effect because we had a priori expectations about differences between sites based on previous research (Garland and Kimbro 2015). For each analysis, we created a series of nested linear models that ranged from simple to complex, including a null model (intercept = 1) with only random effects, models with random effects and each fixed factor independently, and models with random effects and additive and interactive effects between fixed factors. Because there was a negative relationship between cohort diversity and genetic relatedness ($y = -0.03x + 0.10$, $R^2 = 0.47$, $P < 0.01$), we excluded any models that included a cohort diversity \times genetic relatedness interaction, but we included the additive effect of cohort diversity + genetic relatedness to examine which metric(s) of genetic diversity best predicted each demographic trait: cohort diversity, genetic relatedness, or the combination of both. For recruitment, the analysis also included a random effect of time. For short-term and long-term survivorship, the data were analyzed using logistic regression, applying a binomial error distribution and a logit link function. After

constructing these nested models and fitting them using maximum likelihood, we used Akaike's information criterion corrected for small sample size (AIC_c) to identify the most parsimonious model(s) (Burnham and Anderson 2002). In this procedure, models were ranked according to their Akaike weight (w_i), which was calculated as the model likelihood normalized by the sum of all model likelihoods; values closer to 1 indicate greater support of the model (Johnson and Omland 2004). The difference in AIC_c between the best model (i.e., the model with w_i closest to 1) and alternative models (ΔAIC_c) was also calculated; models with $\Delta AIC_c < 2.0$ were considered to have substantial support (Richards 2005).

Because our analysis of growth and recruitment only included the predator-exclosure units, candidate models with treatment were omitted from consideration. In addition, because of extremely low colonization in the southern site (biweekly average of 0–2 recruits/tile; see *Results* for details), we assessed the effects of cohort diversity and genetic relatedness on recruitment in the northern site only. If the best model for each demographic trait included the additive effect of cohort diversity and genetic relatedness, we used partial regression analysis to assess how well each of these metrics of genetic diversity independently predicted survivorship, growth, or recruitment (Quinn and Keough 2002). The survivorship data were logit transformed for this analysis.

There was a significant effect of cohort diversity on long-term survivorship (see *Results*), so we used the tripartite partition method (Fox 2005) to differentiate among diversity mechanisms: trait-independent complementarity (TIC; equivalent to complementarity sensu Loreau and Hector 2001), dominance (DE), and trait-dependent complementarity (TDC; with DE + TDC equivalent to selection sensu Loreau and Hector 2001). It was not possible to apply this method of partitioning to the effect of cohort diversity on recruitment because recruits could not be assigned to specific cohorts (see Appendix S4: Diversity Partitioning and Fig. S1 for details).

RESULTS

Survivorship

In the short term (i.e., the first 3 weeks of the experiment), the best model for survivorship included a three-way interaction among site, predator-exclosure treatment, and cohort diversity ($w_i = 0.845$; see Appendix S3: Table S1 for model selection summary). Survival was highest in the absence of predation (i.e., cage) across both sites. In the presence of predation (i.e., the open tile), oyster survivorship was reduced by 90% at the northern site and by 61% at the southern site (Fig. 1a). Compared to the effects of site and predation, the effect of cohort diversity on survivorship was weak, especially at the high-predation northern site (Fig. 1b).

In the longer term (i.e., 6 months), the best model for survivorship in the absence of predation (i.e., cage)

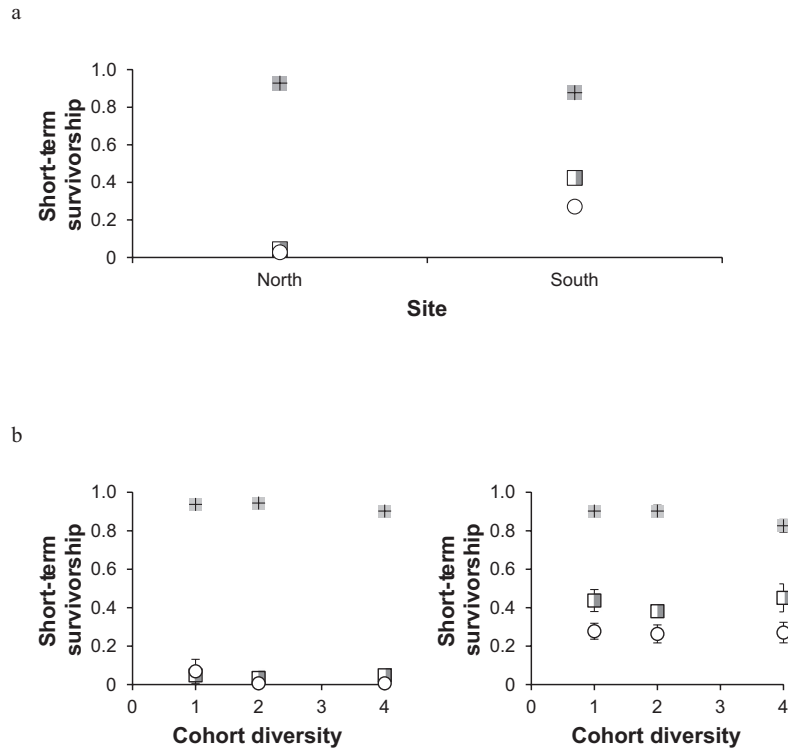


FIG. 1. (a) Short-term survivorship of juvenile oysters (i.e., proportion surviving after the first 3 weeks of the experiment) in each predator-exclosure treatment (cage, gray squares with cross; cage control, white and gray squares; open, white circles) at the northern and southern sites. (b) The relationship between cohort diversity and short-term survivorship for each predator-exclosure treatment at the northern (left panel) and southern (right panel) sites. Values are mean \pm SE.

included the additive effects of site, cohort diversity, and genetic relatedness ($w_i = 0.998$). In the northern site, where survival was higher (mean \pm SE = 0.80 ± 0.02), there was little difference in survivorship across cohort diversity treatments (Fig. 2a), and the relationship between relatedness and survivorship was relatively weak (Fig. 2b). In the southern site, where survival was generally lower (mean \pm SE = 0.47 ± 0.04), the one-cohort treatment had higher mean survivorship and greater variation in survivorship than the two- and four-cohort treatments (Fig. 2a), and there was a negative relationship between survivorship and genetic relatedness (Fig. 2b). In the partial regression analysis across sites, the residuals of both cohort diversity and genetic relatedness were negatively associated with the residuals of long-term survivorship (cohort diversity $F_{1,70} = 6.03$, $y = -0.40x - 2.57 \times 10^{-16}$, $R^2 = 0.08$, $P = 0.02$; genetic relatedness $F_{1,70} = 7.83$, $y = -8.84x + 9.16 \times 10^{-17}$, $R^2 = 0.10$, $P = 0.007$; Fig. 2c), indicating that each diversity metric independently explained a portion of the variation in survivorship after accounting for variation due to the other metric.

Partitioning the effects of diversity on long-term survivorship revealed that the predominant mechanism differed across sites. In the north, there was a negative dominance effect, indicating that cohorts with low survivorship in monoculture had high survivorship in

polyculture. In the south, there was a negative trait-independent complementarity effect, indicating that cohort survivorship in mixture (i.e., two- and four-cohort treatments) was worse than expected based on survivorship in the one-cohort treatment (see Appendix S4: Diversity Partitioning and Fig. S1 for details).

Growth

The best model for growth over the 6-month experiment included the additive effects of site, cohort diversity, and genetic relatedness ($w_i = 0.908$), which reflects higher growth in the northern site (Fig. 3a), slight differences in growth between cohort diversity treatments within sites (Fig. 3b), and a weak positive association between growth and genetic relatedness ($y = 1.85x + 1.38$, $R^2 = 0.03$, $P = 0.16$; Fig. 3c). The residuals of genetic relatedness were positively associated with the residuals of growth ($F_{1,70} = 3.89$, $y = 3.42x + 1.31 \times 10^{-17}$, $R^2 = 0.05$, $P = 0.05$; Fig. 3d), while the residuals of cohort diversity were not associated with the residuals of growth ($F_{1,70} = 1.78$, $y = 0.12x + 2.27 \times 10^{-16}$, $R^2 = 0.02$, $P = 0.19$).

Recruitment

Because of extremely low recruitment at the southern site (Fig. 4a; biweekly average recruitment = 1.33 ± 0.19

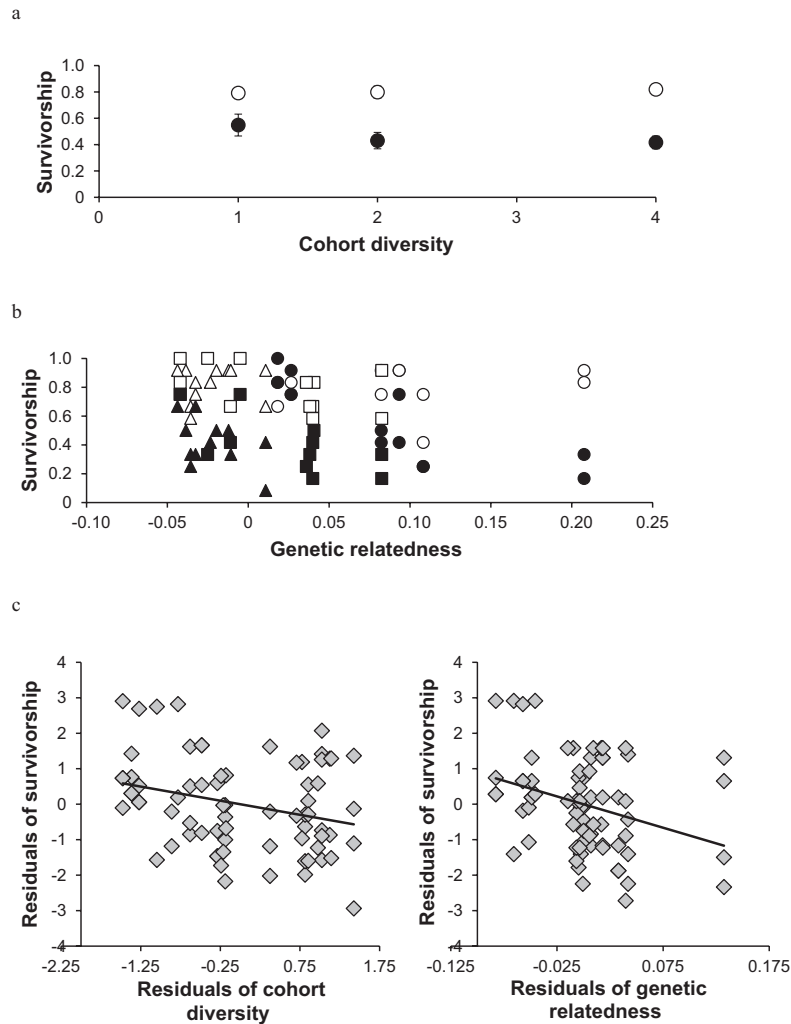


FIG. 2. The relationship between long-term survivorship and (a) cohort diversity at the northern (open symbols) and southern (solid symbols) sites; and (b) genetic relatedness ($y = -3.59x + 0.82$, $R^2 = 0.03$, $P = 0.143$; circles, squares, and triangles represent the one-, two-, and four-cohort diversity treatments, respectively). (c) Partial regression plots of the residuals of cohort diversity (left panel) and the residuals of genetic relatedness (right panel) vs. the residuals of growth.

[mean \pm SE]), we did not include it in our model selection analyses. In the northern site, the best model for recruitment included cohort diversity ($w_i = 0.604$), reflecting higher recruitment in the four-cohort treatment than in the one- and two-cohort treatments (31% more recruits in the four-cohort treatment compared to the one-cohort treatment; Fig. 4b). In addition, a second model including the additive effect of cohort diversity + genetic relatedness also explained recruitment in the northern site ($\Delta AIC = 1.6$, $w_i = 0.278$), yet the negative association between recruitment and relatedness was not significant ($y = -139.74x + 97.98$, $R^2 = 0.05$, $P = 0.19$; Fig. 4c).

DISCUSSION

In our experiment, we detected clear differences between sites for all demographic traits of *C. virginica*,

with higher growth and greater recruitment in the absence of predation (i.e., cage) at the northern site and differences in short-term survivorship across predator-exclosure treatments between sites (Figs. 1a, 3a, and 4a). The effects of intraspecific diversity, cohort diversity, and genetic relatedness on oyster demography varied depending both on the demographic trait and the local environmental conditions (i.e., site). For example, in the presence of predators, particularly at the high predation northern site, cohort diversity had comparatively little effect on short-term survivorship (Fig. 1b). However, in the absence of predators (i.e., cage), both cohort diversity and genetic relatedness strongly predicted long-term survivorship (Fig. 2c). In addition, genetic relatedness was more strongly (positively) associated with growth (Fig. 3d) and cohort diversity was more strongly (positively) associated with

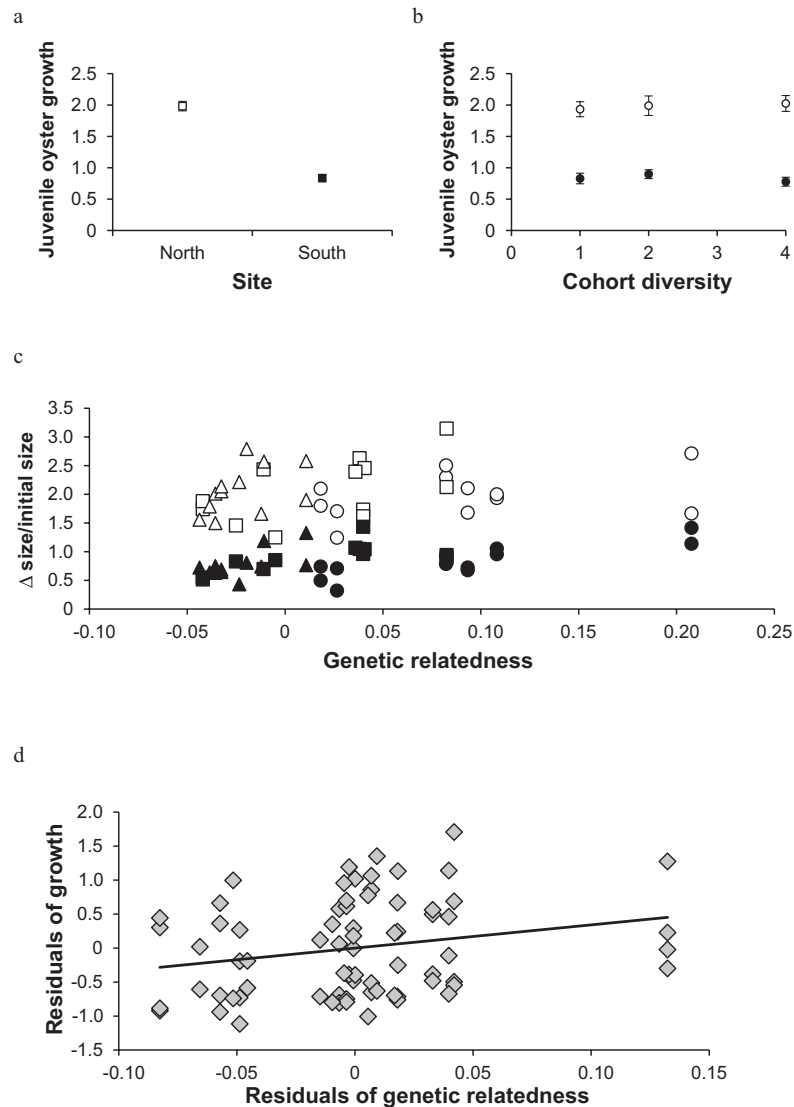


FIG. 3. Juvenile oyster growth (i.e., [change in size from the beginning to the end of the experiment]/[initial size]) measured in mm (mean \pm SE) (a) at each site and (b) for each oyster cohort diversity treatment; open and solid symbols represent the northern and southern experimental sites, respectively. (c) The relationship between genetic relatedness and oyster growth at each site; circles, squares, and triangles represent the one-, two-, and four-cohort diversity treatments, respectively. (d) Partial regression plot of the residuals of genetic relatedness and the residuals of growth.

recruitment (Fig. 4b). Thus, while the effects of oyster genetic diversity may be relatively weak in comparison to strong top-down effects at high predation sites, diversity may play a critical role in determining oyster vital rates and thus population dynamics at low predation and/or high stress sites. Consequently, incorporation of oyster cohort diversity into aquaculture practices, which commonly exclude predators during early life history stages (Dumbauld et al. 2009), may be particularly beneficial.

The majority of studies testing the effects of genetic diversity on population productivity, community diversity, ecosystem function, and recovery from disturbance (e.g., Hughes and Stachowicz 2004, Reusch et al.

2005, Crutsinger et al. 2006, Johnson et al. 2006, Hughes et al. 2008) have manipulated genotypic richness. However, recent research on the role of genetic relatedness (Stachowicz et al. 2013, Avolio et al. 2015) and phenotypic variation (Ellers et al. 2011) suggests that additional metrics of intraspecific diversity may further inform our understanding of the mechanisms underlying biodiversity–ecosystem function relationships (Avolio et al. 2012), similar to studies examining how phylogenetic relatedness and functional diversity influence the effects of species diversity on community and ecosystem processes (e.g., Cadotte et al. 2009, Best et al. 2012). The results of our experiment support these findings: cohort diversity was included in the best models for growth,

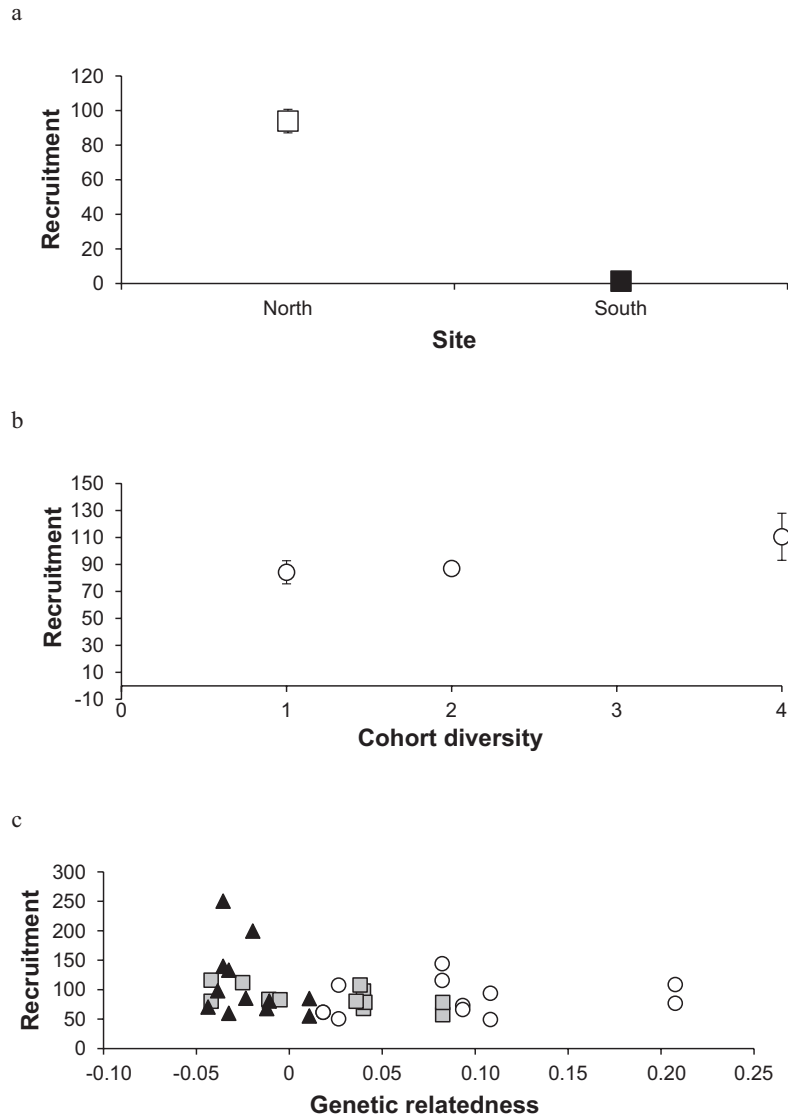


FIG. 4. Recruitment (i.e., number of recruits counted once every other week from the onset of the recruitment period to the end of the experiment; mean \pm SE) (a) at each site (open and solid symbols represent the northern and southern experimental sites, respectively) and (b) for each oyster cohort diversity treatment at the northern site. (c) The relationship between genetic relatedness and recruitment at the northern site (white circles, gray squares, and black triangles represent the one-, two-, and four-cohort diversity treatments, respectively).

recruitment, and survivorship, but genetic relatedness was also a predictor of both growth and long-term survivorship. Furthermore, despite the fact that cohort diversity and genetic relatedness co-vary, the shape and magnitude of their relationship with vital rates differed, indicating that examination of multiple metrics of diversity may further our understanding of the mechanisms underlying these patterns because they capture different aspects of intraspecific diversity.

The effects of oyster genetic diversity on survivorship in the short-term were negligible compared to the effects of predation (Fig. 1), with higher survival in the southern site than the northern site in the presence of predators

and higher survival in the northern site than the southern site in the absence of predators. This pattern is consistent with our a priori expectations, as it is difficult to conceive a mechanistic explanation for an effect of juvenile oyster genetic diversity on predation resistance, particularly given the narrow range of survivorship within each site and predator-exclosure treatment combination. In contrast, the effects of oyster diversity on long-term survivorship in the absence of predation (i.e., cage treatment) were sometimes substantial, consistent with results from a wide variety of species (e.g., bryozoan: Aguirre and Marshall 2012b; flour beetle: Agashe 2009, flowering perennial: Vilas et al. 2006). In addition, they depended on

the abiotic environment (Fig. 2a,b), nicely matching expected patterns based on differences in predation pressure (higher in the northern site), physical stress (lower in the northern site), and resource availability (higher in the northern site) across sites (Garland and Kimbro 2015).

Differentiating among mechanisms underlying diversity effects, such as complementarity and selection (Loreau and Hector 2001), can help identify the processes linking genetic diversity and demographic traits. In our study, the predominant mechanism differed between sites (see Appendix S4: Diversity Partitioning and Fig. S1), similar to species richness manipulations in which the effects of diversity and the underlying mechanisms depended on the environmental context (Fridley 2003). Effects of cohort diversity on long-term survivorship at the southern site were due to significant, negative trait-independent complementarity effects, suggesting competition or other negative interactions among cohorts (Fox 2005). In this case, resource limitation may have resulted in increased competition among cohorts in mixture, producing a negative effect of cohort diversity at this site (Garland and Kimbro 2015). In contrast, effects of cohort diversity at the less stressful northern site were due to significant, negative dominance effects, suggesting cohorts with low survivorship in monoculture had high survivorship in mixture, at the expense of other cohorts (Fox 2005). The relative importance of complementarity and dominance likely reflects differences in physical stress and resource availability that resulted in distinct cohort interactions at each site. Further, this may have implications for intraspecific diversity, as negative complementarity with resource limitation may lead to a decline in intraspecific diversity as a result of competitive exclusion, whereas negative dominance under less stressful conditions may contribute to the maintenance of diversity. Further work is needed to identify the precise mechanisms underlying effects of cohort diversity and genetic relatedness in this system and how they may vary across different environments.

In addition to effects of oyster cohort diversity, genetic relatedness influenced long-term survival in the physically stressful, resource-limited southern site, with survivorship decreasing with increasing relatedness (Fig. 2b). In contrast, there was no relationship between genetic relatedness and survivorship at the northern site. This pattern is consistent with the expected context-dependency of diversity effects, with diversity playing a more important role under stressful environmental conditions (e.g., Mulder et al. 2001, Hughes and Stachowicz 2009, Agashe and Bolnick 2010, Caesar et al. 2010, Steudel et al. 2012). However, this result also highlights that including only one metric of intraspecific diversity in predicting population responses to environmental change may not be sufficient given the differential importance of cohort diversity and genetic relatedness for short-term and long-term survivorship across sites and environmental conditions. Further, metrics such as cohort

diversity and genetic relatedness are imperfect and not completely redundant proxies for the functional variation that directly influences survivorship. At present, the relationship between genetic relatedness and trait diversity remains largely unresolved (e.g., Stachowicz et al. 2013 vs. Abbott and Stachowicz 2016) and may vary across systems. Characterizing the links among genotypic/cohort richness, genetic relatedness, and phenotypic trait variation will enhance our ability to predict the effects of genetic diversity on vital rates like survivorship (Avolio et al. 2012, Hughes 2014).

In our experiment, we found a positive relationship between genetic relatedness and oyster growth in the absence of predation, particularly at the southern site, which had higher physical stress and lower resource availability (Garland and Kimbro 2015). These results contrast with studies examining the effects of diversity and relatedness in bryozoan and ascidian populations founded by siblings (high relatedness and low diversity) vs. non-siblings (low relatedness and high diversity) that identified a positive effect of genetic diversity on size and survival (Aguirre and Marshall 2012a,b). Yet, they are consistent with the positive effects of eelgrass genetic relatedness on population (rather than individual) growth across the range of relatedness values observed in our study (Stachowicz et al. 2013). At higher levels of genetic relatedness ($R > 0.4$), eelgrass production declined (Stachowicz et al. 2013); this unimodal pattern may also occur in oysters, yet the degree of relatedness needed to observe the decline ($R > 0.4$) is perhaps unlikely to occur in sexually reproducing species like oysters. Additional investigation is needed to determine whether the strength and direction of genetic relatedness effects and the predictive power of genotypic richness vs. genetic relatedness differ for clonal vs. non-clonal and plant vs. animal species (Aguirre et al. 2013, Stachowicz et al. 2013, Avolio et al. 2015).

In taxa with complex life histories, the effects of intraspecific diversity on colonization success may play a key role in long-term population dynamics. Because chemical cues from adult oysters influence larval settlement (Smee et al. 2013), there is potential for oyster intraspecific diversity to influence recruitment in multiple ways: first, compared to cues from a single cohort, the cues from multiple oyster cohorts may vary more in type, timing, and/or amount, increasing the likelihood that spat detect a positive, attractive settlement cue; and second, spat settlement is generally higher on living vs. dead shell (Smee et al. 2013), thus effects of diversity on survivorship may in turn influence recruitment. At the northern site, we found generally higher recruitment in the four-cohort, cage treatment (Fig. 4b), similar to previous studies on a range of species, including barnacles (Gamfeldt et al. 2005), oysters (Smee et al. 2013), bivalves (Hedge et al. 2014), ascidians (Aguirre et al. 2013), and the plant *Arabidopsis thaliana* (Crawford and Whitney 2010), that observed increasing colonization with increasing genetic diversity of the established adult population. Colonization also varied with relatedness within

each level of founding cohort diversity, highlighting the potential interdependence of different metrics of diversity (Fig. 4c). However, genetic relatedness alone was a relatively weak predictor of recruitment, suggesting that the identity of oyster cohorts, rather than the genetic relatedness of the cohorts *per se*, may be contributing to this pattern (cf. Vellend et al. 2010). Alternatively, the nature of the diversity–recruitment relationship may depend not only on the diversity of individuals in the existing assemblage, but also on the diversity of individuals settling (Gamfeldt et al. 2005), though we did not measure genetic diversity of recruits in this experiment.

The positive effects of oyster cohort diversity on recruitment occur across multiple oyster life stages (i.e., using a diverse assemblage of adult oysters [Smee et al. 2013] or juvenile oysters [this study] in the founding cohort). Further, “high diversity” oyster populations, consisting of adult oysters from three bays, produced more and larger offspring than “low diversity” oyster populations, consisting of adult oysters from one bay (Smee et al. 2013). Collectively, these results are indicative of a positive feedback loop between processes generating and maintaining genetic diversity (Hedge et al. 2014) in oyster populations across multiple life history stages. Accordingly, the effects of population decline and the corresponding loss of genetic diversity may create a negative feedback loop if mechanisms that generate and sustain intraspecific variation (e.g., recruitment and settlement) are adversely affected (Smee et al. 2013).

Human activities alter the genetic structure of oyster populations, both directly via selective harvesting and indirectly via environmental change (Allendorf et al. 2008). Given the global loss of oyster reef habitat and rapid decline in oyster population size (Beck et al. 2011), understanding how genetic diversity loss affects ecological function is critical (Smee et al. 2013), particularly to inform management practices, restoration efforts, and aquaculture methods (Kettenring et al. 2014). In addition, the effects of genetic diversity on oyster demographic traits varied across sites, reinforcing that consideration of environmental conditions in management design and aquaculture practice is also important. Further, while cohort diversity was included in the best models for all demographic traits, genetic relatedness was also a strong predictor of growth and long-term survivorship. For habitat-forming species like the eastern oyster and eelgrass, genetic relatedness may be particularly important in determining the effects of genetic diversity on ecological processes given close interactions with conspecifics (Kamel et al. 2012, Stachowicz et al. 2013) and thus merits consideration in aquaculture design. Further examination of the relationship between genetic diversity (especially relatedness, which is time-consuming and expensive to measure) and phenotypic trait variation (which is sometimes faster and easier to measure) may facilitate incorporation of genetic relatedness into management, restoration, and aquaculture (Hughes 2014).

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