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Cross-Breeding for Improved Growth and Disease Resistance in the Eastern Oyster

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Introduction

The Eastern oyster, *Crassostrea virginica*, has historically supported a major fishery along the Atlantic and Gulf coasts of North America. Although commercial landings of oysters along the East Coast have been in significant decline for decades, oyster aquaculture has the potential to provide for a sustained commercial harvest as well as to generate a product that is superior to wild-caught oysters. Traditional culture methods rely on the capture of wild spat or seed which are then moved to grow-out sites. Such culture methods are extensive and simply rely on the manipulation of wild stocks. The development of hatchery technologies for the production of oyster seed has alleviated some of the uncertainty inherent in natural supply of seed. In addition to sustaining a vital oyster aquaculture industry, the hatchery-based production of oyster seed allows for the development of genetically improved lines of oysters with characteristics substantially different from their wild ancestors.

Selective Breeding of Eastern Oysters

Genetic improvement is one of the foundations upon which increases in agricultural production are based. Artificial selection has been applied by animal and plant breeders for centuries through the selection of individuals demonstrating unique or high quality traits as parents for the next generation. When a substantial portion of the variation for traits of interest has a genetic basis, artificial selection can produce measurable changes from one generation to the next. Eastern oysters and many other bivalves are prime candidates for selective breeding. They are highly fecund and populations generally harbor substantial genetic variation for commercially important traits. Selective breeding has been applied to bivalves only for a few decades but it has become a key component in efforts to increase the production of farmed shellfish.

One facet of oyster culture that complicates efforts for genetic improvement is the diversity of grow-out conditions (Figure 1). In the northeastern U.S., these range from warm, low salinity, estuarine waters to cool, high-salinity oceanic conditions. As a consequence, oysters grown at different locations often demonstrate dramatically different growth patterns, shell shape, color and taste. This variation is reflected in the diversity and popularity of "brands" of Eastern oysters like Watch Hills, Bluepoints, Glidden Points, and Moonstones, to name a few. Although farmers growing and marketing such brands may rely on genetically improved seed, brands are not synonymous with genetically improved lines of Eastern oysters.

Figure 1. The conditions under which Eastern oysters, *C. virginica*, are cultured in the Northeast varies dramatically from location to location. Nursery and grow-out sites can be sited intertidally, subtidally or in floating bags with environmental conditions varying from near freezing water during cold northern winters to warm extremes during southern summers. Selective breeding programs have typically relied on mass selection for faster growth and disease resistance and produced lines that are particularly well adapted on a local basis. To produce lines that have a broader appeal on a regional basis, selective breeding programs must account for the large gradient in environmental variation and disease pressure that occurs in the Northeast.

A number of broodstock development programs have been established with the intent of generating lines of Eastern oysters with faster, more uniform growth and improved disease resistance. Early progress in programs at the Virginia Institute of Marine Sciences, the National Marine Fisheries Service Milford Laboratory, Rutgers University Haskin Shellfish Research Laboratory (HSRL), the Frank M. Flower & Sons Inc. and the University of Maine, was the focus of an NRAC Fact Sheet by Stan Allen, Pat Gaffney and John Ewart published in 1993. Our intent is to report on recent progress in industry-driven programs seeking to develop lines that are well adapted for use in the northeast and on recent efforts using cross-breeding between northeastern lines to bring about improved performance.

Selection for faster growth rate has been one component of many breeding programs and resulted in lines that demonstrate enhanced growth under local conditions (Figure 2). For example, size-based selection has reduced time to market for the University of Maine Flowers Select Line (UMFS) by nearly 50% compared

Figure 2. Sized-based selection has been at the heart of efforts to develop faster growing Eastern oysters, *Crassostrea virginica*.

to unselected oysters when grown under cold-water conditions typical of culture sites in Maine. In side-by-side grow out trials at these same sites, oysters from both the UMFS line and the Frank M. Flower & Sons Inc. of NY grew on average 5-20% larger than oysters from HSRL's Northeastern High Survival Line (NEH), a line developed under culture conditions typical in southern New England.

In many locations, however, the incidence of disease is the most serious impediment to increased production and revenue from the culture of Eastern oysters. There are several diseases of concern in the northeast region, including MSX, SSO and Dermo, which result from infections by the protistan parasites *Haplosporidium nelsoni*, *H. costale*, and *Perkinsus marinus*, respectively, as well as Roseovarius Oyster Disease (ROD) which is caused by the bacterium *Roseovarius crassostreae.* Comprehensive coverage of these diseases can be found at http://www.vims.edu/research/departments/eaah/programs/shellpath/oie/index.php, the web site of the Virginia Institute of Marine Science's Environmental and Aquatic Animal Health Office of International Epizootics Reference Laboratory. Figure 3 depicts these diseases.

By and large, selective breeding programs for the Eastern oysters have relied on using survivors from a disease challenge as the founders for a particular line. For example, Susan Ford, Hal Haskin, and colleagues produced several lines of oysters demonstrating MSX resistance by breeding oysters that had survived an MSX outbreak in Delaware Bay in the late 1950s. In the late 1980s researchers in Maine began working with oysters obtained from the Frank M. Flower & Sons Inc. which

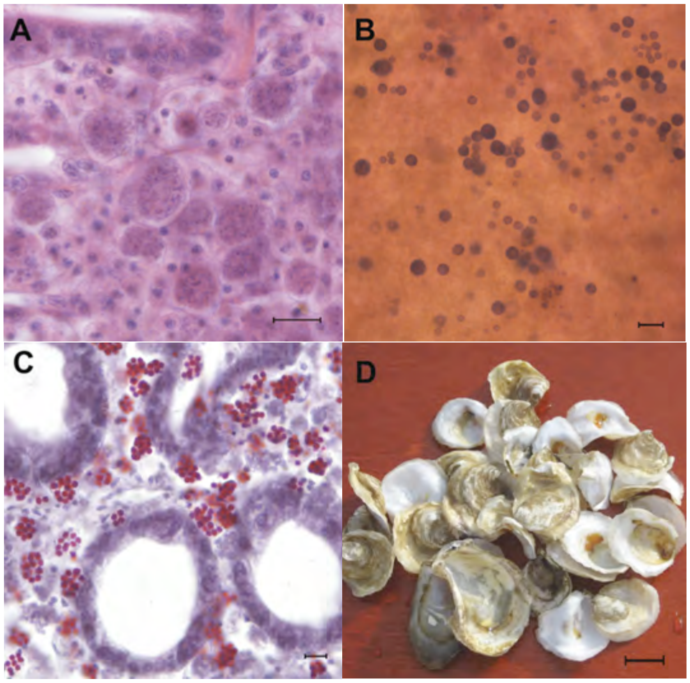

Figure 3. Diseases causing significant mortalities in northeastern aquacultured oysters. A. MSX, *Haplosporidium nelsoni*, paraffin section, H&E stain B. Dermo, *Perkinsus marinus*, Ray's Thioglycollate Medium C. SSO, *Haplosporidium costale*, paraffin section, H&E stain, and D. ROD, Roseovarius Oyster Disease, seed oyster shells with characteristic shell checks and conchliolin rings. Scale bar in panels A, B, and C equals 20 micrometers, and in panel D equals 1 centimeter.

1980s researchers in Maine began working with oysters obtained from the Frank M. Flower & Sons Inc. which had already seen some selection for resistance to ROD. The resulting UMFS oysters have demonstrated improved survival even when compared to Flower's oysters when grown side by side at sites in Maine where ROD is endemic. More recently, selective breeding by the State of Connecticut Bureau of Aquaculture in partnership with Connecticut oyster growers used oysters that survived a major MSX-epizootic in 1997 and 1998. This line, called the Clinton line, has also been exposed to ROD and oysters surviving both diseases were spawned to generate a line that has fast growth, MSX resistance, and ROD resistance.

Available Lines for the New England Region

Although local hatcheries may be working independently to develop their own oyster lines, there are four principal lines which have been in continuous development for high growth and survival under the wide diversity of culture conditions encountered in the Northeast.

The aforementioned Haskin Lab's NEH line has demonstrated resistance to MSX and Dermo and has high growth potential under warmer, low salinity conditions often found in southern New England. On the other hand, the University of Maine's UMFS line has superior performance when cultured at sites where ROD is endemic and high growth performance under colder water, high salinity conditions typical in northern New England. Oysters from the Frank M. Flower & Sons Inc. (New York) have been a stalwart for the industry throughout the northeast. The Flower's company has consistently used the largest oysters that survived disease outbreaks in several locations to develop a line with ROD and MSX resistance and high growth potential in both southern and northern New England, although it does not appear to be as well adapted to cold water conditions as the UMFS line. Finally, the Clinton line has shown resistance to MSX and ROD along with growth performance equal to that of the industry favorite NEH line in southern New England. (Table 1).

Table 1. Improved lines of Eastern oysters covered in this Fact Sheet.

Line	Environmental Conditions	Disease Resistance
NEH	Warm, low salinity	MSX/Dermo
Flowers	Warm, low to high salinity	MSX / ROD
UMFS	Cold, high salinity	ROD
Clinton	Warm, low to high salinity	MSX / Dermo / ROD

"Environmental conditions" refers to the temperature and salinity range where each line performs best.

It is important to note that these disease-resistant lines have been primarily developed for use in aquaculture settings. While several lines have shown increased resistance to one disease or another, for the most part they were selected for fast growth in order to reach market size before disease outbreaks can cause serious crop losses. Thus, these lines are not necessarily "long-lived" and their suitability for restoration projects has not been evaluated.

The use of Eastern oyster lines with resistance to disease has reduced the impact of disease. However, resistance to one disease does not necessarily confer resistance to all diseases of concern. For example, the ROD-resistant UMFS line experienced 100% mortality when grown at the Haskin Shellfish Lab's Cape Shore, NJ, site where Dermo and MSX are prevalent while the MSX- and Dermo-resistant NEH line demonstrated poor growth and <30% survival in field trials in the Damariscotta River, ME, where ROD is endemic. Because MSX, Dermo, SSO and ROD continue to be of concern to farmers throughout the northeast region, there is an interest in developing oyster lines with superior yield due to high growth potential and *combined* resistance to all four diseases. One approach to developing resistance to multiple diseases is to take a line demonstrating resistance to one disease and impart selection for resistance to a second disease. Calvo and colleagues found that dual resistance to MSX and Dermo could be bred into the Delaware Bay line (DEBY) of oysters by growing this MSX resistant line in a portion of the Chesapeake Bay where both MSX and Dermo are prevalent. Although their success suggests that combined resistance to MSX, Dermo and ROD could be achieved by challenging a MSX and Dermo resistant line with ROD and breeding the survivors, our observations suggest that mass selection for resistance to ROD in the NEH line or Dermo and MSX in the UMFS line will require a large base population in order to ensure that there is adequate genetic variation remaining in each line after selection.

Cross-Breeding for Improved Performance

Line crossing also can be used to develop lines with resistance to multiple diseases. Cross-breeding has played a prominent role in the development of several existing lines of Eastern oysters. In the early 1990s researchers at HSRL crossed three MSX-resistant lines from the mid-Atlantic and a wild stock from Delaware Bay to produce the Haskin CROSBreed line. The Haskin NEH line, mentioned above, was produced by crossing MSX resistant varieties from Long Island, including progeny from oysters grown by the Frank M. Flower & Sons Inc. in Oyster Bay, New York and the Ocean Pond Corp. in Ocean Pond, New York. Crossing between lines can achieve two things. First, it can reduce inbreeding and the loss of performance that often occurs as inbreeding accumulates. Most oyster broodstock development programs actively seek to avoid inbreeding. Due to the effects of selection and reduced parentage necessitated by hatchery limitations, most improved lines of Eastern oysters have reduced genetic variation relative to the ancestral wild populations. Second, cross-breeding can take advantage of unique alleles contributed by each of the parental lines whose interaction in hybrid offspring results in improved performance.

We have undertaken a series of field trials with the goal of examining whether crosses between existing lines can produce a line of hybrid oysters with enhanced resistance to all three diseases at the same time. So far, results from these field trials indicate that the survival for oysters from an F1 (first filial generation) cross between the NEH and UMFS lines is intermediate to that of the parental lines at the Cape Shore, New Jersey site where MSX and Dermo are now always present. The survival for the backcross lines, generated by crossing F1 oysters back to one or the other of the parental lines, was intermediate to the F1 and the respective parental line. These results provide a clear indication that Dermo-resistance has a genetic basis and that resistance is retained in hybrids. At the same time, hybrids have improved growth, relative to the NEH and UMFS lines, even in colder, northern New England waters. At the same time, we have found that the Clinton line, with putative MSX and ROD resistance, has performed as well as or better than the NEH line at sites throughout southern New England. Our results suggest that the development of lines with resistance to multiple diseases and suitable for the Northeast are indeed possible through the cross-breeding of existing lines.

Future Directions

To date, genetic improvement programs for Eastern oysters have typically relied on mass selection with occasional cross-breeding between lines. One distinct advantage of such an approach is that it allows for the development of local varieties with unique attributes. Alternative approaches may increase the pace of genetic improvement. For example, it is possible to purposely inbreed oysters and use cross-breeding between inbred lines to capitalize on heterosis (hybrid vigor), as has been used so successfully in maize and other crops. The geneticist R.A. Fisher described inbreeding and hybridization for trait improvement as a three stage process with stage one being the choice of founding individuals for a broodstock program, stage two being the creation of inbred lines with genetic uniformity, and stage three being the actual crossing between inbred lines. He placed an emphasis on "deliberately planned multiplicity" in which numerous inbred lines are crossed in order to identify genotypes with superior combining ability under any given set of circumstances. In this regard, maize breeders have historically worked with thousands of inbred lines in order to identify the best genotypes but, once identified, they can be reproduced at will. This approach requires many generations of inbreeding as well as the production of hundreds to thousands of inbred lines in order to test for combining ability. Thus Fisher's approach is cost prohibitive and beyond the capacity of most Eastern oyster breeding programs.

Family-based selection, wherein individuals are chosen as broodstock based on the performance of their offspring, is another alternative that is much more efficient than mass selection when dealing with group traits, such as survival and yield. It can also be applied to other traits that are not traditionally the focus of broodstock programs, such as shell thickness to ensure that fast growing oysters don't shatter when shucked, or shell shape, so that shape and cupping are optimized for consumer appeal. Ideally, family-based selection is combined with molecular genotyping of individual oysters within the breeding program. Genotyping allows the program to confirm the pedigree of broodstock and use pedigree information to minimize inbreeding while maximizing selection gain. In addition, incorporation of information on the genes affecting disease-resistance and other important traits in oysters will support gains through marker-assisted selection. Family-based selection has been adopted with a high degree of success by the Molluscan Broodstock Program, (http://hmsc.oregonstate.edu/projects/mbp/). Given the similarity in life history for *C. gigas* and *C. virginica*, it is highly likely that a family-based approach could work well for Eastern oysters, too.

Adoption of alternative approaches does not necessitate starting from scratch with wild oysters. There are fast-growing and disease-resistant lines, such as those discussed above, which are currently maintained by university- and state-supported labs, and by several private companies. Many of these lines can serve as founders for either family-based selection or a program based on inbreeding and hybridization. It is important to note that some of the available lines of Eastern oysters may not be genetically independent. For example, oysters from Maine and oysters from Stonington, Connecticut were used to found the lines with fast growth and ROD resistance propagated by Frank M. Flower & Sons Inc. Some of these oysters were subsequently re-imported into Maine as founders for the UMFS line currently favored by several growers in Maine. Such "recycling" of broodstock needs to be carefully managed in order to avoid unintentional inbreeding. To be a viable alternative, cross-breeding requires a long-term commitment to the propagation and maintenance of parental lines.

Industry surveys have repeatedly identified selective breeding as a top priority and research at public universities and government-supported labs has addressed this need. Unfortunately, programmatic adjustments, economic difficulties, and other administrative changes at public institutions can jeopardize long-term breeding programs and the perpetuation of lines that have been in development for decades, often with substantial industry involvement. We believe that cross-breeding between extant lines serves a vital need, the development of stocks with enhanced survival when faced with multiple disease pressures, to support the continued growth of commercial oyster aquaculture in the Northeast. While academic institutions will likely continue with such line development, we encourage the northeastern oyster culture industry to consider the development of commercial broodstock repositories and multiplier hatcheries to assist with the propagation of genetically improved lines of Eastern oysters and ensure the industry's long-term access to these lines.

Contact Information for Specific Lines Mentioned in this Fact Sheet

Clinton line:

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Additional Reading

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Glossary

Artificial Selection – is the process of *Selective Breeding* of plants and animals through the choosing of specific parents to contribute gametes to the following generation in order to produce a population with more desirable traits.

Backcross – the mating of a hybrid with one of the parental genotypes.

Genetics – the science that deals with heredity and variation in living organisms.

Genotype – the genetic constitution of an individual.

Heterosis (hybrid vigor) – the phenomenon where hybrid offspring have higher performance that the parental strains that were crossed to produce the hybrids.

Hybrid – the offspring of a cross between two different species, races or varieties (including lines).

Inbreeding – mating between individuals that are more closely related than expected by random chance.

Line – a group of individuals that form a closed breeding population that are reproductively isolated from other breeding populations.

Marker-Assisted Selection - the process where there is improvement in a trait of interest due to selection on genetic markers associated with the trait and not due to selection on the trait, itself (indirect selection).

Pedigree - A record of ancestry for individuals in a line or closed breeding population often based on information from breeding records or genetic markers.

Phenotype – the observable characteristics or outward appearance of individuals, resulting from the action and interaction of the individual's genotype and the environment in which it develops.

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