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Genetic Considerations for Hatchery-Based Restoration of Oyster Reefs : A summary from the September 21-22, 2000 workshop

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A summary from the September 21-22, 2000 Workshop
held at
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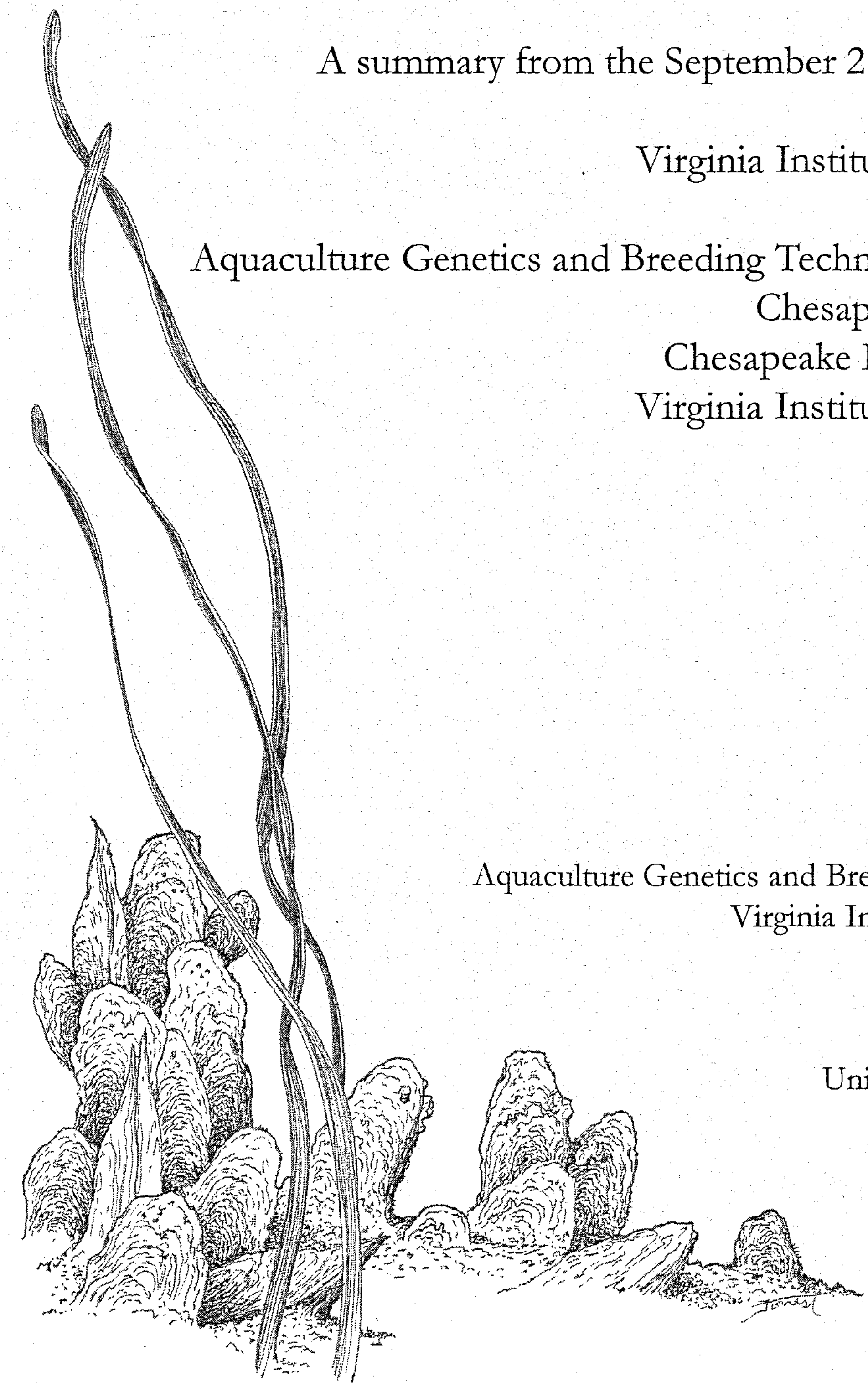

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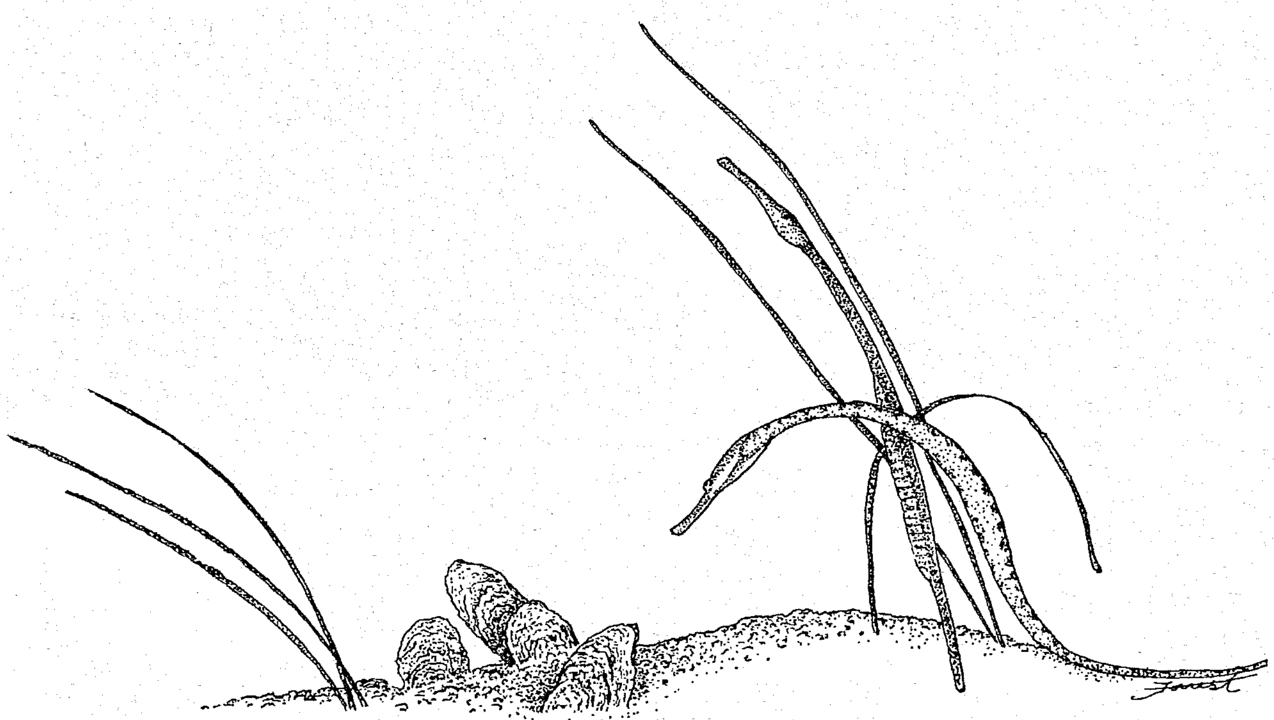
"Genetic Considerations for Hatchery-Based Restoration of Oyster Reefs"

A summary from the September 21-22, 2000 Workshop

The following is a summary of issues and considerations surrounding the use of hatchery stocks for restoration of public oyster reefs. This summary stems from a workshop conducted at the Virginia Institute of Marine Science (VIMS). The original goal of the workshop was to try to develop a consensus, or at least a general agreement, on genetic policy(ies) for stocking oyster reefs. To do this, the first day of the workshop was devoted to placing the genetic concerns "on the table" in the context of both Maryland and Virginia oyster replenishment and restoration programs. The conclusions from the first day of presentations revealed that there are a number of scenarios for hatchery-based restoration/replenishment and that the genetic considerations varied among them. Other genetic considerations were common to the whole Bay. This document summarizes a great deal of discussion, and consequently some detail is omitted.



Main Points and Conclusions

Operating assumptions:

The Workshop members endorse and adopt the principles set forth in the "Chesapeake Bay Oyster Restoration" consensus document (Chesapeake Research Consortium, June 1999, 5 pp.). Specific issues addressed in the CRC consensus that are parallel to the genetic considerations of the workshop are as follows:

- Three-dimensional reefs are important for oyster reproductive success and hatchery produced seed can be used to initiate recruitment.
- Reefs stocked with oysters for the purposes of restoration must be permanent sanctuaries.
- The goal of setting aside and restoring 10% of historic productive reef acreage is supported, with the implicit assumption that massive hatchery production may be necessary to accomplish this goal.
- Increased spat set is an implicit outcome to reef stocking programs (a goal in CRC, 1999) and has the consequence of spreading genes from hatchery stocks.
- Demonstrating the effectiveness of reef sanctuary programs (a goal in CRC, 1999) can be realized by monitoring, using genetic markers from hatchery populations.

Consensus points:

- Stocking programs will be important for jump starting biogenic potential of newly constructed or depopulated reefs in some areas.
- The diseases MSX and Dermo are a major limitation for development of large, highly fecund spawning stocks throughout most of the Bay, especially the southern, high and moderate salinity areas.
- Selectively bred disease-resistant strains may have widespread potential for "*genetic rehabilitation*" of southern, highly disease-impacted oyster populations.
- Continued development and use of selected disease resistant stocks is warranted for the purposes of restoration (as well as for use in oyster aquaculture).
- It is appropriate to zone restoration/rehabilitation efforts according to ecological parameters, primarily salinity and disease prevalence.
- Effective population size of wild populations is an essential parameter to predict genetic effects, but is unknown.

Implications for hatchery operation stemming from genetic considerations:

- Use of hatchery stocks for restoration and replenishment will entail careful selection of brood stocks chosen for specific applications, genetic characters (diversity, disease resistance, etc.), or both.
- High effective population size in the hatchery needs to be maintained through carefully controlled spawning procedures.
- Amplification of brood stocks for spawning is critical to hatchery operations, and therefore, advance anticipation of the numbers and types of stocks is essential.
- Levels of hatchery production today are generally an order of magnitude or two too low to effectively provide the stocking power needed for restoration/replenishment.

Descriptive Summary

Conclusions from background presentations:

There are two primary objectives to hatchery supplementation, and these are not mutually exclusive— restoration of functional oyster reefs for their ecological value and restoration of the traditional oyster fishery. Hatchery supplementation to restore functional oyster reefs (as in Virginia) requires that reefs themselves receive sanctuary status to enable recruitment to the surrounding areas for development of a fishery. Supplementation for replenishment (i.e., on public oyster grounds as in Maryland) is "put-and-take." Nonetheless, hatchery supplementation for either purpose engenders long-term genetic consequences because hatchery stocks have significant spawning potential before death by harvest or disease.

Maryland and Virginia have different problems defined primarily by salinity and responses of oyster populations to Dermo- and MSX-disease during high salinity intrusions. Maryland, by and large, has less need for disease resistant stocks than Virginia does, although in years of high salinity, diseases can cause major mortality. Most Virginia waters are constantly subject to one or both diseases.

Areas of the Bay differ dramatically in whether they can retain a self-sustaining population through auto-recruitment. Some areas have documented auto-recruitment (can seed themselves), others are thought to be reasonably well identified, but for most, it is unclear whether they are open or closed and whether they depend on auto- or allo-recruitment.

For genetic purposes, the Bay should be divided (zoned) based on salinity characteristics. Further areas of special interest—especially areas that retain larvae and are auto-recruiting—should be identified and set aside as preserves.

Any restoration effort, for whatever purpose, depends on the availability of suitable habitat. For replenishment, bottom must be prepared. For reef restoration, reefs must be constructed or rehabilitated. If spawning and recruitment are expected, surrounding areas must be prepared accordingly. Therefore, habitat restoration is an essential element of any and all possible programs.

Recurrent stocking on newly formed or depopulated reefs may be necessary to get the system started. The hatchery capability for this scale of effort is generally lacking, especially in Virginia.

Recovery strategies:

There seem to be a number of ways to look at oyster stocking approaches militated by the salinity considerations in the Bay and the general divergent philosophies in Maryland and Virginia about hatchery supplementation.

Hatchery production using wild brood stock for replenishment

Focus: Maintain genetic diversity

Presently, wild brood stocks from various sources are being used for replenishment efforts in low salinity areas in Maryland. There was considerable concern about the nature of this activity because of underlying genetic concerns. In short, these concerns center on reducing the genetic variability of wild stocks by swamping them with alleles from hatchery stocks with reduced genetic variation. To quantify the risk of "genetic pollution" by hatchery stocks, an estimate of the numbers of wild parents contributing to the overall oyster population is needed, in both the hatchery and in the wild. At present, this is unknown. Our recommendations are couched with the caveat that these are interim recommendations for Maryland stocking programs and for a more precise strategy, an estimate of numbers of wild breeders is required.

***Recommendations*:**

1. Oysters stocked into replenishment areas, despite the fact that they are destined for harvest, must be considered a *in situ* brood stock source.
2. The effective population size (a calculation of the genetic contribution that breeding parents are making to the next generation) of spawns produced in the hatchery must be kept as high as possible.
3. Effective population size in the hatchery can be maintained by using as many spawners as possible in equal ratio of male to female, and endeavoring to keep family sizes equal.
4. Brood stock should not be obtained from sites where replenishment is underway. That is, brood stocks should come from sites and systems outside the recruitment shadow of stocked areas. Using alternative stocks will prevent matings among related individuals and prevent inadvertent fixation of alleles in the population.
5. Replenishment areas should be stocked by progeny from parents of multiple stocks. A set of 3-6 stocks deriving from various locations might be considered as sources of brood stock for the hatchery. Each year, a different brood stock could be spawned with the seed destined for a specific replenishment area. Stocks would be rotated every year. Use of multiple stocks will help prevent loss of rare alleles and help maintain overall genetic diversity in the replenishment area.
6. This strategy is more appropriate to areas with low disease pressure.

Issues in need of clarification:

1. Effective population size in wild oyster populations figures prominently in determining the effect of hatchery supplementation on wild populations and this is unknown at present.
2. What is the range of genetic variability in so-called natural populations of oysters and does it differ among locales within the Bay?
3. What levels of effective population size are practicable in the hatchery?
4. Is there a correspondence between the estimated effective population size calculated from the number of parents used and the actual effective population size of the spat after larval rearing and setting? In other words, does differential survival among families significantly effect effective population by the time the oysters are ready for planting?

Wild set enhancement for replenishment or restoration

Focus: Maintain genetic diversity

Another strategy for stocking oyster reefs or replenishment areas is the use of natural set from the wild. This strategy is essentially equivalent to what commercial oystermen do when they gather seed and distribute it to their oyster grounds for grow out. A variation on this theme could include the intentional collection of spat on artificial collectors, followed by a period of cultivation before moving them to a designated reef area. In this way, recruitment on "restored habitats" could be jump started with populations that are genetically wild and unperturbed from their natural state. Technically, wild set enhancement is not complicated, however it would require significant expansion of current efforts and perhaps development of some new bulk handling techniques for large scale replenishment or restoration. Wild set enhancement is more appropriate for areas with low levels of disease pressure since wild oysters likely will succumb where diseases are prevalent.

Recommendations:

1. A cost-benefit analysis of this strategy versus hatchery supplementation is needed. If it turns out that hatcheries are more expensive per spat than large-scale collection and movement of wild seed, then serious consideration should be given to expanding *wild set enhancement*. Cost-benefit must include an evaluation of the likelihood of obtaining wild set in predictable fashion.
2. Careful analysis of where *wild set enhancement* would be most useful, vis a vis salinity and disease prevalence regimes, is required.
3. Genetically speaking, wild set enhancement is the more conservative approach and obviates the problems associated with effective population size in the hatchery.

Issues in need of clarification:

1. Are there portions of the Bay—specifically in areas where *wild set enhancement* is warranted—that have predictable natural sets that would allow spat collection?
2. Is technology for catching, handling, and nursery care scalable to levels needed for *wild set enhancement*?

Hatchery production using wild brood stock for restoration

Focus: Ineffectiveness of wild stocks

There seems little value in restoration programs in the Chesapeake Bay based primarily on hatchery production from wild brood stock. In Virginia, longevity of oysters becomes a major issue. Stocking seed produced in the hatchery has the double whammy of limiting genetic variability and producing disease-susceptible oysters. Disease susceptible spat will yield adults with lower fecundity, countering the objective of self-sustaining, reef based brood stock. The use of wild set may be more cost effective and avoids potential genetic pitfalls.

***Recommendations*:**

1. Unless proven of value for their longevity in the face of disease pressures (i.e., naturally disease-resistant populations), wild brood stock likely will be of limited use for restoration in disease prone areas of the Bay.
2. From a genetic perspective, recurrent stocking of seed derived from wild brood stock is identical to "Hatchery production using wild brood stock for replenishment" above. If wild stocks are used for restoration, stocks from various origins should be rotated.

***Issues in need of clarification*:**

1. Effective population size in wild oyster populations figures prominently in determining the effect of hatchery supplementation on wild oysters and this is unknown at present.
2. Are there "naturally disease resistant" stocks in the wild?

Disease-resistant, hatchery based enhancement (*genetic rehabilitation*) for restoration

The most creative discussion in the workshop arose from our discussions of the issues surrounding *genetic rehabilitation*. That is, the wild oyster populations, having suffered a number of insults over the last 50 years especially, were deemed in need of rehabilitation through the use of disease resistant strains. A good deal of progress has been made in developing disease resistant strains and they are generally available for aquaculture. Now it seems they may play a significant role in some parts of the Bay, particularly where disease pressures are persistent.

For *genetic rehabilitation*, the value of programs relying heavily on hatcheries is to amplify specific stocks with disease resistance. The advantage of using disease resistant stocks is that they potentially forward the goals of restoration by enabling functional oyster reefs as well as the traditional fishery. Disease resistant hatchery stocks would promote ecological restoration somewhat by enabling oysters to live longer, re-establishing overlapping year classes of adults; fisheries restoration would be served because oysters would be longer lived for harvesting and provide spat for continuing recruitment to designated fishing zones.

In most systems but the most depopulated, stocking disease resistant seed will eventually hybridize (interbreed) with wild populations. The desired outcome of hybridization is introgression of disease resistant alleles into the natural population. Introgression will have a positive benefit if it contributes to the welfare (fitness) in subsequent generations of oysters. This benefit is most likely in the character of disease resistance, giving rise to increased longevity and higher fecundity.

The brightest scenario is that the level of introgression and gene flow from disease-resistant stocks to subsequent generations could be controlled. In reality, several obstacles prevent a precise application of an introgression (or *genetic rehabilitation*) strategy. A specific model for controlling introgression of favorable traits into a wild population, regardless of species, is lacking. There is inherent unpredictability to the population dynamics of oyster recruitment, e.g., proximity of adults for spawning, synchronicity of spawning, larval distribution, variance in reproductive success. At present, there are limitations to the extent to which disease-resistant strains can be amplified through hatchery propagation, i.e., magnified from a few hundred brood stock to a few hundred million spat.

Despite the limited understanding of the parameters of the *genetic rehabilitation* strategy, there was a sense of congruence in the workshop that the so-called wild oyster—especially where diseases were prevalent—was in a downward spiral and that trial implementation of the *genetic rehabilitation* strategy was warranted.

***Recommendations*:**
1. Disease resistant stocks should be stocked in closed/retentive systems where autorecruitment rates are expected to be high, establishing a "disease resistant stock preserve." Autorecruitment will a) enable the magnification of the stocks through natural recruitment and b) allow monitoring of the system to help parameterize the *genetic rehabilitation* strategy.
2. Progeny from the "disease resistant stock preserve" could then be used as part of a larger secondary stocking program by collecting spat and relocating them to other newly developed reef systems.
3. Genetic markers of disease resistant alleles should be developed to monitor differential rates of introgression within and among "disease resistant stock preserves" and in secondary stocking programs.

***Issues in need of clarification*:**
1. Effective population size in wild oyster populations figures prominently in setting parameters for the controlled introgression (*genetic rehabilitation*) strategy and this is unknown at present.
2. Theories of gene flow are abundant, but specific models that apply to oyster population dynamics are lacking.
3. Technologies for stocking of secondary reefs (i.e., from seed derived from "disease resistant stock preserves") presumably would be identical to that used for the *wild set enhancement* strategy, both undeveloped at present.
4. Molecular markers have been developed and will provide the foundation of tracking larval dispersal. But linkage to disease resistant genes has not been examined and more marker development is needed to find those that mark disease resistant genes specifically.

Workshop Participants

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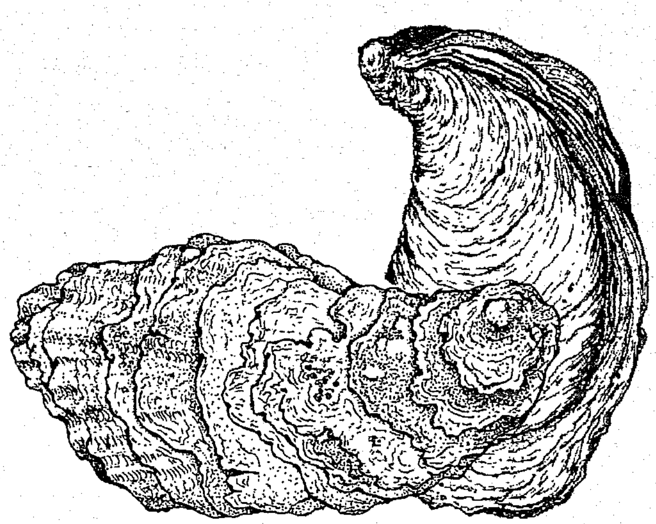

Glossary

Allele (allelic) – one of a number of alternative forms of a given gene. Each allele may affect the function of that gene and therefore the function of the organism.

Allo-recruitment – Recruitment is the addition of new members to a population under consideration. Allo-recruitment refers to the addition of new members from spawning individuals other than from the population under consideration.

Auto-recruitment – the addition of new members from spawning population under consideration. Auto-recruitment would occur if oysters on reefs provided progeny that would settle on the source reef, or nearby.

Biogenic (potential) – Biogenesis is the production of a living cell from another living cell. In the context of oyster restoration, biogenic potential refers to the ability of a reef system to produce other oysters in and around the reef.

Effective population size – the average number of individuals in a population that contribute genes to succeeding generations. Effective population size will always be smaller than the total number in a population because not all individuals will contribute progeny. In oysters, effective population size is believed to vary widely because of the wide variety of environmental conditions that determine recruitment success and because of the huge fecundity in some individuals.

Family (family size) – a set of parents together with their children. Family sizes can vary widely in oysters because each male x female pairing can derive millions of children. Successful families, for whatever reason, can greatly outnumber unsuccessful ones.

Fecundity – potential fertility. Specifically, the term refers to the quantity of gametes, generally eggs, produced per individual over some time period.

Gene flow – the exchanges of genes between different populations of the same species. Gene flow occurs from migration of individuals from one population to the next and can change the frequencies at which genes are found in the recipient population. Gene flow among oyster populations used to maintain homogeneity among all oysters in the Bay. Now, gene flow may be severely restricted from lack of contiguous reef structure.

Genetic markers – a gene used to identify an individual that carries it, or as a probe to mark a chromosome or gene location. Genetic markers take various forms and can be used to monitor the migration of individuals – in oysters, this would be the larvae – from one place to another, such as from a reef to surrounding substrate.

Genetic variability – the heterogeneity of alleles in a population. At any one gene, many alleles are possible. Across many genes, overall variability can be characterized as an attribute of the population. It is widely agreed that such genetic variability (allelic diversity) is beneficial by enabling organisms to adapt to a range of environmental conditions.

Introgression – the incorporation of genes from one population into the gene pool of another. The first step in introgression is the formation of hybrids between the two populations. Afterward, hybrids tend to breed subsequently with the more abundant population. This process results in a population of individuals that look mostly like the abundant parent but who also possess some characters of the other population.

Recruitment shadow – that area around the primary spawning population where progeny may appear. The recruitment shadow of oyster reefs is dictated by the length of time larvae spend in the water column as well as their incipient potential for dispersal from water currents, tides, etc.

Replenishment – the intentional stocking of designated areas for subsequent harvesting by the fishery. Replenishment is widely practiced in Maryland where hatchery seed is distributed into designated areas.

Restoration – in its literal sense, restoration would imply bringing something back to its original state. With oysters, restoration in the literal sense is likely not possible. Most of the activities now are associated with rehabilitation of areas that will promote oyster biogenic activity. These activities include replenishment, reef rebuilding, adding substrate to sedimented areas, and creation of sanctuaries.

Stock (of oyster) – the natural genetic unit of a population determined by its isolation from other populations. Stocks of oysters vary over a fairly large geographic scale. It is unlikely that there is any genetic difference among populations of oysters within the Chesapeake Bay, although things may have changed with the decline of stocks and reduction of gene flow in the last 50 years. Stocks are a evolutionarily determined entity.

Strain (of oyster) – an artificial genetic unit of a population determined by the breeding structure of how these individuals were propagated. Strains obtain by closing the life cycle so that progeny grow up to become parents of the next generation. Generally, new material is not allowed in and strains become more genetically distinct and less genetically variable. Artificial selection may be applied to accelerate the genetic differentiation of a strain, and this is how disease resistant stocks are produced.

Wild (oyster) – a naturally occurring oyster that is unchanged from its natural (normal) genetic state. Oysters in the Bay are more or less wild, although several events have conspired to change its natural state: movement of oysters within the Bay and from outside the Bay, selection pressures brought on by diseases, and selection pressures brought on by fishing pressure, all of which are in some way the result of human influence.