The following supplements accompany the article

Local adaptation of a marine invertebrate with a high dispersal potential: evidence from a reciprocal transplant experiment of the eastern oyster *Crassostrea virginica*

M. O. Burford[1,3,*], J. Scarpa[2], B. J. Cook[1], M. P. Hare[1]

[1]Department of Natural Resources, Cornell University, Ithaca, NY 14853, USA
[2]Harbor Branch Oceanographic Institute at Florida Atlantic University, Fort Pierce, FL 34946, USA

[3]*Present address:* Department of Applied Ecology, North Carolina State University, Raleigh, NC 27695, USA

*Corresponding author: mbreiski@ncsu.edu

Marine Ecology Progress Series 505: 161–175 (2014)

SUPPLEMENT 1

Genetic parentage analysis

Material and methods of parentage analysis

To confirm that each progeny population was spawned from multiple parents and to rule out differences in parental contribution between regions for a given progeny population, we conducted a microsatellite parentage analysis. If proportional contributions of parents within a cross were different across regions (by chance or selection) then regional differences in phenotypic measures could be a family effect rather than representative of mean population effects. Therefore, within each progeny population (N×N and S×S) we tested for differences in parental contributions between surviving oysters in the northern and southern regions. We extracted genomic DNA from the muscle tissue of each specimen using a DNA Extraction Kit (Qiagen) and quantified template DNA using a Nanodrop8000 (Thermo Fisher Scientific) following both manufacturer's protocols. From the extracted genomic DNA, we amplified 4 microsatellite loci (*Cvi.12v*, *Cvi.5v*, *Cvi2i.4*, & *Cvi2i.23*; GenBank Acc. No. DQ205722, DQ205720, AY644658, AY644660, respectively; Reece et al. 2004, Carlsson et al. 2006) using polymerase chain reaction (PCR). The PCR reactions followed the manufacturer's reagent protocol (Invitrogen) and used approximately 10 ng of template DNA in a 7.5 ml reaction. Using fluorescently labeled primers (Perkin Elmer, ABI) for the PCR reactions, we followed cycling conditions that included initial denaturation for 95°C at 1 min, 30 cycles of 95°C for 30 s denaturation, 55, 47, or 51.5°C for 20 s annealing, and 72°C for 10 min final extension. We conducted fragment amplification and analysis at the Cornell Laboratory Core on an ABI3730 automated sequencer and scored the microsatellites using GeneMapper (v4.0; Applied Biosystems) to assign genotypes. We used the genotypes generated by these 4 microsatellites to assess parental contribution of adults, from the 10-by-10 mating, to the progeny in the reciprocal transplant that survived the experiment. With 20 adults per mating and unique genotypes generated from 4 loci, we hand-scored the parents for each individual. The analysis of parental contribution was a conservative estimate, as we did not analyze contribution during the larval or settlement phase, but only after the completion of the experiment and, therefore, we would expect some parents from the 10-by-10 crosses to contribute slightly more than others by chance. We analyzed all 20 adult brood stock and approximately 32 F_1 survivors per genetic cross. For the parentage analysis we used

individuals for which we could assign a parent based on a minimum of 3 of the 4 microsatellite loci, and only allowed the minimum if there was no other possible parent. We conducted a 2-tailed, paired *t*-test (Sokal & Rohlf 1995) to test for significant differences in parental contribution of the 20 adults per progeny populations in the north versus the south, a test of regional difference in family effect.

Results of brood stock contribution

Our goal was to test whether any observed differences in parental contribution between regions for a given progeny population was significant. We were able to diagnose parentage for 31 N×N progeny (15 in the north and 16 in the south) and 30 S×S progeny (12 in the north and 18 in the south). Of the 20 adults we used to produce the N×N progeny, 16 contributed to progeny that survived the reciprocal transplant. For the S×S cross, 13 of the 20 brood stock contributed to the progeny that survived the transplant. We found no regional differences in the parental contribution for the 2 progeny crosses (paired *t*-test between regions, N×N, $p = 0.23$; S×S $p = 0.49$).

Phytoplankton community analysis

Material and methods of the phytoplankton analysis

We collected phytoplankton samples every other week at each of the experimental sites to estimate differences in food sources (e.g. dinoflagellates versus diatoms) between the 2 regions. In general, the eastern oyster consumes a diet comprised predominately of mixed plankton species (Langdon & Newell 1996). Previous research indicated that juvenile oysters had higher relative growth when fed a diet of several diatom species of plankton than flagellated species (Enright et al. 1986, Langdon & Newell 1996). We obtained samples in open water immediately adjacent to the experimental slate using a 3.05 m rope attached to the handle of a 10 l bucket that we sank until the bucket top was at the same depth as the lower edge of the experimental slate (approximately 30 cm off the bottom sediment). Therefore, we sampled the phytoplankton community in contact with experimental oysters. We poured a 5 l sample from the bucket through a 20 micron filter, and using 1 mm filtered seawater washed the contents of the filter into a 50 ml Falcon tube. We repeated this process 3 times at 5 min intervals (total volume 15 l) and, using filtered seawater, resuspended the combined sample (15 l) to 50 ml in a Falcon tube. Finally, we added Lugol's Iodine to the sample (approximately 0.5 to 1 ml) and stored the sample in a dark cool container. We processed samples within 2 months.

To identify and quantify the phytoplankton, we dispersed 1 ml aliquot of the resuspended sample evenly across a counting slide (Sedgewick Rafter Counting Cell slide). Using a light microscope, we obtained a quick qualitative overview to ensure that the central vertical column roughly represented the entire sample. If it did not, we discarded the aliquot and took another sample. We counted all phytoplankton in a total of 6 squares per slide, identified individuals to genus when possible using a variety of sources including Cupp (1943), and cross-referenced identifications with previous studies completed in the Indian River Lagoon (Phlips et al. 2004, Badylak & Phlips 2004). For phytoplankton that lacked identifying marks, we classified them by morphospecies (e.g. unknown centric diatom, or chain diatom). For the final analysis we placed individuals in 2 larger groups: diatoms and dinoflagellates. We calculated the average number of diatoms and dinoflagellates across sites within a region, and also calculated the regional differences across sites within region and across the 5 sampling periods.

We performed a 2-tailed, paired *t*-test (Sokal & Rohlf 1995) to test for differences in average numbers of diatoms versus dinoflagellates over time within each region, because we had no *a priori* expectation of higher diatoms or dinoflagellates in either region. We conducted the *t*-test with each of the

5 sampling time points to confirm differences in the food sources for the outplanted oysters. As phytoplankton abundance and proportions vary during the course of a season, and given that we only had 5 sample times, we focused on average regional differences rather than differences at specific experimental sites.

Results of the phytoplankton analysis

We analyzed a total of 40 phytoplankton samples, 4 sites in each of the 2 regions (north and south) for a total of 5 weeks that began 11 and 12 June, and ended 5 and 8 August 2008, respectively. For both regions, there were higher densities of phytoplankton in the earlier and late samples and relatively low densities at the midpoint sample (third week, 9 and 10 July 2008, respectively). We also found differences in the proportion of diatoms versus dinoflagellates in the phytoplankton analysis (Fig. 2). In the northern region there was significantly higher numbers of diatoms than dinoflagellates overall, because the latter were consistently scarce in the samples (paired *t*-test, $p = 0.009$). In the southern region we found greater temporal variation in the proportions of diatoms and dinoflagellates and did not find a significant difference in abundances between the 2 classes of phytoplankton (Fig. 2; paired *t*-test, $p = 0.324$). Dinoflagellates outnumbered diatoms in the southern region during 2 of 5 sampling periods and diatoms consistently exceeded dinoflagellate counts in the north.

LITERATURE CITED

Badylak S, Phlips EJ (2004) Spatial and temporal patterns of phytoplankton composition in subtropical coastal lagoon, the Indian River Lagoon, Florida, USA. J Plankton Res 26:1229–1247

Carlsson J, Morrison CL, Reece KS (2006) Wild and aquaculture populations of the eastern oyster compared using microsatellites. J Hered 97:595–598

Cupp EE (1943) Marine plankton diatoms of the west coast of North America. Bull Scripps Inst Oceanogr Vol 5, University of California Press, Berkeley, CA

Enright CT, Newkirk GF, Craigie JS, Castell JD (1986) Evaluation of phytoplankton as diets for juvenile *Ostrea edulis* L. J Exp Mar Biol Ecol 96:1–13

Langdon CJ, Newell RLE (1996). Digestion and nutrition in larvae and adults. In: Kennedy VS, Newell RIE, Eble AF (eds) The eastern oyster *Crassostrea virginica.* Maryland Sea Grant, College Park, MD, p 231–269

Phlips EJ, Badylak S, Young S, Kelley K (2004) The occurrence of potentially toxic dinoflagellates and diatoms in subtropical lagoon, the Indian River Lagoon, Florida USA. Harmful Algae 3:39–49

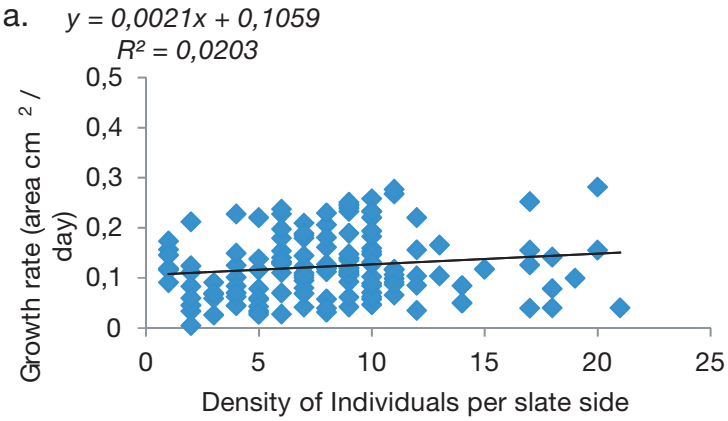
Reece KS, Ribeiro WL, Gaffney PM, Carnegie RB, Allen SK Jr (2004) Microsatellite marker development and analysis in the eastern oyster (*Crassostrea virginica*): confirmation of null alleles and non-Mendelian segregation ratios. J Hered 95:346–352

Sokal RR, Rohlf FJ (1995) Biometry. WH Freeman, New York, NY

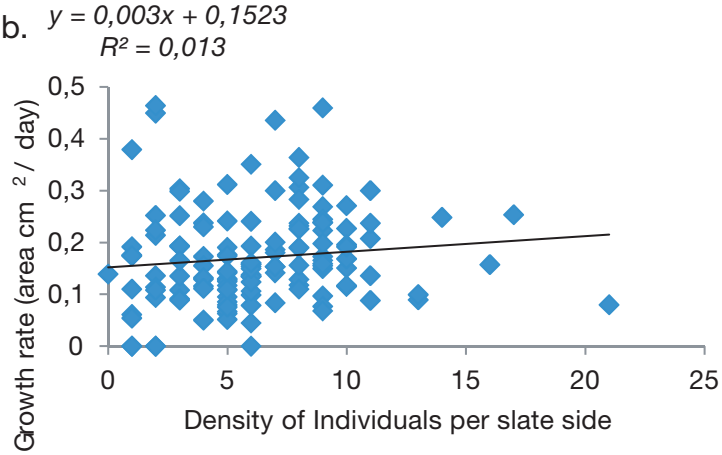
SUPPLEMENT 2

Fig. S1. Analysis of outplanted *Crassostrea virginica* N×N and S×S progeny populations density per settlement plate side versus growth rate at (a) Week 2, (b) Week 6, and (c) Week 11 of the reciprocal transplant in 2008

a.



b.



c.

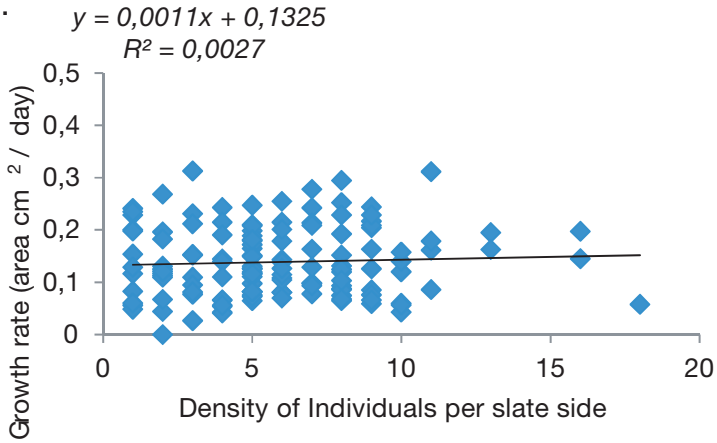


Fig. S2. Weekly survival rate of the outplanted *Crassostrea virginica* N×N and S×S progeny populations in 2008 in the northern and southern regions averaged over all 4 experimental sites