Aquaculture 326-329 (2012) 61–67

Contents lists available at SciVerse ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

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

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ARTICLE INFO

Article history:
Received 21 June 2011
Received in revised form 14 November 2011
Accepted 20 November 2011
Available online 26 November 2011

Keywords:
Eastern oyster
Crassostrea virginica
Selective breeding
Yield
Growth
Survival

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.

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 Borrero, 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

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doi:10.1016/j.aquaculture.2011.11.030

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 interline hybrids (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 lutherii*, 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-l 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 *I. galbana*, *P. lutherii*, *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 microcultch. 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 mm^2 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.