Growth and survival for genetically improved lines of Eastern oysters (Crassostrea virginica) and interline hybrids in Maine, USA

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Abstract

The production of cultured Eastern oysters (Crassostrea virginica) in the northern New England states and Canadian Maritime Provinces is hampered by a short growing season, relatively cold water temperatures, and outbreaks of Roseovarius Oyster Disease (ROD). A breeding program at the University of Maine has produced the University of Maine Flowers Select (UMFS) line by selecting for oysters with improved cold water growth performance and resistance to ROD. We conducted two grow-out trials comparing the survival, size, and yield for the UMFS line to two other genetically improved lines of Eastern oysters to assess the suitability of this line outside of the Damariscotta River, where it was developed. In the first trial, oysters were deployed in August just prior to when ROD outbreaks typically occur in Maine among small, seed oysters. We observed substantial differences in yield in this field trial, particularly at study sites located on the Damariscotta River. These differences were due to variation in line-specific survival. The second field trial was deployed in June when ROD has less of an impact on seed oysters. Mortality in this second trial was lower than in the first trial and there was a corresponding higher dependence of line-specific yield on variation in growth. However, there was no line which consistently grew better and had higher yield at all sites. Based on our results, we suggest that breeding programs for Eastern oysters may benefit from focusing on the additive nature of survival variation among lines and placing less emphasis on the relatively subtle variation in line-specific growth.

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1. Introduction

Natural populations of the Eastern oyster, Crassostrea virginica, historically supported a major fishery along the Atlantic and Gulf coasts of North America. Over the past century this fishery has been in decline due to the effects of overfishing, deteriorating coastal water quality, and disease (Mackenzie, 2007). The culture of Eastern oysters has been viewed as a means to offset declines in the wild fishery and sustain a vital oyster industry in the Northeast (Allen et al., 1993). In addition, the hatchery-based production of seed to support oyster culture has facilitated selective breeding programs seeking to develop domesticated oyster stocks with superior growth and disease-resistance.

In northern New England and the Canadian Maritime Provinces, the production of C. virginica has often been limited by a short growing season and relatively cold water temperatures. Production in some locations has also been negatively impacted by Roseovarius Oyster Disease (ROD). Although the symptoms and effects of ROD were first observed among hatchery-reared seed in New York (Bricelj et al., 1992), oysters with ROD symptoms and ROD-associated mortality have since been reported from New York to Maine (Davis and Barber, 1994; Ford and Borroto, 2001; Lewis et al., 1996; Maloy et al., 2007a). This disease, caused by the bacterium Roseovarius crassostreae, results in stunted growth, highly uneven shell margins, deposition of excess conchiolin on the inner surface of the shell, and crop losses as high as 90% (Boettcher et al., 2005; Davis and Barber, 1999).

A selective breeding program was initiated at the University of Maine in 1986 using broodstock obtained from the Frank M. Flowers Oyster Company in Long Island, New York. After two generations of size-based truncation selection, the University of Maine Flowers Select line (UMFS) demonstrated significantly better growth when compared to control, non-selected animals from the same founding population and to local wild stocks of oysters. Because the program focused on breeding survivors from ROD outbreaks, this line has also demonstrated resistance to ROD (Barber et al., 1998).

Although initially most of Maine’s oyster farms were located along the Damariscotta River, the industry has expanded and oyster farms can now be found in several estuaries along the coast of Maine. This expansion raises concerns about the prevalence of line by environment interactions for growth, disease resistance and yield, and the suitability of the UMFS line for farms located outside of the Damariscotta River. The genetically-based performance in marine bivalves, including blue
mussels (Innes and Haley, 1977; Mallet et al., 1986), hard clams (Rawson and Hilbish, 1991) and oysters (Newkirk, 1978) is often influenced by the culture environment, leading to genotype or stock by environment interactions. We conducted two grow-out trials to objectively examine the performance of the UMFS line outside of the Damariscotta River. Because of a difference in the timing of deployment, oysters in the first trial were more likely to be exposed to ROD compared to the oysters in the second trial.

In both trials we compare the relative performance of the UMFS line to that of the Rutgers University Haskin Shellfish Research Lab’s Northeastern High Survival Resistant Line (NEH) as well as a line developed by the Frank M. Flowers Co. (FMF; New York). The former line has demonstrated resistance to the protistan diseases MSX and Dermo (Ford and Haskin, 1987; Guo et al., 2003) and high growth potential under warmer, low salinity conditions often found in southern New England. The Flowers Company has consistently used the largest oysters surviving disease outbreaks in several locations to develop a line that is putatively resistant to ROD and MSX and has high growth potential in both southern and northern New England (Sunila, pers. comm.). Our study was also designed to determine whether additional gains in growth and survival could be obtained through interline crossing. Oyster seed from the UMFS, NEH, and FMF lines, as well as between hybrid lines (UMFS×NEH and UMFS×FMF), were grown in side-by-side common garden field trials in which we examined the relative growth, survival, and yield of each line at multiple sites in Maine.

2. Materials and methods

2.1. Conditioning and spawning

Fifty adult oysters (~50–65 mm shell height) from the UMFS, NEH and FMF lines were used as broodstock for our common garden trials. Broods were placed into static conditioning tanks in the bivalve quarantine facility at the Darling Marine Center, Walpole, Maine on April 10, 2003. The water temperature in each tank was held at 22–24 °C at ambient salinity (28–30 psu) and pH (8.05) throughout a six-week conditioning period. The water in each tank was changed every other day during conditioning and the broods were fed a mixed diet of Isochrysis galbana, Tetraselmis sp., Pavlova lutheri, and Chaetoceros muelleri at a food ration of ~3% based on the estimated dry weight of the broods in each tank (Helm and Bourne, 2004).

All broods were strip-spawned on May 20, 2003 by lightly scoring the gonad of each oyster with a sterile, disposable scalpel. Eggs were gently massaged from the gonad of female oysters into 1-l beakers containing 1 μm filtered seawater (FSW; 28–30 psu, pH 8.05) held at 22–24 °C. Sperm were collected “dry” as they exited from the gonad and stored on ice in a microcentrifuge tube until all animals had been stripped. The egg suspensions were washed through a 150 μm sieve, retained on a 20 μm sieve, rinsed and resuspended in 1-1 of FSW. The quality of eggs was assessed microscopically and the concentration of eggs from each female was estimated by directly counting the number of eggs in 1 ml of suspension.

The eggs from each line were pooled, mixed and split into two (FMF and NEH) or three replicate (UMFS) 20 l buckets containing an equal number of eggs. A small aliquot of “dry” sperm from each male was placed in FSW (22 °C, 30 psu, pH 8.05) and sperm activity verified by microscopic examination at high power (100×). Equal aliquots of active sperm for all males from a given stock were combined and diluted 100-fold in FSW. The dilute sperm suspension from the UMFS males was used to fertilize one set of eggs from UMFS females at an approximate sperm to egg ratio of 1000:1 (as per Gaffney et al., 1993). Similarly, the sperm from FMF and NEH males were used to fertilize FMF and NEH eggs, respectively.

We also constructed two hybrid crosses between oyster lines. The sperm from UMFS males were used to fertilize eggs from both FMF and NEH females. Reciprocal hybrid crosses were constructed by fertilizing UMFS eggs with sperm from FMF and NEH males. Due to hatchery space limitations, a hybrid cross between the FMF and NEH lines was not constructed. The reciprocal hybrid lines were kept separate until they had developed into D-stage larvae at which time they were combined to create one UMFS×FMF hybrid line and one UMFS×NEH hybrid line. The fertilized eggs were stocked into 300 l conical tanks at a density of 40–50 ml⁻¹ and thinned to 10 larvae·ml⁻¹ when the water was changed at approximately 24 h post-fertilization.

Larvae were raised at 24 °C (ambient salinity, 28–30 psu and pH 8.05) and fed a mixed diet of L. galbana, P. lutheri, Pavlova sp. (CCMP459) and Thalassiosira pseudonana (strain 3H). Larval culture tanks were drained down every other day and the larvae in each tank were gradually thinned to a density of 1–2 individuals·ml⁻¹ prior to setting. Algal rations were maintained at 10,000 cells·ml⁻¹ during the first day post-fertilization, increased daily to 50,000 cells·ml⁻¹ by day eight post-fertilization and reached a maximum of 150,000 cells·ml⁻¹ by day 18. Feeding was reduced to 50–75,000 cells·ml⁻¹ during the time oysters were set.

Larvae obtained competency between June 7 and June 12, 2003 at which time they were set in screened (180 μm) floating wooden trays containing microcultur. The trays were held in larger holding tanks at 24 °C (ambient pH and salinity) that were cleaned and refilled with filtered seawater every other day. Spat were graded into separate size classes each week. Oyster spat that showed no sign of growth after two successive gradings were discarded. Post-set animals were pulse-fed a mixed diet of live algae including Tetraselmis chuii, C. muelleri, and Rhodomonas sp., in addition to those used during larval culture, at a ration that was the equivalent of 4–5% algal dry weight relative to animal live weight per day (Helm and Bourne, 2004) with a maximum cell concentration of 200–250,000 cells·ml⁻¹ at any given time. Post-set oysters were cultured in a recirculating upweller tank, supplied with FSW at 24 °C (ambient pH and salinity), pulse-fed a mixture of live algae supplemented with algae paste (Reed Mariculture, Campbell, CA, Shellfish diet 1800) and grown until they were retained on 2 mm size mesh screens. This protocol was employed to ensure that oysters were pathogen-free prior to deployment.

2.2. Field deployment and monitoring: 2003 field trial

Seed oysters from each line were deployed at five sites along the coast of Maine (Fig. 1) on August 5, 2003. Approximately 1000 oysters from each stock were placed into four replicate plastic vexar bags at each test site (4000 seed total per line per site). We initially deployed oysters in window screen inserts (~1 mm² mesh) within the vexar bags. All of the oysters deployed in 2003 were cultured in surface bags at lease sites belonging to industry growers who were responsible for the cleaning and maintenance of replicate bags. The oysters were maintained at each site through November of 2003 at which time the cooperating growers were responsible for overwintering oysters according to their normal protocol. Methods of overwintering included sinking oyster cages to the bottom, “dry” storage in chiller-controlled rooms, or holding the cages in indoor flowing seawater tanks receiving ambient seawater. Oysters from each line were redeployed at lease sites from April to November 2004, overwintered for a second time, and then redeployed in April of 2005. The single exception was the oysters held at the Damariscotta River B site which were not redeployed in 2005. The first field trial was terminated in October of 2005. The size of individual oysters (wet weight and shell height) and mortality in each replicate were monitored regularly throughout each field season. Oysters in each replicate bag were thinned periodically to maintain a consistent volume and biomass in each replicate (~10 kg total). Final grow-out densities were approximately 125–300 oysters per bag depending on the final size of animals at each site.
2.3. 2004 field trial

A second field trial was initiated in 2004. The same three parental lines (UMFS, FMF and NEH) and two interline crosses (UMFS × FMF and UMFS × NEH) were constructed for this second trial. Broodstocks were conditioned beginning in late January 2004 and spawned on March 24, 2004. The conditioning, spawning, larval rearing and nursery protocols used in 2004 were identical to those used for the 2003 experiment. In late May and early June 2004, seed oysters from each line were deployed in quadruplicate at six grow-out sites (Fig. 1) at the experiment. In late May and early June 2004, seed oysters from each line were deployed in quadruplicate at six grow-out sites (Fig. 1) at the same densities used in 2003. The oysters in the second field trial were overwintered from November 2004 till April 2005, redeployed in April and maintained at individual lease sites till the experiment was terminated in October to late November 2005.

2.4. Data analysis

To characterize site to site variation in water temperature, we deployed temperature loggers at each site. Sites situated farthest east, such as the Narraguagus River site (NR, Fig. 1) and those closer to the open ocean such as the Darling Marine Center site, tended to have colder average water temperatures during the grow-out season when compared to upriver sites on the Damariscotta River (DRA and DRB). We examined the average growth rate for each line as a function of site-specific differences in water temperature for both field trials (Fig. 2). Growth rate was estimated by taking the difference between the final and initial size of 50 oysters in each replicate bag and dividing through by the number of days the replicates were deployed at each site. Variation in water temperature was calculated as the difference in cumulative degree days (CDD) for a given site relative to the cumulative degree days at the warmest site, the Damariscotta River A site. The significance of the relationship between growth rate and temperature was determined by least squares linear regression in SYSTAT (ver 12, Systat Inc. 2007).

To assess the relative performance of each line over a typical production cycle, we examined line-specific variation in total cumulative mortality, final wet weight, and yield. Yield was expressed as survival · final weight · 1000 for each replicate bag at each site. This latter parameter provides an estimate of the final biomass (in kg) harvested for each line per 1000 oysters deployed at the start of the experiment. The statistical significance of any differences in line performance (survival, growth, or yield) was assessed by analyses of variance (ANOVA). We used a series of single factor ANOVAs to test for line effects on a site by site basis using the General Linear Model routine in SYSTAT (ver 12, Systat Inc. 2007). In each case, we considered oyster line to be a fixed effect because our primary interest is in direct comparisons among the specific lines deployed in our study. When a significant line effect was detected, we conducted post-hoc pair-wise comparisons between lines (Bonferroni correction). We used both a Lilliefors test and an examination of the residuals to test for homoscedasticity of error variance and determine the appropriateness of each ANOVA model. We used an arcsine transformation for cumulative
mortality and a logarithmic transformation for yield to reduce heteroscedasticity in the error variance and improve the fit of the ANOVA models for these two variables.

We tested for line by environment interactions in each field trial by two-way ANOVA where line and site were considered to be fixed factors and in which we paid particular attention to the significance of the line by site interaction terms. To account for site-specific differences in wet weight that may have been caused by variation in the length of time that the oysters were deployed at individual sites, we used an ANCOVA approach that incorporated the length of time in the field as a continuous variable. We also estimated daily yield by dividing the adjusted yield for each replicate at each site by the length of time the oysters were deployed at each site, as per Dégremont et al. (2010). The appropriateness of the two-way ANOVA or ANCOVA models was assessed using the same methods used for the single factor ANOVA models, described above.

3. Results

The growth of the oysters in our study was considerably slower at sites which had a reduced number of cumulative degree days relative to the Damariscotta River A site. A linear regression model with growth rate as dependent variable and the difference in cumulative degree days as the independent variable was significant for both field trials (2003, \(P<0.05\); 2004, \(P<0.01\)). However, more of the variance in growth rate was explained by the variance in temperature in the 2004 trial (\(R^2 = 0.761\)) than in 2003 trial (\(R^2 = 0.172\)). The weaker relationship in 2003 was primarily driven by poor growth at the New Meadows River (NM) site. When this outlier was removed, the slope and \(R^2\) values for both years were similar. Even so, most of the variation in growth was between sites while there was little among-line variation in average growth at each site.

3.1. 2003 field trial

We observed substantial mortality during the 2003 field trial (Fig. 3A). Average mortality was highest (~55%) at both sites on the Damariscotta River A site. A linear regression model with mortality as dependent variable and the difference in cumulative degree days as the independent variable was significant for both field trials (2003, \(P<0.05\); 2004, \(P<0.01\)). However, more of the variance in growth rate was explained by the variance in temperature in the 2004 trial (\(R^2 = 0.761\)) than in 2003 trial (\(R^2 = 0.172\)). The weaker relationship in 2003 was primarily driven by poor growth at the New Meadows River (NM) site. When this outlier was removed, the slope and \(R^2\) values for both years were similar. Even so, most of the variation in growth was between sites while there was little among-line variation in average growth at each site.

The average yield differed substantially among sites and was highest at the Damariscotta River A and Baggaduce River sites; cumulative mortality at the end of the experiment was lowest for the FMF and the UMFS × FMF hybrid lines and highest for the NEH line, although pair-wise comparisons of mortality were significant only at the Damariscotta River B site.

There was a high degree of variation in final wet weight among sites. Oysters at the Damariscotta River A site had an average wet weight exceeding 80 g per individual (Fig. 3B) and shell heights of nearly 100 mm (data not shown). In contrast, oysters at the New Meadows site had an average weight <10 g and average shell height <45 mm, even after over two full growing seasons. There was little line-specific variation in wet weight at the end of the 2003 field trial. The main exception was at the Baggaduce River site where the FMF line was significantly larger than the NEH line.

The average yield differed substantially among sites and was highest at the Damariscotta River A and Baggaduce River sites in the 2003 field trial (Fig. 3C). The average yield at the other three sites was generally less than 25% of that observed at the former sites and was driven by poor growth at the New Meadows and St. Georges River sites and poor survival at the Damariscotta River B site. Significant, line-specific variation in yield was detected at the Baggaduce River site where the FMF and UMFS × FMF lines had significantly higher yield than the NEH line. In contrast, differences in yield at the Damariscotta River A site were driven by variation in mortality, although pair-wise comparisons between lines were not significant (experiment-wide \(P\)-value of 0.05). Similarly, line-specific variation in yield was evident at the Damariscotta River B site at the end of two growing seasons with the yield for the highest surviving lines, UMFS and UMFS × FMF, exceeding that of all other lines.

There was no evidence of a line by site interaction for standardized wet weight during the 2003 field trial (Table 1), suggesting that the relative growth of each line was consistent across sites. Both the line and site main effects on standardized wet weight were significant. Post-hoc comparisons indicated that oyster growth was significantly faster at the Damariscotta River A site and across all five sites the UMFS × FMF hybrid line grew significantly faster than the UMFS and NEH lines. We detected a significant line by site interactions for cumulative mortality, a result that is consistent with the substantial changes in line-specific mortality among sites, and for daily yield. Similarly, we detected a significant site by line interaction effect in our two-way
There were dramatic among site differences in average wet weight that is due to poorer than average growth. Between-line differences in yield were detected at only two sites, the Damariscotta River and A and Bagdaduce River sites. At the former site, the UMFS and hybrid lines (UMFS×FMF and UMFS×NEH) had better yields than the NEH line. The FMF and hybrid lines had the highest yield at Bagdaduce River site but these latter differences were not significant in post-hoc tests. We detected significant line by site interactions for both cumulative mortality and standardized wet weight (Table 1), results that are consistent with the site-specific variation among lines for these two variables. Even so, the line by site interaction term in the two-way ANOVA for daily yield was not significant two variables. Even so, the line by site interaction term in the two-way ANOVA for daily yield was not significant for the 2004 field trial (Table 1), indicating that the relative line-specific yield was consistent across sites and that the variation in line-specific mortality and growth essentially balanced each other.

ANOVA for daily yield. This latter observation was consistent with the dramatic changes in the relative yield for each line we observed across sites in the 2003 field trial. Because of the significant two-way interactions, we did not attempt to interpret the site or line main effects for either of these two variables.

### 3.2. 2004 field trial

Cumulative mortality was generally lower in the 2004 field trial compared to the 2003 field trial, particularly at the upriver sites on the Damariscotta River (DRA and DRB). However, mortality at the Narraguagus site (NR) was over 2.5-fold higher than at the other sites and may have been due to higher rates of siltation experienced at this site during the early stages of deployment when oysters were held in fine mesh inserts. Line-specific variation in cumulative mortality was detected at the Damariscotta River A site where mortality for the UMFS was significantly lower compared to mortality for the NEH line. The lowest mortality at the Darling Marine Center and Narraguagus River sites was observed for the UMFS×FMF and UMFS×NEH hybrid lines, respectively, though these differences were not significant after adjusting for multiple comparisons.

There were dramatic among site differences in average wet weight for oysters from all five lines. Oysters at the Damariscotta River A site reached market size (>75 mm shell height; data not shown) after only two seasons of growth. The wet weight of oysters at this site (>60 g) exceeded that observed at all other sites and was six-fold higher than the weight obtained by the same lines at the Darling Marine Center and St. George River sites (<10 g, Fig. 4B). Among-line differences in final weight were detected at the Damariscotta River A site where the UMFS, UMFS×FMF and UMFS×NEH lines were significantly larger than NEH oysters at the end of the experiment while the UMFS line was significantly larger than the UMFS×NEH line at the Narraguagus River site. In contrast, the FMF and UMFS×FMF were the fastest growing lines at the Bagdaduce River and St. George River sites, although these among-line differences were not significant in post-hoc, pair-wise comparisons.

The patterns in final size and mortality, described above, contributed to large differences in yield. Adjusted yield at the Damariscotta River A site (~48 kg; Fig. 4C) was two to sixteen-fold higher than at the other five sites. Despite low mortality, we obtained intermediate yields at Bagdaduce River and New Meadows River sites, a result that is due to poorer than average growth. Between-line differences in yield were detected at only two sites, the Damariscotta River and A and Bagdaduce River sites. At the former site, the UMFS and hybrid lines (UMFS×FMF and UMFS×NEH) had better yields than the NEH line. The FMF and hybrid lines had the highest yield at Bagdaduce River site but these latter differences were not significant in post-hoc tests.

We detected significant line by site interactions for both cumulative mortality and standardized wet weight (Table 1), results that are consistent with the site-specific variation among lines for these two variables. Even so, the line by site interaction term in the two-way ANOVA for daily yield was not significant for the 2004 field trial (Table 1), indicating that the relative line-specific yield was consistent across sites and that the variation in line-specific mortality and growth essentially balanced each other.

### 4. Discussion

Our study examined the relative performance of three genetically improved lines and two interline crosses at sites representing the range of environmental variation under which oyster culture is practiced in Maine. With the exception of the Darling Marine Center site, all of the locations at which we deployed oysters have been and most
still are active farms. Even so, these sites differed substantially with respect to temperature during the growing season and we detected a positive and statistically significant relationship between the variation in growth rate and cumulative degree days across sites. However, the strength of this relationship was weak, particularly for the 2003 field trial. In this field trial, the rather poor growth at the New Meadows River site is particularly evident despite the relatively warm water temperatures at this site. We were not able to consistently monitor other aspects of water quality at each site and the oyster growers and partners in our study typically exert little influence over environmental conditions at their lease sites. Thus, the lack of a strong effect of temperature on growth performance is not surprising given the variety of other factors that can affect oyster growth, including food quality, seston concentration, salinity, and culture methodology.

A major focus of our project was to determine whether there were line by site interactions for oyster growth and yield. Significant differences in the growth performance of genotypes or lines across culture environments has been observed for C. virginica and the congener Crassostrea gigas (e.g., Evans and Langdon, 2006; Mallet and Haley, 1983; Newkirk, 1978; Sheridan, 1997; Swan et al., 2007). We observed subtle variation in growth between lines at most sites, although at some sites variation in growth translated into detectable differences in yield. For example, we found that the fastest growing lines, FMF and UMFS × FMF, had the highest yield at the Damariscotta River site in both field trials. We also observed a similar pattern in growth-dependent yield at the New Meadows River site and faster growth for the UMFS and UMFS × FMF lines at Damariscotta River A site during the 2004 field trial which contributed substantially to variation in yield. We initially anticipated that the UMFS line would demonstrate superior growth because this line has been under size-based selection in Maine waters (Davis and Barber, 1999). For the most part, however, the UMFS line failed to significantly outperform other lines with respect to growth, suggesting that selective breeding has resulted in improved growth for all three of the parental lines included in our study across a broad range of environmental conditions.

Maine’s oyster growers typically send their oysters to the half shell market after they have reached a shell length of 75 mm. The oysters deployed at the Damariscotta River A site, on average, reached this size within a two year culture cycle, a pace that is much faster than farmers in Maine realized before the use of improved varieties of oysters (Barber et al., 1998). The growth of oysters at the Damariscotta River B site was similar to that observed at the former site after the first full field season (2004; data not shown). However, these oysters were not re-deployed for the 2005 season so the overall growth and yield at the DRB site was reduced. On the other hand, oysters deployed at other sites outside of the Damariscotta River would have required an additional one, and perhaps two, seasons of growth to reach market size. Thus, our results suggest that continued improvement in cold-water growth would be beneficial for farmers at these sites.

We also examined whether there was evidence of hybrid vigor our field trials. Hybrid vigor, or positive heterosis, occurs when hybrid lines outperform parental lines because of their mixed ancestry. Hedgecock and colleagues have provided substantial evidence for growth and survival heterosis in C. gigas (Hedgecock and Davis, 2007; Hedgecock et al., 1995). In our 2003 field trial, a significant line effect for growth was, in large part, the result of the superior performance of the UMFS × FMF hybrid line. Even so, the site-specific gain in growth for the UMFS × FMF line was relatively modest, reaching a maximum 10% increase in wet weight at the Damariscotta River A site. This is substantially lower than the 85% increase in adult live weight obtained by Hedgecock and Davis (2007) for hybrids created by crossing inbred lines of oysters. The level of inbreeding is a key component in growth heterosis for oysters. Although captive breeding of C. virginica has led to a loss of rare alleles and overall genetic diversity, particularly within the FMF and UMFS lines (Yu and Guo, 2005; Guo pers. comm.), overall, the UMFS, FMF, and NEH lines still retain appreciable genetic variation. Perhaps more importantly, there was no evidence of increased growth or yield for the interline hybrids in 2004. Thus, hybrid vigor was neither pervasive nor consistent across our two field trials.

Selective breeding programs have been successful at reducing the impact of disease and generated lines of C. virginica embraced by the industry. Among the lines included in this study, the UMFS line is considered to be highly tolerant or resistant to ROD (Barber et al., 1998), but mortality for this line is typically 100% when cultured at sites where Dermo or MSX are endemic (Guo et al., 2003). The NEH line is recognized for having MSX and Dermo resistance while the FMF line has putative resistance to MSX and ROD. In the two field trials reported here, between-line differences in yield were often driven by variation in survival that, in turn, may be based on differences in disease resistance. The association between line-specific survival and yield was particularly apparent at the Damariscotta River sites (DRA and DRB) in the 2003 field trial and where cumulative mortality for the NEH line was nearly 80%. The average mortality for all lines at these two sites was over eight-fold higher than at the New Meadows, St. George and Bagdaduce River sites. The two lines with the lowest mortality at the Damariscotta River sites, UMFS and UMFS × FMF, demonstrated substantially higher yields at these sites during the 2003 field trial and the line-specific mortality at these sites resulted in a significant site by line interaction for survival and yield.

Roseovarius Oyster Disease (ROD) is considered endemic at the Damariscotta River sites included in our 2003 trial (Barber et al., 1998). Mortality associated with ROD is particularly acute among smaller size classes of oysters (Maloy et al., 2007a). For example, Davis and Barber (1999) noted that crop losses were as high as 90% during ROD outbreaks in Maine with peak mortality among oysters <25 mm in shell height occurring in late summer and early fall. Oyster growers in Maine often deploy oyster seed in nursery systems beginning in late May or early June so they will grow beyond 25 mm in shell height prior to the fall when ROD becomes problematic. The oysters in our 2003 field trial, however, were deployed nearly 2 months later than is typically practiced by Maine’s oyster culture industry and substantial mortality during the 2003 field trial was evident within the first 2 months of deployment at the Damariscotta River A and B sites when the majority of oysters were <25 mm in shell height (data not shown). Although we did not conduct a formal analysis of disease prevalence in our field trials, the shell valves of many of the deceased oysters from these two sites displayed evidence of shell curvature and excess concholin deposition, symptoms characteristic of ROD.

The early mortality at the Damariscotta River sites in the 2003 trial was lowest for the UMFS line which was developed using the survivors from diseased oysters in New York and Cape Cod. Thus, Gómez-León et al. (2008) and Markey et al. (2009) have reported that the NEH line had higher survival, relative to locally produced lines, during a field trial in Rhode Island impacted by ROD. Surprisingly, mortality for the FMF line was nearly equal to that of the NEH line and much higher than that of the UMFS line, even though the FMF line has been propagated from oysters that survived both MSX and ROD outbreaks in southern New England (Sunila, pers comm.). Maloy et al. (2007b) have investigated genetic differentiation among strains of R. crassostreae, the etiological agent of ROD, sampled from geographically distant ROD outbreaks. They found that R. crassostreae isolated from any single disease outbreak tends to have the same genotype. However, they also observed that two genotypes were more commonly associated with disease outbreaks in Maine while eight different genotypes were identified from diseased oysters in New York and Cape Cod. Thus, site-dependent susceptibility to ROD may be the result of local variation in the disease agent (R. crassostreae) or environmental conditions leading to the onset of ROD, so that the FMF and NEH lines remained susceptible to ROD in Maine waters. Our results, along with those of Gómez-León et al. (2008) and Markey et al. (2009) suggest that there is a need for further research into the genetic basis of ROD tolerance.
and resistance and the physiological and environmental conditions leading to ROD outbreaks.

The results from our field studies have important ramifications regarding efforts to breed genetically improved Eastern oysters for the oyster culture industry throughout the northeastern U.S. Hedgecock and Davis (2007) have suggested that broodstock development for Pacific oysters could be accelerated through a combination of directional selection on inbred lines and cross-breeding the most productive of these inbred lines. In our study, cross-breeding of genetically distinct but not completely inbred lines of Eastern oysters generated some gains in growth and yield, but these gains were modest in magnitude and inconsistent across sites and years. Based on these results, we suggest that there will be limited opportunity to obtain significant gains in performance through heterosis without the purposeful inbreeding and identification of inbred lines with high specific combining ability, as has been achieved with Pacific oysters.

On the other hand, we observed additive-like effects for survival which translated into substantial differences in yield. This was particularly evident at sites on the Damariscotta River during the 2003 field trial where survival for the NEH line was greatly reduced relative to UMFS, and the UMFS × NEH hybrid line had survival that was intermediate to the two parental lines. A similar pattern was observed with respect to the relative survival for the UMFS, FMF and UMFS × FMF lines. These observations highlight the potential value in continuing to develop disease resistant lines via selection to obtain enhanced survival when there is disease pressure.

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