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Genetic Improvement of the Eastern Oyster for Growth and Disease Resistance in the Northeast

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Introduction

For over a century, harvests of eastern oysters *(Crassostrea virginica)* have been declining in the northeast. This trend has been particularly acute in the mid-Atlantic region where two diseases, MSX and Dermo, have caused catastrophic mortalities. With the reduction in natural populations, growers have increasingly turned to the use of hatchery technology for annual supplies of seed oysters. Today, many commercial shellfish culture operations in the region, especially around Long Island Sound and the New England states, either operate their own hatchery/nursery or purchase hatchery produced seed.

Hatchery technology has been instrumental in freeing growers from unpredictable natural fluctuations in seed supply. For example, hatchery technology and remote setting techniques applied on an industrial scale in the Pacific northwest have revolutionized culture of the Pacific oyster *(Crassostrea gigas)* making Washington state the national leader for oyster production. An equally important potential of commercial hatchery production is yet to be realized: improvement of oyster stocks through genetic manipulation.

This fact sheet reviews some of the breeding methods used to improve shellfish stocks, such as selective breeding and triploid production, and summarizes efforts to develop disease resistant and faster growing strains of the eastern oyster. Some simple suggestions for maintaining the genetic health of production brood stocks are also provided.

Selective Breeding

The natural variation observed in wild populations of shellfish can be exploited through selective breeding. Improvements such as disease resistance or faster, more uniform growth are probably the highest priority for commercial growers. Selective breeding, or artificial selection, is based on choosing specific individuals to contribute to the gene pool of the next generation. The chosen individuals share certain common features, a phenotype, that appear desirable to the person(s) making the choices. The phenotype is a reflection of the underlying collection of genes — the genotype. The expression of the genotype as a phenotype is analogous to the reflection of a face in a pool of water. Under ideal environmental conditions, such as no ripples in the in the pool, the image perfectly reflects the object. Under realistic conditions, i.e., disturbances in the surface of the water, the reflection is a distortion of the original visage. In the same way, the phenotype is a reflection of the genotype that is modulated by the realities of the environment, such as better access by some oysters to food or infection by disease in others. To what degree does the genotype control the phenotype?

Estimates on how much the genotype contributes to the phenotype is the subject of quantitative genetics, which comprises an essential component of breeding programs for agricultural plants and animals and for some aquaculture species, such as salmon and trout. Little in the way of quantitative genetics has been done for eastern oysters or other commercially important bivalve species. Most attempts at selective breeding for shellfish have been practical “by the seat of the pants” programs relying heavily on selecting phenotypes without regard for the underlying genotypic contribution.

However, the “practical” approach worked for agricultural products for thousands of years, and this is likely the best approach for shellfish farmers today for two reasons. First, given the realities of limited institutional funding, the classical quantitative approach of estimating heritabilities that might provide precise criteria for selecting is unlikely. Second, the pace of biotechnology will probably outrun classical approaches, and may produce new technologies for improving shellfish stocks relatively soon. Despite this folksy recommendation for practical breeding, certain genetic tenets should be observed. These are described under” General Hatchery Practices and Management Recommendations” (page 4).

Induced Triploidy

Another method of genetically altering the eastern oyster is through induced triploidy. Triploidy is a condition by which the animal retains three sets of chromosomes (3n) rather than the usual two sets (2n, diploidy). Triploidy in oysters can be readily induced in the hatchery using cytochalasin B, an antibiotic that inhibits the elimination of one set of chromosomes. Under normal conditions, the egg produces polar bodies that bud off from the egg (like pinching off a portion of a balloon). Inside the polar bodies are sets of chromosomes normally destined for elimination. Cytochalasin B* prevents polar body formation and the set of chromosomes are returned to the egg. Thus, the triploid gets two sets from the egg and one from the sperm, and all cells of the oyster remain triploid thereafter. The principal characteristic of triploids is that, relative to diploids, they are virtually sterile. In sterile triploids, stored energy not used for reproduction is generally allocated to body tissue growth. Reduced gonad production also improves meat quality. These attributes are beneficial in aquaculture because reproduction can cause a decline in product quality, contribute to mortality, or impede growth. Triploidy has proven valuable to the oyster industry in the Pacific northwest. Beginning in 1986, research and subsequent pilot scale production in Washington state demonstrated that triploid *(Crassostrea gigas)* oysters grew faster, survived well and maintained superior meat quality during the summer compared to normal, reproductive (diploid) oysters. During the last few years the industry has been gradually increasing its production of triploid Pacific oysters to supply its expanding markets.

**Cytnchalasin B is currently classified by the Food and Drug Administration as an unapproved substance, which will not be of low regulatory prioritry, For additional information, consult the aquaculture extension contact in your state.*

Genetic Improvements to American Oyster Stocks

Disease Resistance

MSX-Disease

In 1957, Delaware Bay began to experience devastating oyster mortalities caused by the Haplosporidian parasite known as MSX. Over the next three years, mortalities continued, but the rate and intensity of infection seemed to attenuate, suggesting that a natural process of selection for disease resistance was occurring. In an attempt to determine if resistance to MSX disease was heritable, i.e., could be transmitted from generation to generation, researchers at Rutgers University led by Dr. Hal Haskin began a series of selection experiments on lab-reared oysters, They were able to determine that resistance was heritable, and their experiments gave rise to what are now about five extant “lines” of MSX-resistant oysters. (A line can be considered a lineage of individuals in a closed breeding population), These lines have been the focus of intense practical interest as oyster aquaculture developed. When MSX disease began to cause mortalities north of Delaware Bay, shellfish hatcheries began to look to the MSX-resistant strains for their obvious commercial potential. In the northeast, two companies are using Rutgers MSX-resistant oysters routinely: F. M. Flowers, New York and Ocean Pond Corporation, New York. For these companies, resistant stocks have been pivotal in avoiding high MSX disease mortalities on several occasions. Other hatcheries

(Mook Seafarm, Maine and Aquacultural Research Corporation, ARC, Massachusetts) have spawned these strains for distribution in field trials of an NRAC sponsored project “Genetic Improvement of the Eastern Oyster for Growth and Disease Resistance in the Northeast.” These findings indicate that resistant stocks are equivalent to wild oysters in the absence of MSX disease, but with disease pressure they excel.

The New Jersey Agricultural Experiment Station has agreed to release the Rutgers MSX-resistant lines as the Haskin Lines (HaLs) to interested growers in the northeastern region on a quid pro quo basis. Specifically, in exchange for using HaLs, growers (hatchery or growout) must agree to act as custodians of the brood stock. While the details of this arrangement are still being formulated, the essential function of a custodian and/or user group will be to keep HaL brood stock distinct from other brood stocks and to keep records of its spawnings and distribution. This relationship between breeder and oyster farmer is mutually beneficial. The benefits to the grower are obvious, The benefit to the breeding program at the Haskin Shellfish Research Laboratory are sanctuary from Dermo disease, and long term maintenance of this unique genetic resource. There will be no licensing fees associated with the use of HaLs. Additional information on the use of HaLs brood stock can be obtained by contacting Dr. Stan Allen, Haskin Shellfish Research Laboratory, Rutgers University, Box B-8, Port Norris, NJ 08349.

Dermo Disease

Some evidence suggests resistance to Dermo disease may be heritable, and that selective breeding programs may succeed. For example, oysters from geographical regions where Dermo is enzootic (normally present) are more resistant than those outside of the range of the disease, suggesting some natural selection for resistance. However, the existence of natural oyster stocks with resistance to Dermo disease or the potential to develop Dermo-resistant brood stock has not been rigorously demonstrated.

Reports of prior attempts to produce Dermo-resistant oyster strains by researchers for the most part, have been anecdotal and no concrete results have emerged. However, two recent attempts to determine the potential for increased resistance to Dermo disease have been initiated. Dr. Gene Burreson at the Virginia Institute of Marine Science (VIMS) is coordinating a selection program using stocks from the Chesapeake Bay region. This program is now in the third generation. At the Haskin Shellfish Research Laboratory, a program was initiated to select for resistance to Dermo disease in the MSX-resistant lines, primarily as a consequence of an epizootic of Dermo disease in Delaware Bay that began in 1990. These lines are designated the High Survival Resistant Lines (HSRLs). Neither the VIMS or Rutgers selection programs have thus far been able to demonstrate that resistance to Dermo disease is a heritable trait or produce strains for commercial scale field trials and use.

For additional information on the history and impact of MSX and Dermo diseases on oyster stocks in the northeast see NRAC Publication No. 200.

Accelerated Growth

Selection for faster growing oysters is another strategy that can provide a practical method for managing around Dermo infections (but not MSX disease) since Dermo disease is more severe in older animals.

Frank Wilde, a commercial grower in Maryland, began developing a line of rapidly growing oysters in 1968. The stock, known as the “Wilde strain”, was selected for fast growth (in both larvae and adults) and deep-cupped shells, in relatively low salinities (12 ppt, parts of salt per thousand parts of water) at his growout site (a tidal creek) in Chesapeake Bay. Selections were made by crossing the fast growers in the new generation with their surviving, fast growing parents. Wilde stock are not resistant to either MSX or Dermo disease, but do grow very quickly. Market size oysters have been produced in six months, under the best of growing conditions. At present, a commercial producer in Maryland is using the Wilde strain in floating raft culture. Cultchless oysters are raised to market size (3 inches) by holding them first for twelve months in waters of low Dermo disease pressure, followed by a two to four month “finishing” period in higher salinity water.

In the 1970’s a selective breeding program was conducted at the National Marine Fisheries Service Laboratory (NMFS) in Milford, Connecticut. The program selected Long Island Sound oysters for rapid growth through four generations producing what is known as the “Milford high-line.” This line is still extant although later generations were perpetuated without further selection for size. Eventually, these oysters may be available to growers if the line could be expanded from the limited numbers of brood stock now alive. A Regional Vocational Aquaculture School at Captain's Grove in Bridgeport, Connecticut most likely will get custody of the lines when the school opens this year (1993).

In 1988, researchers at the University of Maine began a selection program using a native Maine population, the NMFS Milford high-line, stock from a private grower (F.M. Flowers), and a Rutgers MSX-resistant strain. The native Maine population, ironically, has been difficult to spawn in the hatchery, and no selection has been accomplished. However, in 1992, a new line, identified as the "St. Croix line" was begun from a native population in Oak Bay in New Brunswick, Canada. The St. Croix line will be the founder population for a cold water strain of oysters. Both Flowers and the Milford high-line are in their second generation of selection for fast growth. Progress after the first generation was about ten percent improvement, a promising gain for only a single generation. The MSX-resistant lines from Rutgers have not performed as well, possibly because of a recent episode of juvenile oyster mortality in the Damariscotta River.

Triploidy

The first report of induced triploidy in the eastern oyster came from Maine in 1978. However, commercial culture of triploid oysters has achieved the greatest success in the Pacific northwest. As hatchery based aquaculture has grown in the east, field research to determine the technical and commercial feasibility of producing and deploying triploid eastern oysters has been renewed in several locations from Maine to Florida.

In a collaborative project between the University of Delaware and Rutgers University, researchers tested the hypothesis that triploids would be more disease resistant than diploids. They produced all possible combinations of eggs and sperm from both resistant and susceptible brood stock in both diploid and triploid combinations. The eight different combinations were field tested with appropriate replication over two production seasons in Delaware Bay. In the first season, MSX pressure was high and mortalities were observed in all groups. In the second season, Dermo was the major disease factor (although MSX disease was also present) enabling evaluation of the relative resistance of triploids to Dermo disease.

In the first (MSX) season, triploids overall performed somewhat worse than their corresponding diploid crosses. For example, seventy percent of diploid resistant x resistant oysters survived, while about sixty percent of triploids survived. Other triploid crosses also had correspondingly lower survival. In the second (Dermo) season, triploid survival was much worse than that of diploids, in some cases up to three times worse. Researchers at VIMS demonstrated that survival of diploids and triploids to Dermo disease alone was equivalent. These tests suggest that triploids will not be useful for increased disease resistance.

Triploids may play a role in alleviating disease pressure even if they are not resistant to disease *per se.* Researchers at VIMS also have shown that triploid oysters grew faster than diploids in the Chesapeake Bay. By virtue of more rapid growth, triploids were ready for market before the onset of major mortalities during the second year of exposure to Dermo disease. Triploids grown in pilot scale trials in Florida were fifty percent heavier than diploid controls at market size (18 months old). Although Dermo disease was present, triploid and diploid mortality was the same. At one high salinity site, triploids were the only survivors; all the diploids died.

There is an increasing body of evidence indicating that the performance of triploids varies under different environmental conditions. In general, the better the conditions — e.g., ample food, optimum salinity, suitable temperatures, etc. — the better the performance of triploids relative to diploids. The converse seems also true: poorer conditions result in poorer triploid performance. This generalization would account for the difference in results seen between triploid performance in Delaware Bay and Florida. Triploids in Delaware Bay were exposed to two diseases during poor summer growing conditions; those in Florida were exposed to light Dermo disease pressure and good food conditions.

General Hatchery Practices and ManagementRecommendations

All hatchery managers and growers are concerned with the physiological health of their product; few concern themselves with the genetic health of their stocks. The concept of genetic health embodies the maintenance of variation in a population, a concept somewhat at odds with selective breeding.

All populations have a range of genotypes, that is, alternate forms of the same genes in various combinations. The complexity of genotypes gives the population flexibility in its response to a constantly changing environment. For a wild population, this flexibility is essential for long-term survival. For aquaculture, the wide range of genotypes gives rise to a wide range of performance traits, such as size and disease resistance. Selection narrows this range of performance traits so that all individuals perform similarly, e.g., all grow fast or most resist disease.

But selection also narrows the scope of other traits, unintentionally. This unintentional selection may arise from the positive association (correlation) between two traits. For example, maybe one way to grow faster is to mature sexually later in life. So selecting for fast growers will also select for late maturation. The classic situation for bivalves is selecting for fast larval growth in the hatchery. Fast growing larvae are not necessarily the fast growing adults, so discarding little larvae may actually hinder your ability to get shellfish to market early.

Another, more pernicious, effect of selection — and also an inevitable conclusion — is inbreeding. By selecting certain individuals, you are by definition excluding others, usually most others. The selected individuals are more likely to be related to each other since they share features of the genotype that are desirable. Sooner or later, inbreeding will occur. The question for hatchery managers is, how soon? The following guidelines during your spawning and rearing procedures would go a long way to minimizing inbreeding, even if you are not selecting.

- Try to maximize the number of parents in a spawning. It certainly is true that two ripe female oysters will give you enough eggs for a culture tank, but every second larva (spat, adult, future spawner) will be related.
- Try to get an equal contribution from each spawner. A few modifications in your spawning practices will help. The most straightforward, and admittedly painstaking, method is to spawn each animal separately. For some shellfish this is easy, while others are more problematic. Eggs, once spawned, can be divided into equal quantities for fertilization by individual males. After fertilization, gametes can be pooled into a common container again, but now an equal number of eggs was given a chance to be fertilized by each male. In other words, try to eliminate the randomness of encounters among gametes in mass spawns.
- Do not grade larvae, within reason.
- Keep several small, rather than one large, populations of brood stock. Each can be, but doesn't have to be, selected for important traits. Try to establish your future (small) brood stock populations over the course of the season rather than subdividing one population.
- When spawning your small brood stock populations, try to cross them with each other, not with themselves.
- Keep good records.

If a hatchery is not part of your operation and you rely instead on purchased seed for growout, you should make an effort to obtain some basic information on the genetic background of the seed stock you intend to use. Environmental conditions at your growout site, and the potential for exposure to either MSX or Dermo disease, or both, are important considerations. Deploying stocks that are well adapted to local growing conditions, or that have been selected for rapid growth, disease resistance, low salinity tolerance, etc., may make a significant difference, depending on your particular ecological conditions.

You may consider asking a seed supplier, before making your purchase, to provide you with some general background information on the brood stock's origin and how it is maintained, conditioned and spawned. If nothing else, it may tell the hatchery operator that you are paying attention to quality. Other items you may inquire about could include:

- Are brood stock oysters considered to be wild stock or do they represent a selected line(s)?
- For what particular traits are brood stock selected?
- Are any faster growing or disease resistant lines available?
- Are there any particular lines recommended as better suited for local growing conditions or your particular site?
- Can they provide the names of a few (other) satisfied customers in your area?

Alternately, you may be able to provide the hatchery with your own brood stock, selected for particular local conditions.

Future Directions

Will genetically improved strains of oysters be useful in attempts to revive the ailing Atlantic coast oyster fishery? At this point, we cannot predict if, or how long, it will take to develop Dermo-resistant lines. MSX-resistant lines are currently available, but they are as susceptible to Dermo disease as wild stocks, are sometimes less hardy as larvae, and are genetically no better than wild oysters in terms of growth and survival in the absence of MSX disease. MSX-resistant lines at the Rutgers University Haskin Shellfish Research Laboratory are now being crossed to produce new lines of "all-purpose" MSX-resistant oysters that exhibit improved larval survival and growth even in the absence of MSX disease. These new lines may be the starting material for efforts to select oysters resistant to Dermo disease.

One promising alternative to conventional selective breeding involves hybridization between two different species, or interspecific hybridization. This might produce novel genetic combinations with quantum changes in the commercial traits of a species. These traits, e.g., disease resistance, growth rate and hardiness, could then be refined by selection and backcrossing. An important feature of this strategy for aquaculture is the rapid generation of novel genotypes, in contrast to the gradual progress of conventionalbreeding.

Thus far, experimental efforts to accomplish interspecific hybridization and polyploidy among select species of oysters in the genus *Crassostrea* have met with limited success. Hybrid crosses of C. *virginica* with the Suminoe oyster, C. *rivularis,* and the Pacific oyster, C. *gigas,* produced viable larvae, but only for eight to ten days. No hybrid larvae grew past 100 microns and none set. At this point interspecific hybridization in the genus *Crassostrea* doesn't appear to be a potential source for introducing new traits (e.g., disease resistance) into the eastern oyster in the foreseeable future.

The future role of triploids in eastern oyster aquaculture is unclear. Their utility may depend greatly upon exactly where they are used, the triploids excelling in favorable growing areas. One tantalizing prospect is a triploid hybrid oyster, e.g., an animal with two sets of C. *virginica* chromosomes and one set of Pacific oyster (C. *gigas)* chromosomes. Such a construct might closely resemble the prized eastern oyster, yet possess the resistance to both Dermo and MSX disease that C. *gigas is* thought to have. In addition, it would most likely be sterile, which would both enhance its growth and minimize concern about its spread in natural waters. Although such a genotype does not yet exist, evidence from plant breeders suggest that it still remains a possibility. Further research in this direction would be worthwhile, especially considering the apparent range expansion of both MSX and Dermo disease.

Suggested Additional Reading

Allen, S. K. , S. L. Downing, and K. K. Chew. 1989. Hatchery Manual for Producing Triploid Oysters. Washington Sea Grant Program, University of Washington Press, Seattle, Washington. 27 p.

Allendorf, F. W. and N. Ryman. 1987. Genetic Management of Hatchery Stocks. In Population Genetics and Fishery Management, N. Ryman and F. Utter, eds., University of Washington Press, Seattle. 420p.

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Gall, G. A. E. 1989. Inbreeding. In Population Genetics and Fishery Management, N. Ryman and F. Utter, eds., University of Washington Press, Seattle. 420p.

Videotape

Allen, S. K., S. L. Downing, and K. K. Chew. 1989. Triploid Oyster Production. Produced by Washington Sea Grant, Instructional Media Services and the School of Fisheries, University of Washington. Available in 3/4" or VHS format from University of Washington Press, Seattle, Washington.

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Glossary

Artificial Selection: the process of choosing the specific parents, on the basis of one or more desirable traits, to contribute gametes to the following generation.

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

Chromosome: a nuclear body composed largely of DNA and protein and comprising a linear sequence of genes.

Closed Breeding Population: a group of individuals isolated from other external sources of genetic material.

Diploid: having two sets of chromosomes or double the number of chromosomes present in gametes.

Enzootic: a disease that is present, although not necessarily at high levels, in an animal population (equivalent to endemic in humans).

Epizootic: a disease that is rapidly spreading throughout an animal population (equivalent to an epidemic in humans).

Gamete: a mature reproductive cell capable of fusing with a similar cell of the opposite sex to yield a zygote.

Gene: the basic unit of inheritance, by which hereditary characteristics are transmitted from parent to offspring. Each living cell carries a full compliment of the genes typical of the species, arranged in linear order on the chromosomes.

Genetics: the science that deals with heredity and variation.

Gene Pool: the sum total of genes in a breeding population.

Genotype: the sum total of genetic information contained in an organism.

Heredity: 1. the capacity of an individual to develop traits present in parents or ancestors; 2. all the characteristics, morphological or physiological, that are dependent on genetic factors received from parents.

Heritability: the capacity of a trait or characteristic to be passed on to offspring.

Hybrid: the offspring of a cross between two different species, races, or varieties; a crossbred animal or plant.

Inbreeding Depression: reduction in fitness or vigor due to inbreeding of normally outbreeding organisms.

Interspecific Hybridization: crossbreeding or interbreeding between two different species.

Intraspecific Hybridization: crossbreeding or interbreeding among individuals of the same species.

Line: a lineage of individuals in a closed breeding population.

Phenotype: the observable characteristics of an individual, resulting from the interaction between the genotype and the environment in which development occurs.

Polyploid: a cell, tissue or organism having three or more chromosome sets.

Quantitative Genetics: the study of differences between individuals that vary in quantity rather than quality, e.g., body weight versus eye color.

Selective Breeding: see artificial selection.

Strain: a subdivision of a race or a species that possesses a distinctive characteristic or trait.

Triploid: having three sets of chromosomes or triple the number of chromosomes present in gametes.

Zygote: the diploid cell formed by the union of egg and sperm nuclei within the cell.

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