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# Kin aggregations occur in eastern oyster *Crassostrea virginica* reefs despite limited regional genetic differentiation

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ABSTRACT: Larval dispersal, particularly for sessile or sedentary marine organisms, significantly influences the scale of population structure in many species and fundamentally depends on the degree to which larvae from different populations are mixed in the plankton. In general, larval dispersal is thought to lead to well-mixed populations; however, recent evidence shows genetic structure at highly localized spatial scales in several benthopelagic species, raising important questions about realized patterns of larval dispersal and the scale of metapopulation connectivity. Here we use 22 microsatellite markers to characterize multi-scale patterns of genetic structure in the eastern oyster *Crassostrea virginica*, an ecologically and economically important foundation species located along the east coast of North America. At regional scales, we find limited evidence of spatial genetic structuring and weak population differentiation across 4 sites spanning 200 km of coastline. However, despite evidence of larval mixing and limited population structure, we find significant levels of kin structure at the scale of individual reefs, a pattern consistently found across samples. Such localized kin aggregations suggest that oyster larvae have significant larval retention within natal sites or exhibit non-diffusive larval movement, whereby siblings are more likely settle together. Importantly, these results show that larval mixing in the plankton is less extensive than previously believed, which has important implications for our understanding of population connectivity, gene flow, and the appropriate spatial management of marine resources.

KEY WORDS: Eastern oyster · Local retention · Genetic patchiness · Self-recruitment · Marine connectivity · Larval dispersal



## INTRODUCTION

The degree to which individuals are dispersed within and among populations can have substantial influences on range and population dynamics, genetic diversity, community composition, and rates of speciation and extinction in the sea (Roughgarden et al. 1988, Grosberg & Cunningham 2001, Swearer et al. 2002, Hastings & Botsford 2006, Hughes et al. 2008). However, levels of connectivity and dispersal are not clearly understood for many benthopelagic species, mainly due to the difficulty in tracking larvae directly (Cowen & Sponaugle 2009, Anderson et al. 2014). The prevailing thought has been that high levels of gene flow and population connectivity characterize species with the potential for extensive dispersal (Caley et al. 1996). However, for many species, recent behavioral observations, genetic studies, and biophysical models are revealing that larvae can use simple behaviors in the water column to limit dispersal, allowing for high retention of locally produced larvae within their natal populations (Knight-Jones 1953, Behrmann-Godel et al. 2006, Gerlach et al. 2007). As such, an increasing number of studies are

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revealing that even species with lengthy pelagic larval durations can display significant levels of genetic structure over relatively small spatial scales (Selkoe et al. 2006, Iacchei et al. 2013, Concepcion et al. 2014, Ottmann et al. 2016, Selwyn et al. 2016). For example, the California spiny lobster *Panulirus interruptus* shows significant kin structure at multiple sites despite low population differentiation across its 1400 km range (Iacchei et al. 2013). Thus, levels of differentiation among populations can exist at smaller scales, despite little to no genetic structure with increasing spatial distance (Gorospe & Karl 2013).

Genetic studies that estimate rates of retention and migration in metapopulations are therefore confronted by the issue of spatial scale, whereby the absence of genetic structure at a larger scale, despite its presence at a more localized scale, can inflate such estimates (Wiens 1989, Levin 1992, Gorospe & Karl 2013). Although evidence for localized genetic structure and self-recruitment in marine systems is mounting, few studies have considered the potential for a lack of gene flow between groups of individuals located less than several kilometers apart across shorter time scales (Jolly et al. 2003, Taylor & Hellberg 2003, Calderón et al. 2007, Gorospe & Karl 2013, Kamel & Grosberg 2013, D'Aloia et al. 2015). For example, in habitat-forming species, genetic variation within patches can determine productivity and resilience from disturbance and disease, as well as the number and diversity of other species, thereby affecting community structure and ecosystem processes (Whitham et al. 2006, Kamel et al. 2012, Stachowicz et al. 2013). In order to gain a more comprehensive view of the processes influencing a species' population dynamics, it is therefore crucial to examine patterns in the genetic makeup of populations across multiple spatial scales, and in particular, those at which individuals directly interact. Characterizing levels of connectivity and dispersal on these scales should therefore be of critical concern in species that contribute largely to ecosystem function, such as oysters.

The eastern oyster *Crassostrea virginica* provides key ecosystem services, including water quality enhancement, habitat construction, and sediment stabilization (Meyer & Townsend 2000, Cressman et al. 2003, Newell & Koch 2004, Grabowski et al. 2012). A recent assessment of such ecosystem services places its economic value upwards of US$ 99 000 $ha^{-1}$ $yr^{-1}$, not including harvest value, which in the state of North Carolina, USA, totals close to $52 000 $ha^{-1}$ $yr^{-1}$ (Grabowski et al. 2012). Despite their critical role in ecosystem function, oyster populations have experienced a global decline of ~85% in the past century due to overfishing, disease, and habitat destruction (Beck et al. 2011). The decline of oyster stocks has prompted concern over sustainability and considerable restoration efforts. Current management of oysters in North Carolina is explicitly spatial, including oyster sanctuaries, seed beds, and a patchwork of individual oyster leases. The success of spatial management can be improved with an understanding of how oyster subpopulations are connected demographically and genetically by the exchange of larvae, and the degree to which individual subpopulations are self-seeding. However, the extent of connectivity among oyster subpopulations in tidal creeks and estuaries in North Carolina remains unknown.

Previous studies of genetic structure in this species have primarily focused on geographic scales of 10s to 100s of km (Mann et al. 1994, Hare & Avise 1996, Rose et al. 2006, Anderson et al. 2014). For example, biophysical modeling of larval movements in the Pamlico Sound marine protected area (MPA) in North Carolina suggest that *C. virginica* larval dispersal distances may range up to 110 km (Haase et al. 2012), though how these estimates relate to realized dispersal and genetic structure on the scale of an individual reef is unknown. Here, we use microsatellite markers to estimate kinship and genetic structure within and among reefs across multiple scales: from meters to kilometers within tidal creek populations in North Carolina. Quantifying local patterns of reef structure at relatively small spatial scales will clarify the degree to which reefs are connected by dispersal, which is important for the spatial design of fisheries, MPAs, and reef restoration efforts. Moreover, the patterns of genetic structure which emerge within a reef might influence individual and population-level traits, which in turn can shape ecosystem processes.

## MATERIALS AND METHODS

### Study system

*Crassostrea virginica* is an economically and ecologically valuable species (Grabowski et al. 2012) that can be found from the Gulf of St. Lawrence in Canada and along the US Atlantic coast to the Gulf of Mexico (Carriker & Gaffney 1996). In southeastern USA, *C. virginica* reefs occupy a substantial area along the lower fringe of salt marshes and the intertidal bottom of creeks and estuaries (Meyer &

Townsend 2000). *C. virginica* are broadcast spawners, releasing gametes into the water column. After fertilization, larvae have a planktonic phase of 2–3 wk, after which the larvae seek hard substrate and attach to develop into permanently sessile individuals. In North Carolina, primary peaks in reproductive output occur from May to June, with smaller secondary peaks from July to August (Haase et al. 2012, Puckett et al. 2014). In addition to its harvest value, *C. virginica* contributes substantially to ecosystem function by providing services such as habitat production, sediment stability, and water filtration (Beck et al. 2011, Grabowski et al. 2012).

### Sample collection

We sampled 16 reefs along the North Carolina coast from intertidal estuaries in Middle Marsh, Hewlett's Creek, Masonboro Sound, and Lockwood Folly (Fig. 1). All 4 sampling sites are characterized by densely populated, naturally occurring *C. virginica* reefs across relatively flat areas devoid of apparent geographic barriers to gene flow. Adult oysters (>40 mm) were collected from 4 reefs at each site in August 2014 and in March and May 2015. The closest and farthest distances at which 2 reefs were sampled were approximately 3.8 m and 169.5 km, respectively. Individuals were randomly collected within a 9 m radius from the center of each reef, yielding a total sample size of 950 individuals.

### DNA extraction and genotyping

We dissected gill tissue from each individual, suspended the tissue in 95% ethanol, and stored it at −20°C. We then isolated genomic DNA from each tis-

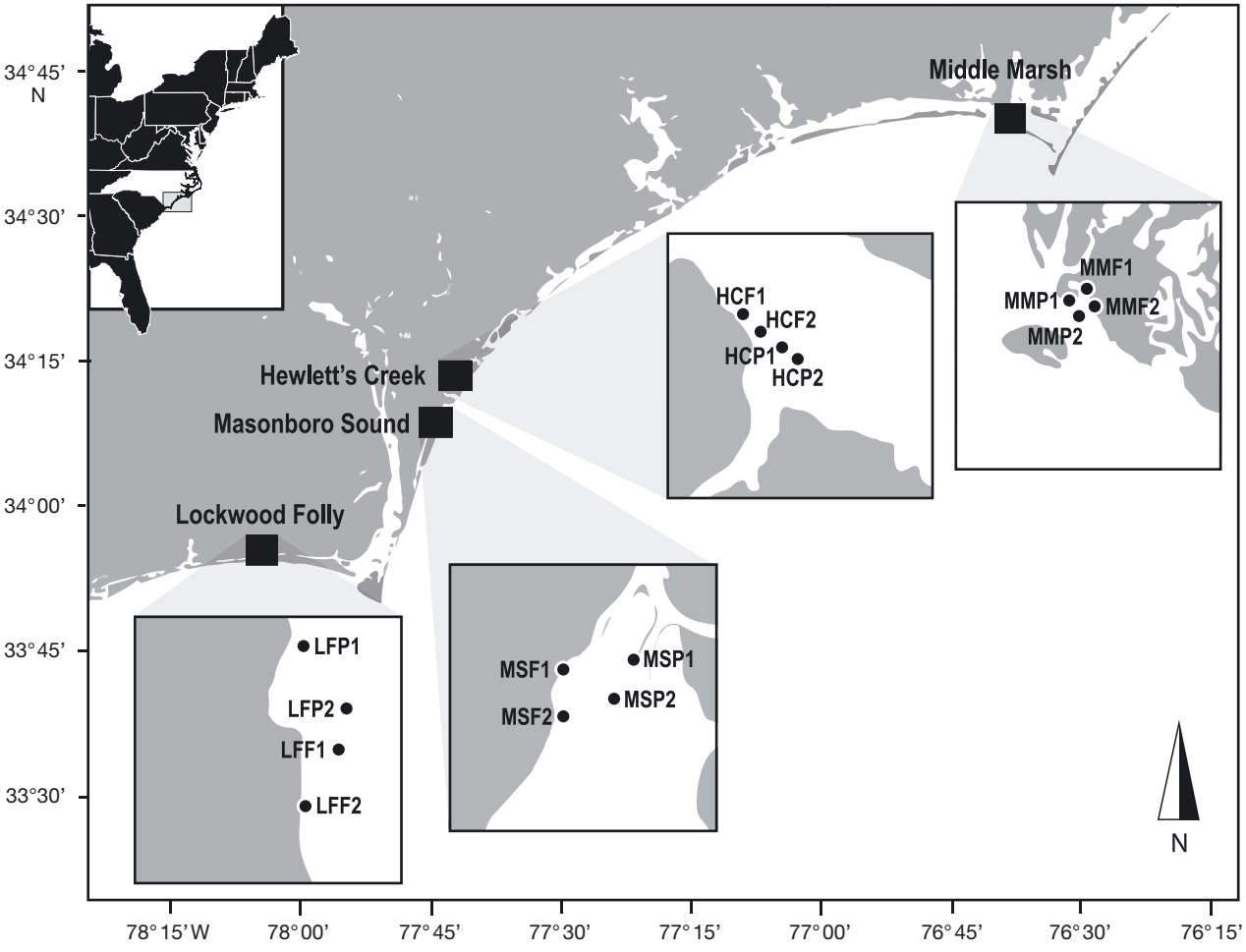



Fig. 1. Sampling sites for eastern oyster *Crassostrea virginica* along the coast of North Carolina, USA. Sites are Lockwood Folly (LF), Masonboro Sound (MS), Hewlett's Creek (HC), and Middle Marsh (MM). Within each site, 2 fringe reefs (F) and 2 patch reefs (P) were sampled

sue sample using a salting-out precipitation method as employed either with the Puregene DNA Purification Kit (Gentra Systems) or the BioExpress Ultraclean Tissue and Cells Extraction Kit (MoBio Laboratories). We amplified 22 microsatellite loci previously developed for *C. virginica* (Brown et al. 2000, Reece 2004, Carlsson et al. 2006, Wang & Guo 2007, Wang et al. 2009, Wang et al. 2010) in 4 multiplex PCRs. Individual primer working stocks contained 1 µl of 10 µM fluorescently labeled forward primer, and 10 µl each of 50 µM unlabeled forward and reverse primers diluted in 80 µl of $ddH_2O$; primers were subsequently combined into 'soups'. The basic PCR reaction contained 3.0 µl of 30 ng $\mu l^{-1}$ genomic DNA, 5.0 µl of 2× Qiagen Multiplex Mix, 0.5 µl of $ddH_2O$, and 1.5 µl of 1 of each of the 4 different primer soups. PCR conditions for all multiplex reactions were as follows: 95.0°C for 15 min, followed by 6 cycles of 30 s at 95.0°C, 45 s at 58.0°C and 1 min at 70.0°C, then 10 cycles of 30 s at 95.0°C, 45 s at 55.0°C, and 69.0°C at 1 min 30 s, then 4 cycles at 95.0°C for 30 s, 53°C for 45 s, and 69.0°C for 2 min, and finally 16 cycles of 95.0°C at 30 s, 45 s at 50.0°C, 68.0°C at 2 min 30 s, ending with a 10 min extension at 68°C. Following PCR, we set up 2 sequencing reactions: one containing 0.5 µl each of PCR product from Multiplex 1 and 2, and one containing 0.5 µl each of PCR product from Multiplex 3 and 4. We added PCR products to 9 µl of formalin containing GeneScan-600 (LIZ) size standard (Applied Biosystems) for genotyping on an ABI Prism 3130XL Genetic Analyzer at the University of North Carolina Wilmington's Center for Marine Science DNA sequencing facility. Fragments were scored using the software STRAND version 2.3.69 (Toonen & Hughes 2001).

### Data analyses

We tested for departure from Hardy-Weinberg equilibrium (HWE) within each reef by locus and over all loci using GENEPOP version 4.2 (Raymond & Rousset 1995). We calculated standard diversity indices, tests for linkage disequilibrium (LD), inbreeding coefficients ($F_{IS}$) according to Weir & Cockerham (1984), and observed and expected heterozygosity ($H_o$ and $H_e$) using ARLEQUIN version 3.11 (Excoffier et al. 2005). Using the PopGenReport package in R (Gruber & Adamack 2015), we calculated allelic richness, rarefied to 58 individuals. We then used MICROCHECKER version 2.2.3 to determine whether any deviations from HWE were due to null alleles or large allele drop-out, as well as to check for stuttering (Van Oosterhout et al. 2004). Since individuals homozygous for a null allele or heterozygous for 2 null alleles will present as missing data, there may be an association between the amount of missing data at a locus and deviation from HWE when null alleles are present. As such, we performed a linear regression between the proportion of individuals which failed to amplify at each locus by reef combination as well as the difference between $H_e$ and $H_o$ to determine if any deviations from HWE might be caused by null alleles.

### Spatial analyses

We used a hierarchical analysis of molecular variance (AMOVA) in ARLEQUIN 3.11 (Excoffier et al. 2005) to partition genetic variance across spatial scales. To further assess the degree of genetic differentiation among reefs, we calculated all pairwise values of population differentiation using Weir & Cockerham's *F*-statistics (Weir & Cockerham 1984), and tested for significance with 10 000 permutations of the data. We also calculated Weir's $F_{ST}$ using the *ENA* correction method for null alleles employed by FreeNA (Excoffier et al. 2005). To test for isolation-by-distance among all reefs and sites, we performed a Mantel test using linearized $F_{ST}$ and the natural log of Euclidean distance using Isolation by Distance Web Service version 3.2.3 (IBDWS; Jensen et al. 2005), and tested for significance of correlations with 10 000 matrix randomizations. The geographic distance (in km) between sites was the shortest over-water path connecting those points.

### Kinship analyses

To investigate spatial patterns of family structure, we calculated kinship coefficients (Loiselle et al. 1995), which are based on the relative probability of 2 alleles being identical by descent between each pair of individuals, using GENODIVE (Meirmans & Van Tienderen 2004). To determine whether kin were more likely to be clustered on the same reef patch, we used the PERMANOVA+ 1.0.2 software add-on in PRIMER7 (Clarke & Gorley 2006) to conduct a 1-way ANOVA on kinship coefficients following the approach of Iacchei et al. (2013). The data were uploaded as a correlation resemblance matrix, and a 1-way ANOVA was performed. To further investigate relationships among oysters within reefs, we binned individuals according to the following levels

of kinship ($k$): 'nearly identical', $0.57 > k > 0.375$; 'full siblings', $0.375 > k > 0.1875$; 'half siblings', $0.1875 > k > 0.09375$; and 'quarter siblings', $0.09375 > k > 0.047$. Here the bounds represent the midpoints between coancestry coefficients (Loiselle et al. 1995, Iacchei et al. 2013). We also conducted a permutation test where the observed number of closely related individuals within reefs was compared to a null distribution of kinship coefficients generated by randomly assigning oysters to reefs (Iacchei et al. 2013).

## RESULTS

### Data analyses

We successfully genotyped 950 individuals at 22 loci from 16 reefs comprising 4 different sites along the North Carolina coast. The mean number of alleles per locus ranged from 11.4 to 17.0 across reefs; similarly, mean allelic richness ranged from 11.6 to 14.3 across reefs (Table 1). $H_o$ (range: 0.52 to 0.71) was lower than $H_e$ (range: 0.75 to 0.79) for all reefs. There were significant deviations from HWE in 155 of the 352 comparisons (44%) after using a Bonferroni correction for multiple tests, and these deviations were largely attributed to homozygote excess, which was observed in 75% of comparisons. Homozygote excess can be indicative of high levels of inbreeding or the presence of null alleles. The ubiquity of homozygote excess in all reefs and across 19 of the 22 loci indicated potentially high levels of inbreeding, rather than the presence of null alleles. All sites had significant $F_{IS}$ estimates (Table 1) and, within each reef, $F_{IS}$ estimates were significant for 16 or more loci (Table S1 in the Supplement; www.int-res.com/articles/suppl/m584p079_supp.pdf). A global test for the presence of null alleles in MICROCHECKER (Clarke & Gorley 2006) revealed that 15 of the 22 loci showed potential evidence of null alleles, although the frequency of null alleles at each locus tended to be low (0.3–5%). Further, we found no relationship between the proportion of missing data and deviations from HWE across loci and reefs ($r^2 = 0.01$, $p = 0.1$). Calculation of Weir's $F_{ST}$ in FreeNA (Weir & Cockerham 1984) also revealed no bias between the data set potentially harboring null alleles and that of the data set in which alleles were generated where nulls were expected. As such, we report all analyses using our original data. Of the 3696 of tests for LD, 356 (9.6%) were significant after correcting for multiple tests, but no specific patterns were found across reefs or loci. The 3 sites with the highest LD were also those with the highest mean kinship values; furthermore, we found a significant positive correlation between mean LD and mean kinship across sites ($r^2 = 0.3$, $p < 0.05$), suggesting that kinship might be driving patterns of LD (see 'Kinship analyses' below).

### Spatial analyses

The global AMOVA revealed extremely slight, but significant, structure among sites, although almost all the genetic variation (99.25%) occurred among indi-

Table 1. Summary statistics for each *Crassostrea virginica* reef averaged over all 22 microsatellites. Observations include: sample size (n), mean number of alleles per locus ($A$), mean allelic richness ($A_R$), mean inbreeding coefficient ($F_{IS}$), observed heterozygosity ($H_o$), expected heterozygosity ($H_e$), percentage of loci pairs in linkage disequilibrium (LD), and mean kinship coefficient ($k$). **Bold:** significant at $p < 0.05$

| Area | Site | Reef type | n | $A$ | $A_R$ | $F_{IS}$ | $H_o$ | $H_e$ | % loci in LD | $k$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Middle Marsh | MMP1 | Patch | 50 | 15.2 | 13.7 | **0.16** | 0.65 | 0.77 | 1.73 | 0.002 |
| | MMP2 | Patch | 48 | 14.7 | 13.3 | **0.21** | 0.62 | 0.78 | 11.26 | 0.000 |
| | MMF1 | Fringe | 48 | 14.1 | 12.9 | **0.21** | 0.63 | 0.79 | 13.85 | 0.002 |
| | MMF2 | Fringe | 34 | 11.4 | 11.6 | **0.14** | 0.68 | 0.78 | 33.33 | 0.004 |
| Hewlett's Creek | HCP1 | Patch | 54 | 13.8 | 12.6 | **0.31** | 0.53 | 0.75 | 20.35 | 0.005 |
| | HCP2 | Patch | 60 | 15.6 | 13.6 | **0.22** | 0.60 | 0.76 | 4.76 | 0.001 |
| | HCF1 | Fringe | 71 | 16.2 | 13.7 | **0.32** | 0.52 | 0.75 | 14.72 | 0.007 |
| | HCF2 | Fringe | 64 | 16.4 | 14.3 | **0.27** | 0.56 | 0.76 | 5.63 | 0.004 |
| Masonboro Sound | MSP1 | Patch | 63 | 16.0 | 13.9 | **0.17** | 0.63 | 0.76 | 5.63 | 0.004 |
| | MSP2 | Patch | 67 | 16.3 | 13.7 | **0.18** | 0.63 | 0.77 | 0.43 | 0.001 |
| | MSF1 | Fringe | 58 | 15.8 | 13.7 | **0.14** | 0.68 | 0.79 | 3.46 | 0.002 |
| | MSF2 | Fringe | 68 | 16.3 | 13.9 | **0.11** | 0.71 | 0.79 | 0.43 | 0.002 |
| Lockwood Folly | LFP1 | Patch | 65 | 16.3 | 14.0 | **0.16** | 0.65 | 0.77 | 6.06 | 0.003 |
| | LFP2 | Patch | 64 | 17.0 | 14.5 | **0.11** | 0.69 | 0.77 | 11.69 | 0.004 |
| | LFF1 | Fringe | 69 | 15.8 | 13.5 | **0.22** | 0.59 | 0.78 | 6.49 | 0.004 |
| | LFF2 | Fringe | 67 | 16.1 | 13.5 | **0.22** | 0.59 | 0.75 | 14.29 | 0.004 |

viduals within reefs (Table 2). Of the 120 pairwise comparisons of genetic differentiation, 26 (22.2%) were significant, only 1 of which occurred within a site (Table 3). We found no correlation between geographic distance and $F_{ST}$ ($r^2 = 0.03$, $p > 0.05$) when all 16 reefs were included. When only considering within-site comparisons, we found a significant positive correlation between geographic and genetic distance ($r^2 = 0.15$, $p < 0.05$), suggesting that distance between reefs might be important at local scales, despite broad mixing across regional geographic scales.

### Kinship analyses

Overall, kinship coefficients within reefs ranged from –0.143 to 0.236, with an overall mean kinship of 0.002. However, the mean kinship among oysters within reefs (n = 16) was 0.003 ± 0.002, which was higher than the mean kinship among oysters located on different reefs (–0.0002 ± 0.002). Indeed, kinship coefficients were significantly greater for within-reef than among-reef comparisons (pseudo-$F_{15, 934}$ = 1.443, $p < 0.001$), supporting the observation that the majority of kin comparisons were between oysters located on the same reef (Fig. 2). There were significantly more kin groupings than expected by chance in all but 1 reef (MMF1). For example, 9 of the 16 reefs showed a greater proportion of half siblings than expected by chance (Fig. 3). Mean within-reef kinship values were significantly positively correlated with the inbreeding coefficient ($r^2 = 0.26$, $p < 0.05$). At a regional scale, within-site (n = 4) kinship coefficients were also significantly greater than those among sites (pseudo-$F_{3, 946} = 2.424$, $p = 0.0001$).

Table 2. *Crassostrea virginica*. Global hierarchical analysis of molecular variance (AMOVA) as a weighted average over all loci

| Source of variation | Variance components | % of variation | p-value |
|---|---|---|---|
| Among sites | 0.03 | 0.32 | <0.01 |
| Among reefs within sites | 0.04 | 0.42 | <0.01 |
| Within reefs | 8.47 | 99.26 | <0.01 |

## DISCUSSION

Across species with intermediate larval durations (1–60 d), there is accumulating evidence that larvae rarely reach their full dispersal potential, contradicting the assumption that most marine populations are genetically homogeneous across broad geographic scales (Jones et al. 1999, Swearer et al. 1999, 2002, Mora & Sale 2002, Grantham et al. 2003, Taylor & Hellberg 2003, Marko 2004, Cowen et al. 2006, Becker et al. 2007, Lopez-Duarte et al. 2012, Iacchei et al. 2013, Ottmann et al. 2016). However, with a larval duration of 14–25 d (Haase et al. 2012), *Crassostrea virginica* shows limited spatial genetic structure and weak population differentiation among 4 sites spanning 200 km of coastline, suggesting a pat-

Table 3. *Crassostrea virginica*. Matrix of geographic distance (km) above diagonal, and pairwise genetic distance ($F_{ST}$) below diagonal. Within-site comparisons in **bold**; significant values in italics. See Fig. 1 for site abbreviations

| | MMP1 | MMP2 | MMF1 | MMF2 | HCP1 | HCP2 | HCF1 | HCF2 | MSP1 | MSP2 | MSF1 | MSF2 | LFP1 | LFP2 | LFF1 | LFF2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MMP1 | | 0.042 | 0.044 | 0.056 | 126.2 | 126.2 | 126.3 | 126.3 | 125.9 | 126.0 | 126.1 | 126.1 | 169.3 | 169.4 | 169.5 | 169.3 |
| MMP2 | **–0.003** | | 0.063 | 0.04 | 126.2 | 126.2 | 126.3 | 126.3 | 126.0 | 126.0 | 126.1 | 126.1 | 169.3 | 169.4 | 169.5 | 169.3 |
| MMF1 | **–0.009** | **–0.003** | | 0.043 | 126.3 | 126.3 | 126.3 | 126.3 | 126.0 | 126.1 | 126.2 | 126.1 | 169.4 | 169.4 | 169.5 | 169.4 |
| MMF2 | **–0.004** | **–0.007** | **–0.006** | | 126.3 | 126.3 | 126.3 | 126.3 | 126.0 | 126.1 | 126.2 | 126.1 | 169.4 | 169.4 | 169.5 | 169.4 |
| HCP1 | –0.005 | 0.000 | –0.003 | 0.003 | | 0.038 | 0.108 | 0.058 | 1.189 | 1.225 | 1.198 | 1.113 | 43.87 | 43.94 | 44.07 | 43.85 |
| HCP2 | 0.002 | –0.009 | –0.014 | –0.008 | **–0.011** | | 0.146 | 0.097 | 1.152 | 1.188 | 1.161 | 1.076 | 43.87 | 43.94 | 44.07 | 43.86 |
| HCF1 | *0.006* | 0.002 | –0.002 | 0.001 | **–0.005** | **0.001** | | 0.05 | 1.295 | 1.33 | 1.30 | 1.217 | 43.84 | 43.91 | 44.04 | 43.83 |
| HCF2 | 0.004 | –0.003 | –0.010 | –0.005 | **–0.011** | **0.001** | **–0.001** | | 1.246 | 1.281 | 1.252 | 1.168 | 43.85 | 43.92 | 44.05 | 43.84 |
| MSP1 | 0.000 | –0.007 | –0.017 | –0.004 | –0.010 | 0.001 | *0.006* | 0.001 | | 0.09 | 0.191 | 0.149 | 43.92 | 43.99 | 44.12 | 43.91 |
| MSP2 | *0.004* | –0.007 | –0.020 | –0.010 | –0.011 | 0.001 | *0.004* | 0.004 | **–0.001** | | 0.114 | 0.123 | 43.84 | 43.91 | 44.04 | 43.83 |
| MSF1 | *0.005* | 0.002 | –0.010 | –0.009 | 0.003 | *0.008* | *0.014* | *0.012* | **0.002** | **0.000** | | 0.101 | 43.73 | 43.8 | 43.93 | 43.72 |
| MSF2 | 0.000 | –0.007 | –0.017 | –0.014 | –0.005 | *0.004* | *0.007* | *0.008* | **0.000** | **0.002** | **0.001** | | 43.79 | 43.86 | 43.99 | 43.78 |
| LFP1 | *0.003* | –0.005 | –0.010 | –0.001 | 0.000 | 0.002 | *0.011* | *0.007* | 0.002 | 0.000 | 0.001 | 0.000 | | 0.092 | 0.225 | 0.160 |
| LFP2 | *0.004* | –0.004 | –0.013 | –0.001 | 0.001 | 0.004 | *0.009* | *0.007* | 0.003 | 0.000 | 0.000 | *0.003* | **0.001** | | 0.137 | 0.236 |
| LFF1 | *0.006* | –0.004 | –0.006 | 0.001 | –0.001 | 0.002 | *0.008* | *0.006* | *0.005* | 0.001 | *0.008* | *0.005* | ***0.005*** | **0.003** | | 0.339 |
| LFF2 | 0.001 | –0.002 | 0.000 | *0.006* | 0.002 | –0.003 | 0.004 | –0.003 | –0.002 | –0.005 | 0.002 | –0.001 | **0.004** | **0.001** | **0.001** | |

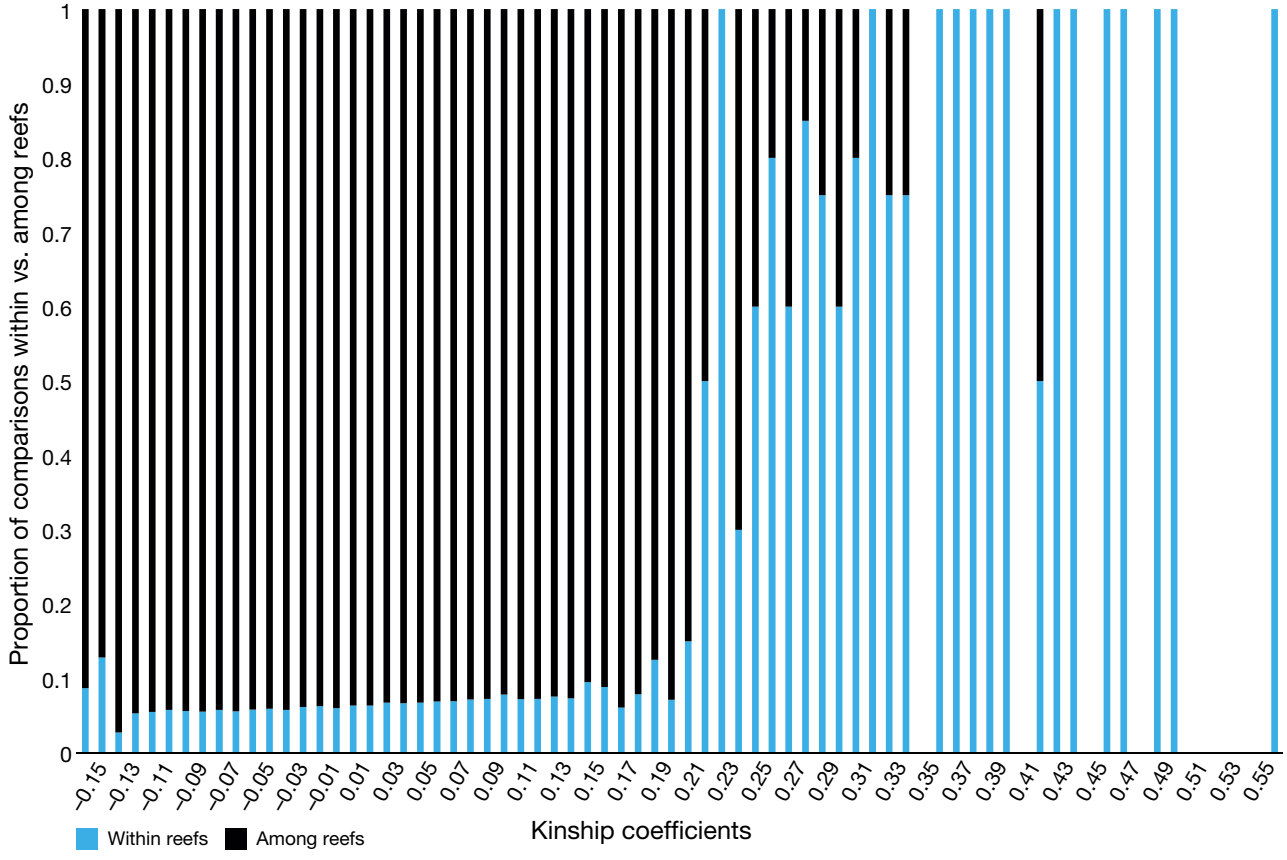
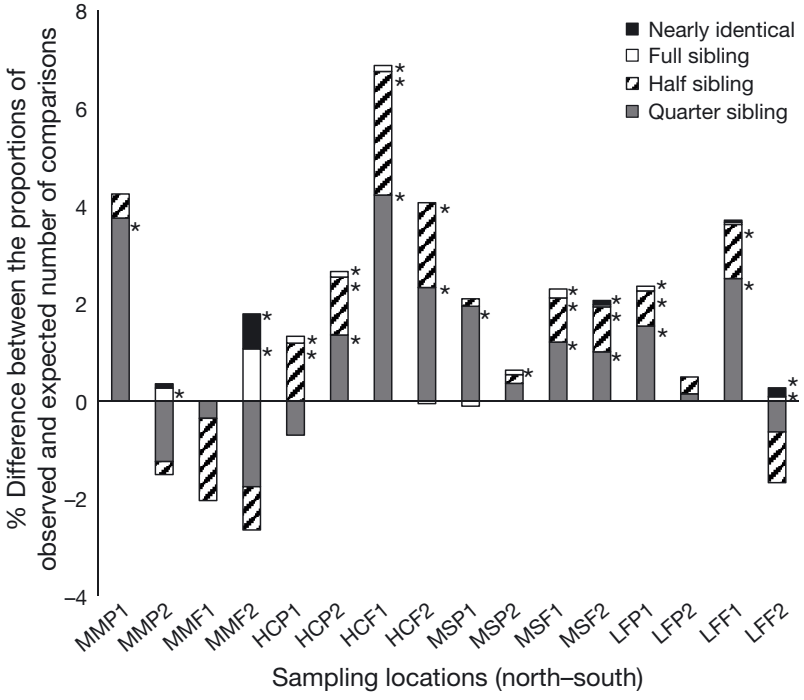



Fig. 2. Proportion of total number of pairwise kinship ($k$) comparisons (n = 450 775) among all *Crassostrea virginica* oysters sampled. Kinship estimates are divided into 0.01 bins (each vertical line represents a particular kinship value) and each value is partitioned into within-reef (blue bars) or among-reef (black bars) comparisons. Absence of bars indicates kinship coefficients that were not found in the current data set




tern of genetic homogeneity, at least over a broad regional scale. Hierarchical analyses of variance revealed that <1% of genetic variation could be explained by differences among sites; almost all the genetic variation occurred among individuals within reefs. This mirrored the isolation-by-

Fig. 3. Proportion of observed kinship comparisons for each *Crassostrea virginica* reef that were more or less than expected for each of 4 kinship categories (nearly identical: $0.375 < k < 0.57$; full sibling: $0.1875 < k < 0.375$; half sibling: $0.09375 < k < 0.1875$; and quarter sibling: $0.047 < k < 0.09375$). Expected numbers of kinship comparisons for each category based on pairwise kinship coefficients that were calculated after randomly shuffling individuals among reefs. *: significant differences at $p < 0.05$. See Fig. 1 for site abbreviations

distance analyses which showed no relationship of increasing genetic dissimilarity with increasing distance. Further, the majority of significant $F_{ST}$ values included comparisons with reefs located in Hewlett's Creek. This site, located in the headwaters of the creek, might experience more limited tidal excursions leading to reduced larval dispersal into the surrounding waters.

Our results differ from previous studies of genetic structure among *C. virginica* populations which have shown discontinuity in genetic homogeneity across broad spatial scales in the Chesapeake Bay and along the Texas and Florida coasts (Hare & Avise 1996, Rose et al. 2006, Anderson et al. 2014). Along the eastern Florida coastline, analyses of mitochondrial and nuclear loci revealed a stepped genetic cline in the transition zone between populations of oysters collected from sites located approximately 40 km apart (Hare & Avise 1996). Further studies of this genetic cline indicate that larval dispersal along the coast should be sufficient to homogenize populations, making limitations to dispersal unlikely and local adaptation a possible contributor to the observed genetic differentiation (Zhang & Hare 2012). Indeed, a reciprocal translocation experiment revealed that local adaptation is a major mechanism in promoting the maintenance of this step cline, with oysters displaying better fitness in their natal environments (Burford et al. 2014).

Importantly, our analyses of genetic structure within and among individual reefs revealed far more significant patterns of genetic differentiation. Across all sites, oysters were more closely related within than between reefs and, for the majority of reefs, we found significantly greater than expected levels of kinship between adult oysters. The close association between mean reef-kinship and reef-specific $F_{IS}$ values supports the idea that stable aggregations of kin could be driving fine-scale genetic structure in *C. virginica*. This suggests 2 possible mechanisms that could be driving this pattern: (1) retention of a significant proportion of kin on their natal reefs, or (2) collective dispersal (Yearsley et al. 2013), whereby closely related larvae travel in 'packets' and settle together (Finelli & Wethey 2003, Siegel et al. 2008, Ottmann et al. 2016). An increasing number of studies support the idea that self-recruitment and limited dispersal are some of the major drivers of genetic structure in marine populations (Cowen & Sponaugle 2009, Ottmann et al. 2016). Local retention can also be prevalent despite high levels of gene flow, if pools of larvae released from a local breeding group do not diffuse randomly, but instead remain aggregated during dispersal and settlement (Bernardi et al. 2012, Iacchei et al. 2013, Yearsley et al. 2013). In a recent study of the effects of larval swimming behavior on dispersal and settlement in *C. virginica*, hydrodynamic and larval behavior models were used to demonstrate that the probability of settlement depends on the local hydrodynamic environment and swimming behavior (Hubbard & Reidenbach 2015). Importantly, their simulations of dispersal indicate that most larvae do not settle very far from their original spawning locations, and that reefs in areas characterized by low flow velocities (bays and more sheltered areas) were more likely to show self-colonization than reefs in those areas characterized by high flow velocities, such as deep channels connected to inlets (Hubbard & Reidenbach 2015). Given the relatively sheltered nature of our study sites, low flow velocities could potentially be contributing to high levels of larval retention and self-recruitment.

Alternatively, in marine species with high fecundity and highly variable reproductive or recruitment success, extremely skewed differential reproductive success, in which only a small proportion of individuals within a population actually reproduce, may foster 'sweepstakes-like chances' of larval survival and settlement success (Johnson & Black 1982, 1984, Christie et al. 2010, Hedgecock & Pudovkin 2011). These effects tend to lead to temporary kin structure, with higher than expected relatedness within cohorts of recruits declining as subsequent cohorts settle over time (Allendorf & Phelps 1981, Hedgecock 1994, Herbinger et al. 1997, Selkoe et al. 2006, Hedgecock et al. 2007, Christie et al. 2010, Hedgecock & Pudovkin 2011). Although these results may reflect sweepstakes effects, the reef-specific patterns of kin structure seen in our study argue strongly in favor of sibling cohesiveness, where siblings are more likely to settle together than disperse across sites (Bernardi et al. 2012, Iacchei et al. 2013). This implies that some pelagic assemblages of sibling larvae remain more-or-less intact as they disperse from their natal site, despite the potential for admixture during the planktonic phase (Veliz et al. 2006, Iacchei et al. 2013).

Another intriguing possibility is that, given that the intertidal zone is characterized by strong environmental gradients, local adaptation might be an important driver of the underlying patterns of genetic structure (Sanford & Kelly 2011). Environmental heterogeneity can produce genetic differentiation across small spatial scales, even among populations that lack physical barriers to gene flow. For example, Tobler et al. (2008) observed phenotypic divergence, high levels of population differentiation, and low

rates of migration in the Atlantic molly *Poecilia mexicana* across habitat types characterized by differences in hydrogen sulfide content and light. Adaptation to a spatially varying environment might result in non-random post-settlement mortality, with larvae surviving better on their natal reef types, leading to the observed patterns of kin structure. We know that fringing reefs differ from seaward reefs in a number of biotic and abiotic factors, including tidal elevation, emersion time, sediment load, salinity, and predation pressure (Shumway 1996, Lenihan 1999, Lenihan et al. 1999). However, links to environmental differences and their influence on patterns of post-settlement mortality in *C. virginica* remain untested.

While understanding the processes generating such patterns of genetic structure remains an open question, the consequences of kin structure on localized scales also warrant consideration. For example, Hanley et al. (2016) showed that while in juvenile oysters, cohort diversity and kinship were negatively associated with long-term survivorship, kinship did show a positive association with growth. Conversely, Smee et al. (2013) found greater settlement of oyster larvae on experimental assemblages of adult oysters with high genetic diversity than on experimental assemblages of adult oysters with low genetic diversity. Given that oyster reefs have experienced a significant global decline due to overharvesting, coastal development, and environmental degradation, and that oyster reef restoration has often produced equivocal results (Beck et al. 2011, Grabowski et al. 2012), other factors such as kin genetic structure and genetic diversity should be considered in efforts aimed at population recovery.

Our kinship analyses show that across North Carolina, the majority of sampled reefs contain an excess of closely related individuals, despite population-level data showing limited regional differentiation or isolation-by-distance patterns. This suggests that either self-recruitment or coordinated larval delivery is driving small-scale genetic differences in a species with a moderately long larval duration, and complements the growing number of studies that show genetic differentiation over small spatial scales in marine populations (Veliz et al. 2006, Christie et al. 2010, Iacchei et al. 2013, Selwyn et al. 2016). The small scale at which potential breaks in connectivity are occurring in this system should thus be considered during the placement of constructed reef reserves and the design of MPAs. Shorter dispersal distances should favor the delineation of MPAs that are closer to one another in order to facilitate connectivity between reserves (Sunday et al. 2014). Connectivity among populations of habitat-forming species can determine levels of genetic variation that are essential to the promotion of aspects associated with population performance such as growth and biomass (Kamel et al. 2012), persistence in the face of habitat fragmentation and environmental alterations due to climate change (Cowen & Sponaugle 2009), and biodiversity of facilitated species (Whitham et al. 2006). Understanding the extent of connectivity between populations, and how realized larval dispersal alters connectivity and gene flow, will be essential to the recovery and long-term sustainability of the eastern oyster fishery.

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