Performance of selectively-bred lines of eastern oyster, *Crassostrea virginica*, across eastern US estuaries

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First to evaluate multiple oyster lines across diverse sites

Contributors

DAP assisted in field evaluation, conducted data analysis, and wrote the manuscript. BTV confirmed propriety of statistical analysis, conducted the mixed effects analysis and assisted in writing the manuscript. RJC and JP conducted field evaluation at RI sites, collected disease acquisition data, and assisted with data analysis. SKA Jr. coordinated activities in VA (e.g. production of southern lines, field evaluation at the VA site) and assisted in writing the manuscript. JMS assisted in coordinating activities in VA. XG, CL, ML, and GD conducted field evaluation in NJ. PR coordinated activities in ME (production of northern lines, field evaluation at the ME site). MGC coordinated the entire project, and assisted in writing the manuscript.

Abstract

Eastern oyster, *Crassostrea virginica*, aquaculture has expanded greatly in recent years, but further growth of the industry is constrained by disease-related losses. Oyster breeding programs supporting the oyster aquaculture industry along the east coast of the US have targeted resistance to three prominent diseases: MSX, Dermo, and ROD, caused by *Haplosporidium nelsoni*, *Perkinsus marinus*, and *Roseovarius crassostreae* respectively. Consequently, selected oyster lines possess some level of resistance and/or tolerance but the extent to which these lines, derived from various programs, perform across diverse growing environments used by industry has not been tested. The performance of six selected eastern oyster lines was evaluated at five sites along the east coast of the US (Maine to Virginia) to 1) identify differences in performance among lines at each site, and 2) identify lines that perform well across all sites. Performance measures included growth, mortality, and yield over a 15-month evaluation period. During unusually high mortality events, subsets of oysters were processed for disease diagnosis. Growth trajectories were similar among lines within a site, but varied significantly across sites (78% of random variance explained). Oysters grown in Rhode Island were largest while oysters grown in Maine were smallest at the end of the study. Mortality varied greatly among lines at each site as well as among sites. Line $\times$ site interaction explained 61% of the total random variance in the mortality data. In Maine, extensive mortality was observed early in the year for all lines, coincident with increased ROD prevalence. In New Jersey and Virginia, unusually high mortality was evident in the UMFS, Clinton, and NEH-RI lines during the final months of the experiment when the prevalence of both Dermo and MSX were 100% and <50%, respectively. NEH, DEBY, and hANA lines were less affected, demonstrating that lines selected to perform better in their native site surpassed those selected outside the area. Despite large and significant line $\times$

62 site interaction effects for mortality and yield, NEH, DEBY, and hANA performance was above
63 average across all sites. These findings have important implications for oyster breeding
64 strategies and industry practices.

65

66 **Keywords**

67 *Crassostrea virginica*, performance, selection, $G \times E$ interaction, disease, aquaculture, oyster,
68 breeding

69

70 **Abbreviations**

71 MSX-Multinucleated Sphere X, ROD-*Roseovarius* Oyster Disease, SSO-Seaside Organism,
72 CM-Cumulative Mortality

1. Introduction

The eastern oyster, *Crassostrea virginica*, has been an ecological, economic, and cultural mainstay of eastern US estuaries for centuries. Overfishing during the 18th and early 19th centuries reduced native populations to a fraction of historic levels, particularly in the Northeast (Kirby, 2004; Beck et al., 2011; Ermgassen et al., 2012). Aquaculture has begun to fill the production void created by the collapsed fishery; however, contemporary issues, such as the quantity and quality of available oyster seed and the introduction and spread of oyster pathogens, continue to constrain the industry's potential (Naylor et al., 2000; Jackson et al., 2001).

Hatcheries are a critical element of eastern oyster culture. Because natural recruitment in northeastern estuaries can be highly unpredictable and often absent entirely (Thompson et al., 1996; Oviatt, 2004), oyster growers are dependent on hatchery-produced seed to stock their farms. In addition to providing the vast number of seed required to sustain a profitable aquaculture enterprise, hatcheries are in an opportune position to positively impact oyster culture by improving existing oyster germplasm through selective breeding (Allen et al., 1993).

Disease resistance represents a high priority target for selective breeding throughout the Northeast and Mid-Atlantic regions. Since the 1950's, eastern oyster populations have suffered losses from three principal diseases: MSX, Dermo, and *Roseovarius* Oyster Disease (ROD). Exposure of farmed oysters to the causative agents of disease is inconsistent and unpredictable over space and time (Powell et al., 2011). Therefore the use of selective breeding for disease resistance and/or tolerance is necessary to minimize the impact(s) of disease on the culture industry (Dégremont et al., 2015).

MSX, which is caused by the parasite *Haplosporidium nelsoni*, was first detected between 1957 and 1959 as an epizootic resulting in the death of > 90% of infected oysters in the

lower Delaware Bay (Ford and Haskin, 1982). Although the parasite was initially confined to the Delaware and Chesapeake Bays, it has since invaded estuaries as far north as the Damariscotta River, Maine (Messerman et al., 2012) and as far south as South Carolina (Bobo et al., 1997). For much of the latter half of the 20th century, Dermo disease (caused by the parasite *Perkinsus marinus* and responsible for > 50% mortality in affected populations), was primarily a problem in the southeastern US and the Gulf of Mexico; however, by 1992 the disease had spread to nearly every region where eastern oysters are cultured (Ewart and Ford, 1993; Ford and Smolowitz, 2007). The bacterium *Roseovarius crassostreae*, known to cause ROD, has caused annual losses ranging from 40-90% in Maine, Massachusetts, and New York since 1988 (Maloy et al., 2007; Barber et al., 1996). The activity of all three diseases is modulated in part by temperature and/or salinity and early management strategies were based on limiting the exposure of juveniles to the disease seasonally (Ford and Tripp, 1996).

By the mid-1960s, it became evident that the progeny of survivors of the initial MSX outbreak were more resistant to the disease than naïve oysters (Ford and Haskin, 1987). This observation led to the inception of the first oyster breeding program in the Northeast targeting disease resistance and within five generations of selection, the negative effects of MSX in Delaware Bay were largely neutralized (Haskin and Ford, 1979; Guo et al., 2008). When Dermo disease expanded into northern waters, it was clear that MSX-resistant oysters were not Dermo resistant (Burreson, 1991). Therefore, selection for Dermo resistance was incorporated into the Haskin Shellfish Research Laboratory (Rutgers University) breeding program in 1992, and the result is the Northeast High survival (NEH) line (Guo et al., 2008). In the late 1990s, a second program, the Aquaculture Genetics and Breeding Technology Center (ABC) at the Virginia Institute of Marine Science, was founded to develop genetically improved (e.g. disease resistant)

oyster stocks for industry (Frank-Lawale et. al., 2014). Wild Delaware Bay oysters have been selected over multiple generations in the York River, VA, where exposure to both MSX and Dermo is consistent from year to year, to create DEBY, an oyster line with dual resistance (Ragone-Calvo et. al., 2003). A third breeding program, initially targeting fast growth in colder waters, was established at the University of Maine in 1986. The founding population for this program was a line developed by Frank M. Flowers Oyster Company and was subsequently selected in Maine. The University of Maine Flowers Select (UMFS) line is derived from the largest 20% of Flowers survivors after 18 months of growth at a location where ROD is enzootic (Davis and Barber, 1999). These and other selected oyster lines are currently used by industry to minimize disease impact.

Performance of selected eastern oyster lines is routinely evaluated at their respective sites of origin to enable continued genetic improvement, but only a handful of studies have examined how well they perform across sites and geographic regions (but see Davis and Barber, 1999; Guo et al., 2003; Rawson and Feindel, 2012; Frank-Lawale et al., 2014). This is an important question for the oyster aquaculture industry in the eastern US because there are few selected lines available and many different growing environments. Eastern oysters thrive in waters ranging from -2° to 36°C and 5 to 40 ppt. Adult growth is positively correlated with temperature while survival is likely influenced by the effects of temperature and salinity on the distribution of disease-causing organisms (Shumway, 1996).

With the occurrence of oyster culture across varied environmental conditions comes a strong possibility for Genotype $\times$ Environment ($G \times E$) interactions. $G \times E$ interactions occur when two or more genotypes respond to different environments in different ways (Futuyma, 1998). Predictability of yields across localities can be affected by $G \times E$ interactions in

agricultural species. Although the presence and magnitude of $G \times E$ interactions for specific traits should be considered when developing a breeding strategy, attempts to quantify and characterize them have been modest (Annicchiarico, 2002).

The purpose of this study was to evaluate the performance (mortality, growth, and yield) of six selectively-bred eastern oyster lines from various programs at five grow-out sites ranging from Maine (ME) to Virginia (VA) to 1) identify differences in performance among lines at each site, and 2) identify lines that perform well across all sites. Differences in performance among lines within sites reflect the success of selection and the suitability of specific lines for particular growing environments, while the identification of consistent performers across sites reflects whether breeding programs can be centralized or environment-specific programs are required.

2. Materials and methods

2.1. Oyster lines

Six eastern oyster lines routinely used for aquaculture throughout the Northeast and Mid-Atlantic were chosen for evaluation in this study. The origin, site and extent of selection, and environmental conditions (including potential disease exposure) at selection sites are summarized for each line in Table 1. The UMFS, NEHTM (NEH for simplicity), DEBY, and hANA lines are products of multiple generations of selection for performance, while Clinton and NEH-RI are derived from closed populations of survivors of acute, disease-related mortality events and have not been subject to prolonged, consistent, selection efforts.

2.2. Oyster seed production

All material for this study was spawned during the summer of 2012. The original intention was for all six lines to be spawned at the University of Maine's Darling Marine Center shellfish hatchery; however, spawns for the lines of southern origin (NEH, DEBY, and hANA) were not successful. Subsequently, DEBY and hANA seed were produced at the Aquaculture Genetics and Breeding Technology Center (ABC) at the Virginia Institute of Marine Science, and NEH material was obtained by mass spawning approximately 50 broodstock at Muscongus Bay Aquaculture (Bremen, ME).

UMFS, Clinton, and NEH-RI broodstock were conditioned and spawned as in Rawson and Feindel (2012). In brief, 50 oysters from each line were strip-spawned, eggs fertilized and stocked in 300 L conical tanks. Larvae were reared at 24°C, ambient salinity (28-30 ppt) and fed a diet of *Isochrysis galbana*, *Pavlova* sp., and *Thalassiosira pseudonana* until set. Post-set oysters were maintained in recirculating upwellers supplied with 24°C, 28-30 ppt filtered seawater and fed mixed live algae (*Tetraselmis chuii*, *Cheatoceros muelleri*, *Rhodomonas* sp., in addition to those mentioned above) until they reached 2 mm in size.

DEBY and hANA seed were produced according to standard ABC hatchery procedures (Frank-Lawale et al., 2014). One hundred pair matings were created and then pooled for each line by strip-spawning, then stocked in 200 L tanks supplied with filtered, UV-treated, ambient seawater from the York River. Larvae were fed live algae including *Pavlova* sp., *Chaetoceros neogracile*, and *Tetraselmis* sp. The specific composition of the larval diet was adjusted in accordance with developmental stage and size. Post-set oysters were transferred to upwellers supplied with raw York River seawater and maintained until they too reached 2 mm in size.

Prior to distribution, 100 seed from each line were tested and certified as free of MSX, Dermo, SSO, and for lines spawned in ME – ROD. The certification process was conducted by

Kennebec River Biosciences for UMFS, Clinton, NEH-RI, and NEH lines and by the Virginia Institute of Marine Science Shellfish Pathology Laboratory for DEBY and hANA lines. In early August, 2012, certified, 2 mm seed were packed in mesh ‘purses’ (1200 individuals per ‘purse’) and divided among evaluation sites. Each site received 3 ‘purses’ per oyster line. Upon arrival at the evaluation sites, seed oysters were again placed in upwellers until they reached a minimum average shell length of 10 mm and could be transferred to culture bags for field performance evaluation.

2.3. Evaluation sites

The performance of each oyster line was evaluated at five representative sites from some prominent oyster growing regions in the eastern US where the aquaculture industry largely depends on hatchery produced seed. Site locations and characteristics are shown in Fig. 1. and Table 2. In addition to temperature and salinity, the sites chosen for this study differ with respect to disease incidence. ROD poses a consistent and significant threat to juvenile oysters grown in the Damariscotta River, ME (Davis and Barber, 1994), is less predictable at the Rhode Island Bay (RIBay) and Rhode Island Pond (RIPond) sites (Gómez-Chiarri, unpublished), and has not been reported at the New Jersey (NJ) or VA sites. In contrast, the parasites causing MSX and Dermo diseases have been detected consistently in NJ and VA since 1960 and 1990, respectively (Carnegie and Burreson, 2011; Cook et al., 1998), but the impact of these diseases varies from year to year at the three northern sites (ME, RIBay, RIPond) and is typically low at the ME sites (Gómez-Chiarri, unpublished; Marquis et al., 2015).

All six lines were evaluated at RIBay, RIPond, NJ, and VA sites; however, the NEH line was lost at the RIPond site midway through the study. Despite the certifications, DEBY and hANA lines were not deployed in ME due to importation restrictions.

2.4. Grow-out and performance measures

At each test site, each oyster line was deployed in two to three replicate bags containing 1000 oysters per bag and grown using either floating surface bags (ME) or rack and bag culture (RI, NJ, VA). Racks were placed intertidally at NJ and VA and sub-tidally at RIBay, and RIPond. The grow-out period spanned 15 months, beginning in August 2012 and continuing until November of 2013. Oysters were monitored either monthly or bi-monthly excluding the winter months (December through March).

Performance of each oyster line at each test site was assessed by measuring mortality, growth, and yield. Mortality (M) between sampling intervals was determined by counting live and dead oysters in a random sample of 100-150 individuals per bag at early time points and the entire bag at later time points such that:

$$M = \text{Dead Count} / (\text{Live Count} + \text{Dead Count})$$

Because bags containing oysters were thinned during the course of the experiment, estimates of cumulative mortality (CM) were obtained using the equation:

$$CM_t = CM_{t-1} + M_t * (1 - CM_{t-1})$$

where CM_t represents the cumulative proportion of dead oysters at time t , CM_{t-1} represents the cumulative proportion at the previous time point, and M_t represents the mortality at time t (Ford, 2006). Growth measurements included shell lengths (greatest distance from umbo to growing

edge) from a random sample of 25 oysters per bag at each sampling time point and total weight (TW) at the conclusion of the study. Yield (Y) from each bag of oysters was calculated as

$$Y = [(1 - CM_{Final}) * 1000] * TW_{Final}$$

in order to estimate the final biomass harvested from the 1000 oysters deployed at the start of the experiment.

2.5. Disease testing

In months where unusually high mortality was observed for any given line at any given site, a subset of live oysters ($N \geq 20$) were collected and processed for disease diagnosis. Clinical signs (excessive cupping of the lower valve and conchiolin deposits along inner shell surfaces) were scored to confirm ROD infections (0 to 3, with 3 being most severe as per Bricelj et al., 1992; Ford and Borrero, 2001). MSX- and Dermo-causing parasites were detected in oyster tissue using a multiplex quantitative PCR (qPCR) assay. This assay is based on the single-plex qPCR assays developed for *P. marinus* (De Faveri et al., 2009) and *H. nelsoni* (Wilbur et al., 2012) detection and quantitation such that, if present, both parasites are detected in one qPCR reaction. Infection intensities were quantified as log copies parasite DNA/ μ l of total DNA. Intensities > 3.3 log copies/ μ l and > 5 log copies/ μ l represent moderate to heavy *P. marinus* and *H. nelsoni* infections, respectively (Piesz et al., in preparation).

2.6. Statistical analysis

All statistical analyses were performed with R version 3.1.5 (R Core Team, 2015). To assess differences among the oyster lines grown within each of the five sites, five one-way analysis of variance (ANOVA) models ($y_j = \mu_j + \tau_{\text{Line at Site}(j)} + \sigma^2_{\text{Error}(=\text{Bag within Line at Site}(j))}$); for Site $j = \text{ME}$,

RIBay, RIPond, NJ, or VA) were fit to the bag average data for each performance measure (y): shell length, CM, and $\log_{10}(\text{yield}+1)$. Because the UMFS and Clinton lines at the VA and NJ sites expired prior to the final sampling, shell lengths from the sampling date when last they were alive were used to model the effect of line on growth. Post-hoc multiple comparison tests (Tukey's HSD) were applied to identify significant differences among lines contributing to the overall line effect.

A two-way mixed effects ANOVA was used to broaden the scope of inference, by incorporating bag average data from all sites, and address the question of whether the available selected lines can perform consistently well across sites with characteristics similar to those exhibited by the five observed sites. Thus, the sites included in this experiment were treated as a representative, random sampling from a range of potential grow-out sites and all model effects containing the 'site' factor were modeled as random effects using the REML method. The model $\mathbf{y} = \boldsymbol{\mu} + \boldsymbol{\tau}_{\text{Line}(i)} + \sigma^2_{\text{Site}} + \sigma^2_{\text{Error}(=\text{Line} \times \text{Site})} + \sigma^2_{\text{SubSampling}(=\text{Bag within Line} \times \text{Site})}$; where σ^2_{effect} represents the random effect variance component for 'effects' Site, Line $\times$ Site, and Bag within Line $\times$ Site; $\boldsymbol{\tau}_{\text{Line}(i)}$ represents the fixed effect for Line i (i=1-6) combining all sites, and $\boldsymbol{\mu}$ is the overall mean of the performance measure (y = shell length, CM, $\log_{10}(\text{yield}+1)$) across all sites and lines), allowed for the most statistically accurate test of line $\times$ site effect (Bates et al., 2015).

3. Results

3.1 Environmental parameters

Average daily temperature profiles from each site during the peak growing months (April through October) of 2013 confirm that oysters grown at northern latitudes experience cooler

conditions than those grown at southern latitudes. The mean difference in average daily temp between the northernmost (ME) and southernmost (VA) sites was 5.8°C (difference range = 2-10°C). Specific conductivity (SC) was measured at three of the five evaluation sites during the same time period (although not continuously at the RI sites). Measurements reflect a non-overlapping range in SC between RI (37-49 mS/cm; 23.4-32.0 ppt) and VA (20-35 mS/cm; 12.0-22.0 ppt) sites (Fig. 2).

3.2. Patterns of growth and mortality over time

Shell length trajectories of selected lines within each site were similar, but differed among sites (Fig. 3). In ME, growth was slow in the first year but rapid during the second summer. By the end of the study, the mean shell length for all lines grown in ME was 49.58 ± 4.28 mm. Oysters grew in all but the winter months at both RI sites, and thus were bigger than those grown at all other sites at the end of the experiment. On average, RI oysters were the largest (73.50 ± 10.01 mm and 71.70 ± 6.25 mm at RIBay and RIPond, respectively). Most growth occurred between June and September 2013 in NJ. The average shell length for oysters grown in NJ was 49.50 ± 13.78 mm. Patterns of growth in Virginia and NJ were similar, but VA oysters were larger than those grown in NJ and ME (final shell length = 55.60 ± 5.04).

Plots of mortality over time for each oyster line at each site are shown in Fig. 4. As with shell length, different patterns were observed across sites. In ME, most of the mortality occurred between September and November, 2012, with CM in November 2012 highest for Clinton (85.33 ± 10.00 %) and lowest for UMFS (42.33 ± 7.60 %). By the end of the study, mean CM for all lines grown in ME was 81.25 ± 9.00 % with the Clinton line experiencing the greatest mortality (93.67 ± 5.00 %) and UMFS the least (74.33 ± 7.40 %). Observed incremental mortality was

relatively consistent and mean CM for all lines was lowest at both RI sites compared to all other sites at the end of the experiment ($48.00 \pm 16.25\%$ and $48.40 \pm 7.40\%$ for RIBay and RIPond). CM among lines grown at the RIBay site ranged from $30.67 \pm 2.08\%$ to $68.66 \pm 2.52\%$, while at the RIPond site observed CM ranged from $40.00 \pm 7.10\%$ to $55.00 \pm 12.00\%$. Spikes in mortality were evident between August and October 2013 in NJ and September and November 2013 in VA for Clinton, NEH-RI, and UMFS lines. CM averaged across all lines grown in NJ was $65.83 \pm 35.53\%$. In VA, mean CM for all lines was $78.17 \pm 21.70\%$. The large standard deviations in CM at these sites reflect the discrepancy in performance among the northern (UMFS, Clinton, and NEH-RI) and southern (NEH, DEBY, and hANA) lines at these locations.

3.3. Line performance within sites

The within site analyses revealed that shell length varied significantly among lines at the ME ($F = 7.77$, $p = 0.012$), RIBay ($F = 27.34$, $p < 0.001$), and NJ ($F = 81.57$, $p < 0.001$) sites. UMFS and Clinton lines grew best at the ME (54.69 ± 7.70 mm, 50.73 ± 11.56 mm) and RIBay (80.84 ± 10.65 mm, 86.63 ± 13.99 mm) sites, while NEH grew best (68.11 ± 4.98 mm) at the NJ site. Shell length did not differ among lines at the RIPond and VA sites. (Fig. 5).

Differences in CM among lines were not detected at the ME and RIPond sites. At the RIBay site ($F = 25.60$, $p < 0.001$), UMFS ($30.67 \pm 2.08\%$) experienced the lowest mortality while DEBY ($66.33 \pm 5.86\%$) and hANA ($68.67 \pm 2.52\%$) experienced significantly greater mortality than all other lines. In NJ ($F = 319.66$, $p < 0.001$), the NEH line survived best (CM = $21.33 \pm 3.51\%$), DEBY (CM = $35.67 \pm 5.13\%$) and hANA (CM = $45.67 \pm 5.51\%$) survival was intermediate, and all or nearly all of the UMFS (100%), Clinton (100%), and NEH-RI ($92.33 \pm 1.53\%$) oysters died before the end of the study. One hundred percent mortality among UMFS

and Clinton oysters was also observed at the VA site ($F = 452.62$, $p < 0.001$), while DEBY and NEH lines experienced the lowest mortality with $54.00 \pm 2.65\%$ and $58.50 \pm 0.7\%$ CM, respectively (Fig. 5).

As with CM, differences in yield among lines were not detected at the ME and RIPond sites. At the RIBay site ($F = 24.80$, $p < 0.001$), the UMFS line yielded the most biomass (45395.73 ± 3318.87 g/1000 seed) while DEBY produced the least (11988.47 ± 3083.91). Yield was highest for the NEH line (30476.67 ± 2093.59) at the NJ site ($F = 6051.4$, $p < 0.001$) and the DEBY (13706.30 ± 108.95), NEH (11893.63 ± 770.51), and hANA (11782.34 ± 917.40) lines at the VA site ($F = 23142$, $p < 0.001$) (Fig. 5).

3.4. Effect of line $\times$ site interaction on oyster performance

Results of the mixed effects ANOVA are reported in Table 3. With respect to shell length, the line fixed effect was statistically significant ($p = 0.026$), and of the three random effects (site, line $\times$ site, and bag within line $\times$ site), most (78.0%) of the total random variance was explained by site. A relatively low portion (16.5%) of the random variance was attributed to the line $\times$ site interaction. In contrast, large and significant line $\times$ site interaction effects were detected for CM and yield. The percent of total random variance accounted for by the line $\times$ site random interaction effect, $\sigma^2_{\text{Error}(=\text{Line} \times \text{Site})}$, was 61.0% and 69.5% for CM and yield respectively; calculated as $100\% \cdot \sigma^2_{\text{Error}(=\text{Line} \times \text{Site})} / [\sigma^2_{\text{Site}} + \sigma^2_{\text{Error}(=\text{Line} \times \text{Site})} + \sigma^2_{\text{SubSampling}(=\text{Bag within Line} \times \text{Site})}]$. It appears that for CM and yield, the inconsistent performance of UMFS, Clinton, and NEH-RI lines across sites contributes most to the line $\times$ site interaction effect (Fig. 5). Although it is clear from practice and observation that there should be a significant effect of line on CM and yield, the mixed effects ANOVA did not detect one ($F = 1.77$, $p = 0.1739$ and $F = 2.41$, $p = 0.079$ for CM

and yield respectively). This is because the presence of a large and significant line $\times$ site interaction violates the assumption required to obtain a valid F-test for line fixed effect significance; the line $\times$ site interaction effect is expected to represent variability of residual magnitude; not the variability associated with extreme differences in performance of lines from site to site as was observed in this study.

3.5. Relationship between mortality and disease

The timing of mortality in ME was consistent with the occurrence of ROD epizootics, where ROD-induced mortality among juvenile oysters (< 25 mm) typically begins in the late summer months and can continue into the fall (Davis and Barber, 1999). Forty-five percent of Clinton oysters, which are presumed to be naïve to ROD, exhibited clinical signs of ROD in November 2012, about 23% of the cases were ranked as severe. For NEH oysters, 47% exhibited some degree of shell curvature and conchiolin on the inner shell surface, but only 3% of the cases were severe (Fig. 6).

Dermo and MSX diseases impact adult oyster populations during the late summer/fall in both Delaware and Chesapeake Bays (Ford and Haskin, 1982; Villalba et al., 2004). Peak mortalities in NJ and VA coincided with this time period. At the NJ site, *P. marinus* prevalence was 100% in UMFS, Clinton, and NEH-RI lines in September 2013 and infections were moderate to very heavy. By November 2013, of the affected lines, survivors remained from only NEH-RI. At that time, sixty percent of NEH-RI oysters were free of the Dermo-causing parasite, but disease intensity was still moderate to heavy among infected oysters. Both disease prevalence and intensity were lower among unaffected lines (NEH, DEBY, hANA) in November 2013 (Fig. 6). MSX was also present at NJ in late summer of 2013. Sixty-seven percent of the

UMFS oysters tested were positive for *H. nelsoni* compared to 59% and 55% for NEH-RI and Clinton, respectively. Interestingly, all of the NEH-RI survivors tested in November 2013 had MSX infections and most infections were severe. This is in stark contrast to the three high-surviving lines at this site where, when detected, MSX infection intensity was very low (Fig. 6).

In VA, prevalence and intensities of Dermo and MSX diseases were also high in lines with high mortality during the peak mortality period. One hundred percent of oysters tested positive for *P. marinus* in August 2013 and most infections were moderate to heavy. *P. marinus* prevalence remained high within oyster lines surviving through October, but the average infection intensity was reduced by late fall. MSX was present, but detected primarily in UMFS (61% prevalence) and NEH-RI (51% and 90% prevalence for August and October samples, respectively) (Fig. 6).

4. Discussion

Three factors dictate the performance of cultured oyster populations: genotype (line), environment (site), and their interaction ($G \times E$). Genotypes that have been modified through either natural or artificial selection are adapted to specific environments and should perform best under those conditions. In this study, all but the Clinton line were tested at their site of selection. Generally, lines grown at their site of development performed as well or better than all other lines, thereby validating the success of breeding programs at the local level. NEH in particular exhibited dramatically better growth, survival, and yield than all other selected lines at its site of selection (NJ). Similarly, DEBY survived better than all other lines at its selection site in VA. UMFS tended toward better growth, survival, and yield at the ME site, but differences with other lines grown in ME were not always statistically significant. In ME, the lack of large and

significant performance gains in the UMFS line compared to lines that were not selected there can be explained by differences in the extent of selection (only 2 generations for UMFS versus 15 generations for NEH) and/or the intensity of selection (which was not measured).

The effect of $G \times E$ interactions on performance has long been recognized and can present formidable challenges for agricultural breeding programs where crops are grown in multiple, varied environments (Hill, 1975). Differential performance of selected lines across growing localities raises the question of whether breeding programs should focus on developing generalist or specialist lines, i.e. cultivars (Evans and Langdon, 2006). In addition to measuring the magnitude of $G \times E$ interactions, it is important to understand the primary contributors to each interaction in order to devise optimal breeding and planting strategies (Votlas et al., 2002).

Results of the mixed-effect modeling approach used in this study indicate a large and significant effect of $G \times E$ interaction on eastern oyster performance, specifically CM and yield (which is a composite of CM and total weight). Significant $G \times E$ interactions have been reported for several marine bivalve taxa, but the magnitude of the interactions varied among previous reports. For example, Evans and Langdon (2006) evaluated the performance (growth, survival, yield) of multiple *Crassostrea gigas* full-sib families across four grow-out environments within two west coast estuaries and found that, although significant, family $\times$ environment interactions explained less than 10% of the total variation in the data. Similarly, significant $G \times E$ interactions were rarely detected for individual growth traits among full-sib families of the pearl oyster (*Pinctada maxima*) exposed to varied food quality, food abundance, and salinity over the course of 43 days (Kvingedal et al., 2008). By comparison, 37% of the variation observed in juvenile growth rate among full-sib families of the hard clam, *Mercenaria mercenaria*, reared at five locations spanning much of the eastern US coast was attributed to $G \times$

E interaction (Rawson and Hillbish, 1991). Sizable and significant $G \times E$ interactions for mortality, growth, and yield were also detected among distinct *C. virginica* lines deployed at multiple sites within a narrow geographic range with variable disease risk (Rawson and Feindel, 2012; Degremont et al., 2012; Frank-Lawale et al., 2014). Although the ability to detect and quantify significant $G \times E$ interactions is likely influenced by the environmental range of sites used, the extent of genetic variation present in the groups being compared, and the traits being analyzed, the strength of $G \times E$ interactions reported for eastern oyster thus far suggests a need for environment-specific breeding programs for this species. Additional characterization of the $G \times E$ interaction revealed that differential performance (specifically CM and yield) of the two northern lines (UMFS and Clinton) across sites contributed most to the interaction effect. The below average performance of UMFS and Clinton lines overall implies they are maladapted to typical southern growing environments and should not be grown there. In contrast, the above average performance of NEH, DEBY and hANA lines indicates they can perform well in a wider range of environments.

Mortality is controlled by multiple factors. In this study, CM was strongly influenced by disease exposure. Furthermore, interpretation of CM for each line within the context of known or putative disease resistance and observed patterns of disease at each site provided additional insight to whether selected lines can resist parasitic or bacterial infection (resistance) and/or resist pathogen-induced mortality (tolerance) (Roy and Kirchner, 2000). For example, substantial mortality was observed during the second summer of the study in NJ and VA, when Dermo and MSX diseases were active, and the mortality was line-specific. UMFS, NEH-RI, and Clinton experienced far more mortality than NEH, DEBY, and hANA. Based on their selection history, one would expect the UMFS and Clinton lines to be susceptible to Dermo (Table 2) and

they were. For MSX, resistant lines Clinton, NEH, DEBY, and hANA were expected to fare best and ultimately survived MSX exposure with few, if any, *H. nelsoni* infections (Fig. 7). NEH, DEBY, and hANA were presumed to be Dermo-resistant based on previous studies (Ragone-Calvo et al., 2003; Guo et al., 2008). In this study, relatively low mortality supports Ragone-Calvo et al. (2003) and Guo et al. (2008) to some extent, but infection data indicate that the prevalence of *P. marinus* infections within these lines does not differ from susceptible lines (although the disease intensity is lower) (Fig. 7), suggesting that NEH, DEBY, and hANA have developed Dermo tolerance rather than resistance *per se*. Previous studies documenting survival and Dermo infection intensity suggested tolerance versus resistance in both DEBY oysters grown in Maryland and ‘naturally-resistant’ wild stocks grown in Virginia (Abbe et al., 2005; Encomio et al., 2005).

The NEH-RI line was assumed to possess resistance/tolerance to three diseases (MSX, Dermo, and SSO (caused by the parasite *Haplosporidium costale*) and was expected to perform very well. On the contrary, this line exhibited below average growth and survival across sites and unlike its parent line, acquired significant *H. nelsoni* infections. A possible explanation for this observation is that the genetic composition of oysters that survived the SSO epizootic (and were subsequently used as broodstock for the NEH-RI line) was different from the original NEH. The implication here is that mechanisms of resistance to MSX and SSO are not the same. There are several examples in the literature suggesting that established resistance to one disease does not translate to enhanced resistance to multiple diseases in oysters (Dégremont et al., 2015). A recent study examining the response of adult *C. gigas* to the bacterial pathogen *Vibrio aestuarianus* found no significant difference in survival among oyster lines previously selected for ostreid herpes virus (OsHV-1) resistance and control lines (Azéma et al., 2015). Similarly,

Burreson (1991) showed that MSX-resistant eastern oysters were more susceptible to Dermo than unselected lines, prompting selection for oysters with dual resistance. Alternative explanations for the poor performance of the NEH-RI line include a small, genetically-depauperate founding population and/or a substantial shift in environmental conditions between the selection event and the timing of this study. Whether the development of oyster lines with resistance to numerous pathogens that do not typically co-occur is possible or even warranted is still arguable. Even though the parasite causing SSO has been detected in cool, high-salinity coastal waters of the Northeast since the early 1960's, it is typically present in low numbers and epizootics causing significant mortality occur infrequently during the late spring/early summer months (Wood and Andrews, 1962; Stokes and Burreson, 2001; Sunila et al., 2002).

CM in ME was higher than expected, particularly for ROD-resistant UMFS. In a previous field trial, UMFS dramatically outperformed NEH (~ 20% vs. 75% mortality) when they were grown side by side at two ROD-enzootic sites (Rawson and Feindel, 2012), but no significant differences were detected between these lines in this study. The poor survival of all oyster lines grown in ME may be a consequence of deployment time. According to Barber et al. (1996), seed that are spawned and deployed in Maine very early or very late in the season survive best, while seed < 25 mm deployed during July and August experience upwards of 80% mortality. For this study, oyster seed were deployed at all sites in mid-August, when risk of juvenile mortality is highest in ME.

Thus far, breeding programs for the eastern oyster, *C. virginica*, have achieved notable gains in performance (growth, survival, and yield) using a mass selection approach. Based on the evaluation of the mass-selected lines presented here, the success of the approach is undisputed, yet more can be done to benefit the *C. virginica* aquaculture industry in the

Northeast and Mid-Atlantic regions, and probably the Southeast and Gulf regions as well. ABC at the Virginia Institute of Marine Science has recently adopted a family-based approach to selective breeding and genetic parameters (e.g. heritabilities, genetic correlations, breeding values) for survival, growth, and other traits are being estimated (Degremont et al., 2015). Although disease resistance in the eastern oyster is an economically important trait conducive to selective breeding, pathogen load is not typically measured in conjunction with mortality, resulting in a lack of genetic parameter estimates for the trait (Degremont et al., 2015). The estimation of genetic parameters not only contributes to accelerated genetic improvement within specific environments, but also can be applied to shape breeding strategy when there are significant $G \times E$ effects on performance. As outlined in Falconer and Mackay (1996), the same trait measured in several, different environments can be treated as multiple traits. The heritability and correlation of performance for each trait can be used to predict improved performance in environments other than the site of selection, and thus the extent to which differences in environment require locally adapted cultivars.

This study represents an important first step in assessing the performance of available, selectively-bred eastern oyster lines across a broad geographic and environmental range. The data suggest that NEH, DEBY, and hANA lines perform consistently well across sites with and without MSX and Dermo. Line response to ROD could only be evaluated at one site (ME) and unfortunately, line-specific responses were confounded by the absence of DEBY and hANA and universally poor performance of all other lines at that site. At least, UMFS tended toward higher growth and survival at its site of origin. At sites where disease pressure was absent or low (e.g. RIPond, data not shown), performance among lines was indistinguishable, underscoring the importance of disease in driving oyster mortality and yield. A refined understanding of line $\times$

site interactions and their effect on eastern oyster performance should be used to build upon existing breeding programs and assist industry in choosing appropriate seed for specific growing environments throughout the Northeast and Mid-Atlantic.

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Table 1. A summary of the *Crassostrea virginica* lines included in this study. “Environmental Conditions” refers to the typical temperature and salinity regimes at each line’s site of origin as well as potential disease exposure. *The UMFS line was selected for fast growth for 2 generations during the 1990’s; the line was further selected by breeding the survivors of ROD outbreaks.

Line	Origin	Site of Selection	Generations under Selection	Environmental Conditions	Reference
UMFS	Flowers line subjected to size selection in Maine	Damariscotta River, ME	2*	Cold temperatures, high salinity, ROD	Barber et al. 1998
NEH-RI	NEH survivors of SSO outbreak in Rhode Island	Wickford, RI	NA	Warm temperatures, high salinity, Dermo, MSX, SSO	
Clinton	CT wild stock survivors of MSX epizootic	Long Island Sound, CT	NA	Warm temperatures, low-high salinity, MSX	Rawson et al. 2010
NEH	Inter-strain crosses of MSX-resistant strains from the northeast region	Cape May, NJ	15	Warm temperatures, low salinity, Dermo, MSX	Guo et al. 2008
DEBY	Wild oysters from Delaware Bay	York River, VA	13	Warm temperatures, moderate-high salinity, Dermo, MSX	Ragone-Calvo et al. 2003
hANA	Wild oysters from Louisiana crossed with previously selected material (e.g. DEBY, XB)	York River, VA	3	Warm temperatures, moderate-high salinity Dermo, MSX	ABC, 2009

691 **Table 2.** Location and environmental characteristics of evaluation sites. Temperature and
692 salinity range during the peak growing months (April through October) are reported. *Conditions
693 reported for RIPond span July through October 2013.

Site	Site Code	Location	Temp °C	Salinity ppt	ROD pressure	MSX pressure	Dermo pressure
Damariscotta River, ME	ME	44°1'1.2"N 69°32'37.4"W	8-23	27-33	HIGH	LOW	LOW
Narragansett Bay, RI	RIBay	41°34'39.7"N 71°26'4.6"W	6-24	30-32	VARIES	VARIES	VARIES
Ninigret Pond, RI	RIPond	41°21'54.7"N 71°38'49.0"W	10-27*	30-33*	VARIES	VARIES	VARIES
Cape May, NJ	NJ	39°13'58.9"N 75°1'46.9"W	9-26	17-24	NONE	HIGH	HIGH
York River, VA	VA	37°14'53.1"N 76°30'26.3"W	8-28	15-23	NONE	HIGH	HIGH

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Table 3. Results of two-way mixed-effects analyses of variance: line fixed effect and site and line $\times$ site random effects [$y = \mu + \tau_{\text{Line}} + \sigma^2_{\text{Site}} + \sigma^2_{\text{Error(=Line} \times \text{Site)}}$] for y = shell length, cumulative mortality and log-transformed final yield. Line fixed effect significance tested using F statistic with 5 numerator and 17 denominator degrees of freedom. Site and Line $\times$ Site random effect significance tested using χ^2 likelihood ratio test (LRT) with 1 degree of freedom.

Shell Length							
Effect	Variance Component Estimate	% Total Random Variance	df	Random Effect χ^2 value	Fixed Effect F value	F or χ^2 df	p value
Line			5		3.62	17	0.0206
Site	130.30	78.0	4	21.08		1	<.0001
Line $\times$ Site	27.54	16.5	17	33.07		1	<.0001
Bag in [Line $\times$ Site]	9.13	5.5	40				

Cumulative Mortality							
Effect	Variance Component Estimate	% Total Random Variance	df	Random Effect χ^2 value	Fixed Effect F value	F or χ^2 df	p value
Line			5		1.77	17	0.1739
Site	0.01781	28.4	4	2.65		1	0.1033
Line $\times$ Site	0.03829	61.0	17	52.54		1	<.0001
Bag in [Line $\times$ Site]	0.00669	10.6	40				

Final Yield							
Effect	Variance Component Estimate	% Total Random Variance	df	Random Effect χ^2 value	Fixed Effect F value	F or χ^2 df	p value
Line			5		2.41	17	0.0793
Site	0.60179	28.8	4	2.94		1	0.0862
Line $\times$ Site	1.45310	69.5	17	110.57		1	<.0001
Bag in [Line $\times$ Site]	0.03622	1.7	40				

Figure Legends

Fig. 1. Location of sites used to evaluate performance of *C. virginica* lines used for aquaculture in the Northeastern U.S. They include the Damariscotta River, Maine (ME), Wickford, Rhode Island (RIBay), Ninigret Pond, Rhode Island (RIPond), Cape May, New Jersey (NJ), and York River, Virginia (VA). Site coordinates are listed in Table 2.

Fig. 2. Average daily temperature (top panel) and specific conductivity (bottom panel) measured at each evaluation site during the peak growing months (April – October) of 2013.

Fig. 3. Growth trajectories for each *C. virginica* line at each site over the course of the evaluation period. Shell length (in mm) and sample date are plotted along the y- and x-axis respectively. DEBY and hANA oysters were not deployed in ME. NEH bags were lost at the RIPond site midway through the study. All UMFS and Clinton oysters died prior to the final sampling time point at the NJ and VA sites.

Fig. 4. Mortality patterns observed for each *C. virginica* line at each site over the course of the evaluation period. The estimated cumulative proportion of dead oysters (CM) and sample date are plotted along the y- and x-axis respectively. DEBY and hANA oysters were not deployed in ME. NEH bags were lost at the RIPond site midway through the study.

Fig. 5. Shell length, CM, and yield measured at the end of the study for six lines of *C. virginica* deployed at five evaluation sites along the Northeastern US coast. The height of each bar represents the mean of the observed performance measure calculated from two to three replicate bags. Error bars reflect the standard error of the mean. Letters (a-d) above bars indicate statistically significant differences in performance among lines within a site detected by single-factor ANOVA and post-hoc comparison tests. hANA and DEBY lines were not deployed at the

ME site. The NEH line was lost at the RIPond site during the study. UMFS and Clinton lines did not survive to the end of the study at NJ and VA sites.

Fig. 6. Prevalence and Intensity of ROD in ME, Dermo and MSX in NJ and VA during periods of high mortality for *C. virginica*. All lines grown in ME were examined for ROD symptoms in November, 2012. ROD intensity was quantified by ROD score, where a score of 0 indicates no symptoms and a score of 3 represents the most severe symptoms. In NJ, only lines that experienced a spike in mortality (UMFS-S, NEH-RI-S, Clinton-S) were tested for disease in September, 2013. All surviving lines were tested at the end of the experiment in November, 2013 (NEH-RI-N, NEH-N, DEBY-N, and hANA-N). Only NEH-RI was tested at both time points. Similarly, in VA, affected lines (UMFS-A, NEH-RI-A, Clinton-A) were tested for disease in August, 2013. All surviving lines were tested in October, 2013 (O). Both NEH-RI and NEH lines were tested at both time points. Dermo and MSX intensities were quantified as log copies parasite DNA/ul of total DNA. Intensities ≥ 3.3 and ≥ 5 log copies/ul represent moderate to heavy Dermo and MSX infections, respectively.

743 **Fig. 1.**

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Table 1. A summary of the *Crassostrea virginica* lines included in this study. “Environmental Conditions” refers to the typical temperature and salinity regimes at each line’s site of origin as well as potential disease exposure. *The UMFS line was selected for fast growth for 2 generations during the 1990’s; the line was further selected by breeding the survivors of ROD outbreaks.

Line	Origin	Site of Selection	Generations under Selection	Environmental Conditions	Reference
UMFS	Flowers line subjected to size selection in Maine	Damariscotta River, ME	2*	Cold temperatures, high salinity, ROD	Barber et al. 1998
NEH-RI	NEH survivors of SSO outbreak in Rhode Island	Wickford, RI	NA	Warm temperatures, high salinity, Dermo, MSX, SSO	
Clinton	CT wild stock survivors of MSX epizootic	Long Island Sound, CT	NA	Warm temperatures, low-high salinity, MSX	Rawson et al. 2010
NEH	Inter-strain crosses of MSX-resistant strains from the northeast region	Cape May, NJ	15	Warm temperatures, low salinity, Dermo, MSX	Guo et al. 2008
DEBY	Wild oysters from Delaware Bay	York River, VA	13	Warm temperatures, moderate-high salinity, Dermo, MSX	Ragone-Calvo et al. 2003
hANA	Wild oysters from Louisiana crossed with previously selected material (e.g. DEBY, XB)	York River, VA	3	Warm temperatures, moderate-high salinity Dermo, MSX	ABC, 2009

Table 2. Location and environmental characteristics of evaluation sites. Temperature and salinity range during the peak growing months (April through October) are reported. *Conditions reported for RIPond span July through October 2013.

Site	Site Code	Location	Temp °C	Salinity ppt	ROD pressure	MSX pressure	Dermo pressure
Damariscotta River, ME	ME	44°1'1.2"N 69°32'37.4"W	8-23	27-33	HIGH	LOW	LOW
Narragansett Bay, RI	RIBay	41°34'39.7"N 71°26'4.6"W	6-24	30-32	VARIES	VARIES	VARIES
Ninigret Pond, RI	RIPond	41°21'54.7"N 71°38'49.0"W	10-27*	30-33*	VARIES	VARIES	VARIES
Cape May, NJ	NJ	39°13'58.9"N 75°1'46.9"W	9-26	17-24	NONE	HIGH	HIGH
York River, VA	VA	37°14'53.1"N 76°30'26.3"W	8-28	15-23	NONE	HIGH	HIGH

Table 3. Results of two-way mixed-effects analyses of variance: line fixed effect and site and line $\times$ site random effects [$y = \mu + \tau_{\text{Line}} + \sigma^2_{\text{Site}} + \sigma^2_{\text{Error(=Line} \times \text{Site)}}$] for y = shell length, cumulative mortality and log-transformed final yield. Line fixed effect significance tested using F statistic with 5 numerator and 17 denominator degrees of freedom. Site and Line $\times$ Site random effect significance tested using χ^2 likelihood ratio test (LRT) with 1 degree of freedom.

Shell Length							
Effect	Variance Component Estimate	% Total Random Variance	df	Random Effect χ^2 value	Fixed Effect F value	F or χ^2 df	p value
Line			5		3.62	17	0.0206
Site	130.30	78.0	4	21.08		1	<.0001
Line $\times$ Site	27.54	16.5	17	33.07		1	<.0001
Bag in [Line $\times$ Site]	9.13	5.5	40				
Cumulative Mortality							
Effect	Variance Component Estimate	% Total Random Variance	df	Random Effect χ^2 value	Fixed Effect F value	F or χ^2 df	p value
Line			5		1.77	17	0.1739
Site	0.01781	28.4	4	2.65		1	0.1033
Line $\times$ Site	0.03829	61.0	17	52.54		1	<.0001
Bag in [Line $\times$ Site]	0.00669	10.6	40				
Final Yield							
Effect	Variance Component Estimate	% Total Random Variance	df	Random Effect χ^2 value	Fixed Effect F value	F or χ^2 df	p value
Line			5		2.41	17	0.0793
Site	0.60179	28.8	4	2.94		1	0.0862
Line $\times$ Site	1.45310	69.5	17	110.57		1	<.0001
Bag in [Line $\times$ Site]	0.03622	1.7	40				

Figure 1

Figure2

Temperature (degrees Celsius)

Specific Conductivity (mS/cm)

Figure3

Figure4

- ◆--- UMFS
- ▲--- NEH-RI
- Clinton
- NEH
- DEBY
- hANA

Figure5