Chapter 18

Culture: Genetic Improvement

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INTRODUCTION

Genetically based breeding programs have made an enormous contribution to increases in agricultural yield during this century. Estimates suggest that at least 30% of the increase in the rate and efficiency of land-based protein production since 1900 is the result of genetic improvement (USDA 1988). This same improvement of production is possible with any species of aquatic plant or animal provided the life cycle can be controlled. Though there are large differences in the ecology, physiology, and life history characteristics between the eastern oyster, *Crassostrea virginica,* and other domestic animals that have implications for the design of breeding programs, the eastern oyster is one of the species that has a great potential for genetic improvement. In this chapter I will examine the status and potential of genetic improvement in *C. virginica.*

I use the word "breeding" here to refer to attempts to make changes in the genetic composition of an oyster stock with the objective to improve production. Genetic changes can be the result of *selection* that saves certain genotypes and removes others; *hybridization* that introduces new genes or gene combinations (or changes genotype frequencies); *inbreeding* that reduces genetic variation and increases homozygosity; *chromosome manipulation* that changes the number of chromosomes; or newly developed *biotechnological approaches* that can introduce exotic genes with specific effects.

In *selection,* the individuals that have superior performance are bred and, to the extent that this better performance is a result of genetic differences among the individuals, there will be a genetic change in the stock. Selection has been the primary mechanism for making improvements in cultivated species since humans first domesticated plants and animals. Oysters have not been propagated in hatcheries on a consistent basis so there has been little scope for the kind of selection of livestock done by landbased farmers for many generations. In a long-term selection program, the genetic changes in the population are achieved by changing the frequencies of genes and, in the process, allowing new genotypes to be formed by increasing the probability that better gene combinations will occur. The expectation is that there will be continuous and cumulative change over time.

Hybridization is the mating of individuals from different sources (or those that are known to be different genotypes) to produce a new genotype or to increase in frequency an existing genotype. The improved performance may be the result of the combination of favorable genes from the different sources or due to the "hybrid vigor" that may result from the non-additive interaction of the genes, i.e., the combined effect deviates from the expectation of the linear combination of the separate effects.

Inbreeding is the mating of individuals that are more closely related than random individuals in the population. This definition must be understood in

relative terms: inbreeding is relative to the "population," which must be defined. It may be the local oysters in a bay or the species as a whole. In stock improvement programs, inbreeding is usually defined in terms of a common ancestor (parent, grandparent, etc.). The outcome of inbreeding is an increase in the level of homozygosity and, in small populations, a loss of genetic variation. In a breeding program, inbreeding may be used to increase homozygosity, thus making the animals more genetically uniform.

Chromosome manipulations can be made by intervening during the fertilization of eggs to change the normal diploid composition of the zygote. This intervention may result in a change in the number of chromosomes or in the pattern of transmission (see Longwell and Stiles, Chapter 12). The result can be an increase in chromosome numbers (polyploidy) which may result in increased performance due to the presence of additional copies of genes or changes in the level of heterozygosity.

Rapid advances are being made in *biotechnology* that will have many applications to oyster culture in the future. New techniques have been developed that allow the identification and manufacture of natural products such as hormones that can be used in hatcheries. Other methods will lead to the introduction of foreign genetic material into oysters to produce transgenic animals.

Most of the traits that are of concern in oyster culture are likely to be influenced by many genes. The traits themselves are usually measured on a continuous scale so there are no discrete categories. Such traits are called quantitative traits and their genetic study is called quantitative genetics. Though single genes may have large effects on important traits, the focus on quantitative traits in this review is chosen for two reasons. First, in animal breeding most of the progress has been made through the assumption that traits are quantitative. Second, there is little room for the expression of single genes in the morphology of oysters because they do not have fins, color patterns, or other morphological features that are easily categorized into distinct classes. Thus, superior individuals are judged to be so based on traits that have a continuous distribution. These are best treated as quantitative traits.

BREEDING PROGRAMS

Among the first steps in any genetic improvement program are: setting the breeding goals, determining the mating scheme, and choosing the stock (Shultz 1986). The trait or traits that are to be improved must be clearly defined. Mahon (1983) carried out a survey of researchers and oyster producers and found that growth and survival (including disease resistance) were considered most important in an oyster breeding program. The major obstacle for work with oysters is a limitation on the number of traits that can be easily measured on live animals. Some important traits such as meat yield can only be measured by sacrificing potential breeders. In contrast, whole-body weight is a trait that can be measured on individuals. The question then is whether increasing whole-body weight will also increase meat yield. That is, how high is the correlation between meat and whole-body weight? It would be of little value to increase shell weight! Such questions can be addressed with a morphometric analysis. Although a phenotypic analysis will not guarantee that the same relations pertain on a genetic level (i.e., that genes that produce differences in whole-body weight do so by producing more meat), it is a first approximation. Another trait that is impossible to measure on an individual is the probability of survival. It can be measured on groups of animals such as families and this information included in the selection program.

Correlations of traits can be either an advantage or a problem in genetic improvement programs. If two traits are positively correlated such that desired levels of one trait, say growth rate, are positively associated with desired levels of another, perhaps disease resistance, then improvement in one, will indirectly result in improvement in the other, but only if the traits are correlated on a genetic level (i.e., if they are determined by the same genes). For traits of concern in oyster breeding, these associations may be favorable, as might be the case with growth rate and resistance to disease, but much more information is needed. One problem is that if there is a negative correlation, improvement in one trait may result in a decrease in another commercially important trait.

There are potential problems with bivalves in the improvement of meat yield because the meat-to-shell ratio varies seasonally (e.g., Hilbish 1986). However, at any one time there is usually a high correlation (r = 0.9 for the European flat oyster, *Ostrea edulis*) between whole weight and meat weight (Newkirk, unpubl. data). Thus, improvement of whole live weight will probably achieve the desired goal of improving meat production. This can be accomplished by using growth rate (measured as size at age).

The mating schemes possible with *C. virginica* are numerous. Probably the most commonly used method is mass spawning with pooled matings made possible by the external release of eggs and sperm. Particularly when small numbers of individuals are involved, care is needed in the interpretation of results of genetic experiments using mass spawning. The relative contribution of the parents to the final pool of offspring from a pooled mating may be less than that presumed because of differential gamete contributions, uneven fertilizations resulting from differences in fertility or the timing of gamete contributions, or differences in egg viability. Genetic markers using electrophoresis as proposed by Gaffney (1989) may be used to sort out parentage at a later date and DNA fingerprinting may also be very effective (Harris et al. 1991). However, both of these methods require time and access to the appropriate laboratories.

In contrast to pooled matings are those that maintain some pedigree information. The information can vary from detailed records of individual sires and dams for every offspring to the identification of groups of offspring where individual parentage is not known but the groups are known to be offspring of separate parental groups such that inbreeding can be controlled.

There are only a few reports of experimental work with full-sib families (one male and one female parent) of *C. virginica,* and there is no evidence that family identity was maintained beyond the one generation of the experiment (Newkirk et al. 1977; Losee 1978; Mallet and Haley 1983a). Families of the Pacific oyster, *Crassostrea gigas,* have been maintained for several generations at the University of Washington in a selection program for resistance to summer mortality (Hershberger et al. 1984) where the oysters have been selected on the basis of the mortality exhibited by the family. In selection for growth rate in *O. edulis,* Newkirk (1986) kept families for two generations.

The kind of families that can be produced cover a broad range. The simplest is the full-sib family with one male mated to one female. However, because of the flexibility of *C. virginica* other variations are easily produced. Half-sib families with two or more females mated to the same male are possible. Factorial matings where every male is mated to every female are also possible. These variations are of value in experimental work to produce estimates of genetic factors needed in designing breeding programs.

Lack of synchrony in spawning of oysters can cause problems with some experimental designs. In agronomy, an experiment can begin at a specific time because seeds can be planted nearly simultaneously. With species such as *C. virginica* in which spawning can be induced (Gibbons and Castagna 1984), there is some control over spawning but it is almost impossible to obtain specific crosses on specific days. When spawnings are separated by only a few days, there may not be much difference as a result of the differences in timing because setting time will ultimately vary. However, as the difference in setting time increases either due to different spawning times or for other reasons, the comparisons of the groups becomes questionable. Thus, some control over time differences is needed in the design; this can be accomplished with replicates at different times.

A genetic improvement program should start with the best stock available. Although it has not been shown in detail by population genetic studies (Buroker 1983), there are indications that there are physiological races or clines in *C. virginica.* Barber et al. (1991) indicate that oyster stocks originally collected from outside Delaware Bay, but held in the bay, maintain spawning periods consistent with those in the region from which they came, (i.e., northern stocks spawn earlier in the season than southern stocks). Such differences probably exist for a number of traits. Existing genetic differences in such physiological traits should be exploited before effort is put into making similar genetic changes through a breeding program.

One of the first steps in a breeding program should be to evaluate differences in traits of concern

in the potential stock sources and choose starting broodstock from the natural population with the best performance. It may not necessarily be true that the best stock is the local population, and it may be that what is needed is a combination of traits from different populations. Mallet and Haley (1983b) tested pooled matings from three populations and their crosses in two locations. There were significant differences between the within population crosses but the rank differed at the two locations, suggesting that stock performance may be site specific.

APPROACHES TO BREEDING

Here I review the approaches to breeding that have proved useful in agriculture with respect to application to genetic improvement of *C. virginica.* The experience with *C. virginica* and other bivalves will be assessed. The first topic will be inbreeding because it has implications for other approaches.

Inbreeding

Of major concern to a geneticist is the control of inbreeding, which can produce deleterious effects (Longwell and Stiles 1973; Kincaid 1983). The term inbreeding has been used loosely in the oyster literature and although often not used incorrectly at least it is often used misleadingly. By definition, inbreeding is the crossing of individuals of close relationship. This can be the mating of blood relations like sisters and brothers or matings within a small isolated population where the relationship among the individuals is closer than among individuals taken from different populations. In the latter sense, a selected line becomes "inbred" as it is propagated from a small number of oysters, but the average mating may not be of close relatives.

The consequences of inbreeding are two-fold: an increase in homozygosity and a decrease in genetic variation. These consequences go hand in hand in a small population but effects are different. Increased homozygosity can have physiological effects as deleterious recessive genes are expressed and fitness is decreased. This is known as inbreeding depression and its effect on offspring increases with the closeness of relation of the parents. There are only a few reports of inbreeding depression in oysters (Longwell and Stiles 1973; Mallet and Haley 1983a). Haskin and Ford (1987) report no inbreeding depression with respect to mortality, but their oysters were simultaneously selected for increased survival so the effect of inbreeding cannot be separated from the effect of selection. The limited number of published observations make it difficult to assess the magnitude of inbreeding depression in oysters or to generalize. There is a need for more evaluation of inbreeding depression in oysters.

The consequence of reduced genetic variation is the long-term loss of genes that may be useful in genetic improvement. In the relatively small populations that can be maintained in a breeding program, genetic variation must be conserved because once there is no more genetic variation, no more genetic change can be made. Inbreeding in small populations is a function of the number of parents that contribute genes to the next generation and their proportional contribution. If a few individuals contribute a very high proportion of the genes while many individuals contribute a very low proportion, the next generation will be made up of offspring of, effectively, very few parents. In such a stock, inbreeding will increase much faster than in a population where the same number of parents contribute equally. In pooled matings of *C. virginica,* it is very likely that there will be unequal contributions of all parents due to differences in the frequency of the gametes from the different parents, fertility, and survival (Gaffney 1989). If inbreeding is to be kept to a minimum in a randomly breeding population, the rule of thumb is to maintain 30 to 50 pairs spawning each generation. There is no critical number; it is a question of degree of inbreeding that is acceptable.

In describing oysters as "inbred," it should be made clear whether this is a result of random mating in a small population or a result of known or intentional mating of close relatives. If it is a result of small population size, the size of the population should be given. If the inbreeding was intentional through mating of close relatives, the kinds of matings should be described. The degree of inbreeding is important and, if not known precisely, it can be approximated with information on the history of the stock as done by Haskin and Ford (1987).

Inbreeding as a breeding tool increases the frequency of genes in the stock. Without selection the genes that are increased will be a random sample. Even with selection for specific traits, favorable genes can be lost when they do not have a large influence on the variance of the trait. This loss of genes can have a long-term effect in reducing potential gains. A combination of inbreeding and selection may be used to increase the frequency of selected genes, but this may be of short-term benefit because the genes that contribute marginally to the trait being selected may be lost through inbreeding.

The use of inbreeding to improve livestock in agriculture has been limited. Where successful, it has been the result of extensive breeding programs with domesticated and pedigreed stocks using multiple inbred lines. Such stocks are not available with *C. virginica*, and their development would require considerable time and expense.

There has been reference to "inbred" oysters in the literature (Haskin and Ford 1987; Paynter and DiMichele 1990). These authors are not specific whether inbreeding is meant in the sense of the mating of close relatives or breeding in a small population, but in these reports it seems to be the latter. The number of parents that actually produced offspring (as opposed to the number spawned) in each generation has not been reported for the oysters in Delaware Bay (Haskin and Ford 1987) or the Chesapeake Bay (Paynter and DiMichele 1990), so it is difficult to estimate the level of inbreeding and determine whether deleterious effects of close inbreeding are to be expected.

Research on Delaware Bay populations has been underway since the early 1960s after disease decimated the natural stocks. The propagation of selected lines has been by mass spawnings, usually with 4 to 10 individuals per sex per spawning. These oysters have been referred to as "inbred" lines by Haskin and Ford (1987) but at other times the authors speak of "inline" breeding. In Vrijenhoek and Ford (1988) the oysters are referred to as "strains in their fifth and sixth generation of inbreeding." Vrijenhoek et al. (1990) report conservative estimates of the effective population size for five strains of selected oysters at the Rutgers laboratory. The estimates range from 4.1 to 16.2, with 4 out of 5 estimates less than 10. Since these estimates are based on the numbers of oysters spawned, they are likely to be considerably higher than if they could be adjusted for the differential contribution of the parents. In spite of the apparently low and variable numbers of parents used, Vrijenhoek et al. (1990) report no decrease in heterozygosity of electrophoretically detectable loci compared to wild oysters, whereas a decrease would be expected after five to six generations of close inbreeding.

Inbreeding and pedigree of oysters can be controlled because it is possible to keep individual records of oysters throughout their post-metamorphic life. A tag can be attached to either the substrate on which oysters are attached or, when they are larger, the oyster itself (Mallet and Haley 1984 for *C. virginica* and Newkirk and Haley 1982a, 1983 for *O. edulis*). Identity of *C. gigas* families has been kept by setting larvae on shell cultch (Beattie et al. 1980). Keeping individual records can be tedious but will have great advantage in breeding programs. Certainly it will be an advantage in genetic studies to understand the quantitative genetics of the traits to be selected.

The use of lines to control inbreeding is easily accomplished with *C. virginica*. These lines may be propagated by pooled matings, but the males and females will come from different lines; thus the offspring cannot be full sibs. If the maintenance is done by a rotational scheme as described by Kincaid (1977) for fish and Hershberger et al. (1984) for oysters (see p. 666), the level of inbreeding can be kept low without the time-consuming effort of keeping family identity.

Alternative Selection Approaches

There are a few basic selection procedures that can be used in oyster breeding: mass (or individual) selection, between-family selection, and within-family selection. There can also be a combination of between- and within-family selection called combined selection. The differences among these methods are in the emphasis placed on the mean value of the trait in the family (maximum in between-family selection), the deviation of an individual's value of the trait from its family mean (maximum in within-family selection), or the measure of the trait in the individual relative to the population mean (maximum in individ-

ual selection), which gives equal weight to family mean and the within-family deviation. In combined selection, weights are given to both the family mean and the deviations from the family means; this makes optimal use of the two sources of information.

High phenotypic variance seems to be a feature of aquatic organisms in general (Gjedrem 1975, 1983). This has some advantage in a selection program because genetic improvement through selection is, among other things, proportional to the intensity of selection, which is a measure of how much the selected individuals or families deviate from the population mean. With a higher variance of the trait, the intensity of selection can be higher because there are more individuals farther away from the mean value.

Individual or mass selection in oysters is the selection of individuals from the whole population, disregarding any family relationship that may be known. In calculating the individual values, there may be a statistical adjustment for time of spawning or known environmental differences, such as placement of oysters in different holding trays (Newkirk and Haley 1983). The mating of selected individuals could be done randomly with no pedigrees maintained, but this requires careful consideration of the number of reproducing adults in each generation. As mentioned, inbreeding caused by maintaining small population size should be avoided.

When using individual selection for oysters, it would probably be best to structure the stock in separate spawning groups that may be maintained as lines. As mentioned above, Hershberger et al. (1984) suggest a scheme for maintaining oysters in lines. Actually the "line" identity is not maintained completely between generations because matings are made between lines from each generation (males from line A fertilize eggs from line B, males from B fertilize eggs from C, etc.) With such control, inbreeding will be reduced compared to random mating of the selected oysters.

If the synchrony of spawning is ignored in a selection program, higher levels of inbreeding may occur than is anticipated. If, at the time of selection, all the oysters produced in one season are considered for selection by size, the largest oysters may be mostly from the group first spawned, i.e., the oldest oysters (Newkirk 1978a). These older oysters will be only a subsample of the parents used throughout the whole season.

For between-family selection, the performance of different families (usually full-sib families) is ranked and the best families are retained. Randomly chosen individuals from each of the selected families are taken as parents. The number of families has to be very large to allow for very strong selection. With families identified, pedigrees are maintained and inbreeding can be minimized by planned matings. This design has the serious limitations of usually having a much lower selection intensity than alternative designs because to have even 10 breeding pairs when selecting 10% of the families, 100 families must be produced which is a large effort compared to producing a few mass spawned lines with 10 pairs in each.

When raising families, the offspring from one family will be raised together (and separate from other families) for a period during which environmental differences between larval tanks or culture trays may cause differences in the family means. This factor will be in addition to the genetic differences and the random sources of variation which affect all families equally. These environmental differences common to members of one family are difficult to separate statistically without sufficient replication, which is costly and time-consuming. If the environmental differences are great, selection will be inefficient (Falconer 1981).

One source of differences between families is the non-genetic influence of a dam on her offspring, known as the maternal effect. Larval oyster growth and survival is clearly influenced by the physiological condition of the mother (Newkirk et al. 1977; Losee 1978; Lannan et al. 1980; Mallet and Haley 1984; Muranaka and Lannan 1984). However, the maternal influence in *O. edulis*, though continuing into the juvenile stage, has been shown to have a very small effect by the time the oysters have grown to market size (Newkirk and Haley 1982b). This may not always be the case because even though the effects on ultimate size may be small, the effects on early stages may be continued and magnified by competition during grow-out. Certainly the effects on mortality have a permanent influence.

Within-family selection also requires known families, but in this case the best individuals from every family are selected. With species like oysters, high fecundity means that the same strong selection used in individual selection can be used within each family. With pedigree maintained, matings can be made with a relatively small number of families in a way that inbreeding is kept low. Thus, fewer families need be maintained than when between-family selection is used. Because selection is done within the family, any differences between families due to environmental differences or synchrony of spawning are not confounded with the differences between individuals. Within-family selection has been used with *O. edulis* (Jarayabhand and Newkirk, unpubl. data) where the control of spawning is difficult (Newkirk 1986).

Combining between- and within-family selection makes use of the advantages of both. Some families are selected and then the best individuals within those families are selected. Efficient use of this design depends on knowledge of the genetic and environmental sources of variance and when this information is available, combined selection is more efficient than any other method because it makes maximal use of the genetic variation in the population (Falconer 1981). For oysters, combined selection suffers from the same problem as family selection does under most circumstances: a large number of known families must be maintained.

If a large breeding program can be initiated for *C. virginica*, combined selection would probably be the method of choice. For a small hatchery, particularly a commercial hatchery, another method will be needed. Within-family selection has been proposed for small-scale aquaculture by Uraiwan and Doyle (1986) because it provides control on inbreeding for small numbers of broodstock and yet still provides a means to make genetic improvement through selection. As a result of the way genetic variation is partitioned within and between families, within-family selection can only act on half the genetic variation and thus, with all else being equal, it is not as efficient as combined selection and may also be less efficient than individual selection. However, "all else" is never equal! The better control of inbreeding by within-family selection in small broodstocks compared to between-family selection or combined selection compensates for loss of efficiency in selection. This compensation cannot be quantified because the benefit is in maintaining genetic variation for the long term and the value of this benefit cannot be estimated. Furthermore, if only a small number of families can be maintained, the loss in efficiency of selection compared to combined selection will be reduced because the contribution of between-family selection in the combined selection scheme will be small.

Modifications to within-family selection are possible which will make the technique more suitable for the small, commercial hatchery. In the above discussion, "families" have been defined as full-sib families, with one male and one female parent. The same approach as used in within-family selection for selecting the best individual can be applied to groups of oysters produced by a small number of parents. These would be, in effect, mixtures of a few families if the eggs and sperm of a few males and females were pooled. They could be considered as lines as the term is used by Hershberger et al. (1984). The advantages of within-family selection would apply: inbreeding would be controlled and selection would be on a group of individuals (within each line) that had been kept together from spawning in the same way as a family would be maintained. The number of offspring could be very large (thousands) and selection could be very intense.

Experimental Selection of Bivalves

Haskin and Ford (see Haskin and Ford 1987 for a review) have selected *C. virginica* for resistance to MSX disease in Delaware Bay and found that selected strains have survival rates up to nine times those of unselected stocks. Selection was performed for five generations (Haskin and Ford 1987; Vrijenhoek and Ford 1988). This result is a very clear demonstration of what can be done with oysters. Selection for disease resistance in agriculture livestock usually results in slow progress because of low genetic variation for resistance. Thus, the rapid response of *C. virginica* for MSX resistance is very exciting.

Haskin and Ford (1987) suggested that inbreeding has had no detrimental effect on survival of the select-

ed strains. Unfortunately, there is no "control." These oysters have been selected for improved survival, but inbreeding has most likely been increasing. Even if inbreeding decreases survival, as expected from other work and basic genetic theory, there is no way to estimate this effect without at least comparisons with lines that are similarly selected but with no inbreeding. Haskin and Ford (1987) show data on estimates of inbreeding coefficient in relation to mortality, but the inbreeding coefficient is highly correlated with the selection intensity because the more advanced generations (more inbred) are more selected.

The selected Delaware Bay oysters grew more slowly in Maine (in MSX-free waters) than non-selected oysters from Long Island Sound over an 18 month period (Hawes et al. 1990). In another study, oysters derived from the Delaware Bay-selected oysters and maintained at a commercial hatchery on Long Island were compared to an MSX-susceptible stock from Long Island in growth trials in Massachusetts (Mathiessen et al. 1990). In this case the Delaware oysters grew faster than the Long Island oysters and the resistance to MSX was confirmed. (Unfortunately, it is impossible to determine the exact history of the oysters used in these two studies.)

Paynter and DiMichele (1990) have shown that a stock of oysters "selected for over 18 generations by a local oyster grower" have higher growth rates than oysters from the wild stock from which the hatchery stock was taken. The growth rate of the selected oysters at the end of the first growing season was 28% higher than the wild stock and 24% higher during the second growing season. The sizes were not reported, but from the graph I estimated that after two growing seasons the selected oysters were 97 mm and the wild stock 75 mm long. Details of the selection methods used in the commercial hatchery were not reported by Paynter and DiMichele (1990), but it does seem that the performance of the hatchery stock is much better than the wild stock. However, the results reported are only indicative because the number of parents used for each group was very small (two males and three females) and, as the authors point out, a larger sample is needed to make definitive conclusions.

Family selection has improved resistance to summer mortality in *C. gigas* (Hershberger et al. 1984). Cumulative mortality after three generations of selection was around 20% whereas the wild control had a cumulative mortality of 62%. These results are highly indicative of genetic gain, but a measure of the genetic change produced cannot be made because a control population was not maintained for the same population from which the selected families were taken. As the authors indicate, inbreeding may be a problem because the number of families at the start was small (20) and was reduced by selection (it seems that there were five to seven families selected, although the exact number is not given).

Improvement by selection has also been demonstrated in a few other species of bivalve molluscs. Newkirk and Haley (1982b, 1983) have selected for whole-body weight in *O. edulis* and have shown an increase in two generations compared to a control population. Hadley et al. (1991) have reported increase in growth in two out of three replicates of the hard clam, *Mercenaria mercenaria*, selected for whole-body weight for one generation.

Hybridization

Hybrids have been used extensively in plant breeding and to some extent in animal breeding. Two lines of different genotypes are crossed to produce the hybrid which has a commercial value exceeding that of the parental lines. In this case the hybrid is said to have "hybrid vigor" either because the combination of traits makes it more valuable or the non-additive genetic interactions of the heterozygous genotype results in better performance for the trait of concern. In agriculture, breeders have purposefully developed the parental lines so they differ in genotype and when interbred the result is hybrid vigor. Usually the lines are developed by inbreeding which results in lines that are themselves of no direct commercial value. The commercial hybrids in use are a result of selecting among many which have not shown the same high level of performance.

Studies of hybrid oysters have evaluated the heterozygotes naturally occurring at single loci, crosses between geographically separated populations, and crosses between species. Electrophoretically detectable loci are the easiest genes to identify in oysters, and the

genetic variation at these loci make it relatively easy to compare the heterozygotes to the homozygotes in the same population. In experimental crosses, the mating of oysters from geographically separated populations has been used to determine whether there are genetic differences between the populations and whether the "hybridization" of the populations will result in improved growth or survival. Although the genetic differences between populations may not be known, there must be genetic differences between oysters of different species. Thus, if two species can be hybridized, the result must be an oyster that is highly heterozygous. However, to be of practical value it must be determined that the resulting hybrid is actually of more value in oyster culture.

Evidence from electrophoretic studies suggests that there are non-additive effects of some genes that affect growth rate of oysters. Singh and Zouros (1978) have shown that higher levels of heterozygosity at electrophoretic loci are correlated with size in cohorts of *C. virginica.* Oysters with higher heterozygosity also had higher survival (Zouros et al. 1983). Although this is a clear and statistically significant effect, the proportion of the variance in growth rate explained by heterozygosity is relatively low (Foltz et al. 1983), and it is not clear how this information would be used in a breeding program.

Experimental studies on crosses of geographically separated populations have not been conclusive in showing hybrid vigor. Newkirk (1978b) showed that hybrid vigor for larval growth occurs in some of the between-population crosses at some of the salinities in which the larvae were raised. However, there was not a clear demonstration of hybrid vigor in all cases. Stiles (1978) produced between-population crosses but none survived past metamorphosis, whereas the within-population crosses did. Mallet and Haley (1983b) showed variable ranking of the hybrids of three populations of *C. virginica* compared to the within-population crosses when grown for 40 months at two locations, though there was some indication of hybrid vigor.

More work is needed on testing the crosses of geographically separated populations for applied breeding. The assumption that any cross will produce hybrid vigor is clearly not valid. However, specific crosses may be of value commercially. Many will be tested before a few are chosen.

Few attempts have been reported at producing interspecific hybrid oysters. Menzel (1971) and Stiles (1978) have crossed *C. virginica* and *C. gigas* but produced very few offspring. Several recent studies indicate that these two species have little if any propensity to cross (Allen and Gaffney 1991; Downing 1991; Gaffney and Allen 1991). More work is needed in which growth and survival are compared to conspecific controls. The success of the interspecific crossing has to be confirmed by electrophoretic or other genetic analysis because it is possible that the attempts at interspecific hybridization can result in parthenogenetic development, or there could be conspecific sperm introduced by accident.

Applied Chromosome Manipulation

Changes in the chromosome composition of oysters can be very important in increasing production as demonstrated with *C. gigas* (Allen and Downing 1986). (See Longwell and Stiles, Chapter 12, as well as Beaumont and Fairbrother [1991] for a general review of ploidy manipulation in bivalves.) Triploids produced in the hatchery and transplanted to the estuary had less gonadal development during the normal reproductive period, than diploids raised at the same sites. In Washington State, mortality is often high during a summer period, and the reduced gonad development of the triploids resulted in higher survival. Furthermore, growth of triploids continued during this period when the diploids have reduced growth. The result of triploid induction is primarily inhibition of gonad development and reduced utilization of glycogen in gamete production. There is no evidence that triploids have higher growth rates during the non-reproductive periods.

Triploidy itself in *C. virginica* did not result in an increase in growth (Stanley et al. 1984). In this study, triploidy was induced by blocking both meiosis I and II, and oysters in the first group had a slightly higher growth rate as measured by shell length. The explanation given is that by blocking meiosis I, an increase in heterozygosity was produced and this, rather than triploidy per se, produced a higher growth rate. No

data on meat content or reproductive condition were given so it is not certain whether the results observed in *C. gigas* would apply to *C. virginica.*

Induction of polyploidy as it has been developed for *C. gigas* must be done each generation; the genetic changes last for only one generation. While there is no cumulative effect of the genetic manipulation, triploid production may prove to be a valuable tool in oyster culture.

Biotechnology

Genetic engineering and biotechnology encompass a rapidly expanding array of methodologies that will have important applications to the culture of *C. virginica.* A variety of methods are available, some of which, though not making genetic changes in the organism of interest, can be used to increase production, e.g., the cloning of genes to produce hormones that can be used in the hatchery to increase seed production (Paynter et al. 1989). DNA fingerprinting can be used to study population genetics problems in a more sophisticated way than previously possible with electrophoretic analysis (Harris et al. 1991).

New technologies have been developed recently to induce rapid genetic changes by creating transgenic organisms. Work has started with oysters, but it will take time before commercial products are available. In some species where there is a body of knowledge about genetics and physiology, it will be possible to make rapid progress with the new technologies. Transfer of growth hormone genes and regulatory genes has been made between species of different phyla and has resulted in improvement (see MacLean and Penman 1990 for a review) — gene transfer may be possible with oysters as well.

Summary

While a number of methods can be used to make genetic improvement of *C. virginica,* most have only been used on a small experimental scale and must be evaluated further in both experimental studies and in commercial breeding.

Large-scale experiments on oyster genetics have not been possible due to financial constraints. Models such as the Norwegian breeding program for Atlantic salmon (Refstie 1990) are too large for *C. virginica* because of the small size of the culture industry. However, there are experimental designs that can be used with oysters that may prove better than large experiments even if the latter could be done. For example, Stanley et al. (1984) compared the performance of the test groups within each culture unit while comparing the growth of diploid and triploid oysters. Newkirk and Haley (1982b, 1983) compared the growth of different selected groups grown in the same trays.

I believe there has been so little work on the selection of *C. virginica* for culture because of the small number of commercial hatcheries producing seed. There is no value in producing genetically improved stock until a hatchery can increase profits through sales of better seed stock. Until market improvement occurs, there will be little pressure on public institutions or industry for breeding programs.

The experimental work on genetics and breeding of *C. virginica* indicates the potential for genetic improvement for commercial purposes. Selection studies have shown improvements in disease resistance (Haskin and Ford 1987) and at least one commercial hatchery has improved growth rate (Paynter and DiMichele 1990). A number of selection methods and breeding schemes are possible with *C. virginica.* The particular methods used in a commercial program will depend on the facilities and breeding goals. Results in other bivalve species suggest that the potential gains can be considerable (Newkirk and Haley 1983; Hadley et al. 1991). Hybridization and chromosome manipulation will need further study and their application will probably vary with the site and the population of oysters used.

Roosenburg (1976) compared livestock breeding to oyster breeding and made several recommendations which, to me, are premature. He suggested using progeny testing of superior oysters such as is used in cattle breeding. This involves the same limitations as discussed above in producing families: the facilities available are too limiting. The suggestions to form a breed registry and stock certification program are also premature. In cattle breeding, pedigrees are known and the value of an animal is based on its pedigree and the performance of its offspring, in addition to its individual performance. This information is not available for oysters. These suggestions may one day be

taken up by the oyster industry, but there is a considerable amount of work to be done first.

Genetic engineering with new technologies is expected to contribute to improved oysters in the future, but it is unlikely that such improvement will happen soon because considerable research is needed first. However, we cannot assume that the new technologies will eliminate the need for more traditional methods. There are so many genes involved in the production traits of concern that selection will continue to be an extremely important breeding method. Thus, there needs to be an emphasis on traditional breeding programs (USDA 1988).

Unfortunate as it might be, it is unlikely that high levels of oyster production will be maintained by natural seed supplies due to environmental degradation. When farmers of *C. virginica* turn to hatcheries, they will need the benefits of improved stocks. The increased production from improved stocks will justify much of the increased cost of hatchery production. The methods used to improve current agricultural stocks need to be adapted and applied to oysters and, with the new genetic technologies, can contribute to the evolution of oyster fishing to oyster farming.

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Chapter 19

Culture: Application

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INTRODUCTION

Production of the eastern oyster, *Crassostrea virginica*, in the United States has been diminishing for nearly a century. Present North American production (see Mackenzie, Chapter 21) does not equal what once came from the Chesapeake Bay alone (Clark and Langmo 1979). The initial decline of the early fishery in the late 1800s was probably a result of overfishing and ineffectual conservation efforts. Management has consisted primarily of conserving available shell stock for replanting as cultch, protecting spat through cull laws, seasonal closure or protection of productive areas, and restrictions on harvesting gear (reviewed by Kennedy and Breisch 1981). Unfortunately this type of management in itself probably cannot increase oyster production significantly without recovery of habitat and improvement of water quality. Due to anthropogenic activities, pollutants entering estuarine waters create periodic sublethal concentrations of chemicals that interfere with early life history stages of the resident organisms (Davis and Hidu 1969; Stewart and Blogoslawski 1985; Castagna 1987; Haven 1987; Roberts 1987; Hargis and Haven 1988; Roberts et al. 1990). Because these toxins often affect embryogenesis and early larval stages, it is possible to ameliorate the situation by exploiting culture techniques to supplement habitat reclamation and conservation.

The concept of culturing oysters is not new. The European flat oyster, *Ostrea edulis*, may have been the original organism for mariculture, being cultivated at Lago Lurina, Italy, in early Roman times (Dupuy et al. 1977). It is generally believed that the actual farming of oysters as thought of today did not begin until 1624 in Hiroshima Bay, Japan (Fujiya 1970). On the Atlantic and Gulf coasts of the U.S., a large part of the eastern oyster harvest has traditionally been fished on public grounds rather than farmed on privately leased oyster beds (Shaw 1974). Traditional culture of oysters practiced both by private planters and state management agencies follows a general pattern. Cultch, usually stockpiled shells from shucking houses or dredged from fossil beds, is broadcast over reefs and oyster bottoms in areas where larval settlement is generally high due to hydrography and other suitable environmental conditions (Matthiesson 1969; see also Kennedy, Chapter 10 and MacKenzie, Chapter 21). The cultch is usually left undisturbed until the spat grow large enough to survive some of their more numerous predators before being transplanted.

Shortages of oyster seed have become common. Setting failures have been attributed to degradation of seed areas, poor water quality, and reduction of brood stock due to oyster diseases (Matthiesson 1969; Bardach et al. 1972; Kochiss 1974; Shaw 1974; Krantz and Meritt 1977; Kennedy and Breisch 1981). Overfishing of the oyster beds can easily become a major factor in reducing brood stock when environmental degradation occurs.

When seed oysters are about 2.5 to 3.8 cm in shell height (hinge to lip measurement; Galtsoff 1964; Quayle and Newkirk 1989), they are harvested from the seed beds and transplanted to areas where they

grow more rapidly. The transplants are planted far less densely than they had set naturally in the seed area. Depending on the site, food availability, and temperature, the time from setting through grow-out (growth to harvest size) varies from 13 months to 5 years.

Eastern oysters have many characteristics that make them excellent candidates for aquaculture. They are hardy, sessile, suspension-feeding animals that consume food low on the food chain. Oysters are a well established commercial species with a strong market demand and high value (Virginia Sea Grant 1990). The technology is available to grow them, and because of their high economic value, it is biologically and economically feasible to culture oysters of different species from egg to market size.

By using an oyster hatchery, growers can spawn oysters in seawater that has been filtered or treated to improve its quality. Larval development can occur free of competitors or predators in filtered water of optimum salinity and temperature to ensure good growth and survival to the eyed larval stage. Newly set oysters can be grown in a nursery system free from predators, competitors, fouling organisms, and excessive silt loads until they reach a refuge in larger sizes, i.e., large enough for planting in a grow-out area where their size ensures a reasonably high survival.

HISTORY OF HATCHERY DEVELOPMENT

Methods of culturing eggs and larvae of bivalves under laboratory and small-scale hatchery conditions have been tested by many workers over the past century. Costé was probably the first to attempt this around 1858 (Costé 1883). The start of oyster aquaculture in the U.S. can be attributed to Brooks, who demonstrated that spawn could be taken from eastern oysters, gametes fertilized (much in the same manner as fish eggs in fish hatcheries), and the young oysters kept alive until they had absorbed their yolk [sic] (Brooks 1879). Brooks (1880), Ryder (1883), Winslow (1884), and Nelson (1905) reported on efforts to culture *C. virginica* but their attempts were generally unsuccessful (Loosanoff and Davis 1963). Winslow (1884) has given a summary of some of these early efforts.

Interest in artificial propagation of *C. virginica* was revived when Wells (1920, 1927) and Prytherch (1924) succeeded in rearing oyster larvae to metamorphosis. Some other species successfully cultured in these pioneering studies include the Pacific oyster, *Crassostrea gigas* (Hori and Kusakabe 1926; Imai et al. 1950), and *O. edulis* (Cole 1936; Bruce et al. 1940). Several commercially important species, including the eastern oyster, were cultured by Loosanoff and Davis using newer techniques (Davis 1953; Loosanoff 1954; Loosanoff and Davis 1963).

Early attempts to culture bivalves were hampered by a lack of information, technology, and equipment. Small mesh size sieves or efficient filters commonly used today had not been developed. For instance, Wells (1933, 1969) used a milk clarifier, a relatively new invention, to clean suspended material from seawater and to concentrate larvae. Earlier, Filtros Plates® (a type of book filter often used in the food packing industry) were used by Prytherch (1924) to reduce suspended particle loads in the seawater.

CULTURE METHODS

Several culture methods for a number of bivalve species have been described in the published literature. Loosanoff and Davis (1963) give an excellent review of early methods for larval culture. Culture techniques have been described for the eastern oyster by Dupuy et al. (1977) and Krantz (1982); for the Pacific oyster by Breese and Malouf (1975), Wilson (1981) and Wilson et al. (1984); and for the European flat oyster by Wilson (1981). General culture techniques have been described by Walne (1974), Korringa (1976), and Castagna (1983).

Three culture methods most often referred to are the Milford method, the Glancy or Wells-Glancy method, and the Brown Water method (Wells 1933, 1969; Loosanoff 1954; Loosanoff and Davis 1963; Glancy 1965; Hidu et al. 1969; Ogle 1982). The Milford method uses filtered seawater and the addition of cultured unicellular algae whereas the Wells-Glancy and Brown Water methods use centrifuged or filtered seawater without cultured algae. Each method

has certain advantages, and most commercial hatcheries use a combination of these techniques. Here we describe an oyster culture system and methods that have proven successful even in areas with marginal water quality (Fig. 1). We will describe plumbing, water treatment, algal culture, conditioning and spawning of brood stock, and larval culture and settlement. These issues will be followed by a discussion of methods used in nursery and grow out.

Seawater System

In our hatchery, a dual seawater system is used to control fouling within the pipes (Castagna and Kraeuter 1981; Castagna 1983, 1987). By having the pipes duplicated from the intakes to the delivery valves (lines A and B), one intake, pump and seawater line (A) can be used one week and then shut off. The seawater in the system (A) becomes anaerobic and remains stagnant while the duplicate line (B) is used for the next week before the first line (A) is flushed and reactivated. Anaerobic conditions kill any fouling organisms that may have set in the unused seawater line during the preceding week. Such fouling organisms are usually microscopic in size and are easily flushed out as soon as seawater is delivered through the line at the next pumping interval. The intake is lifted out of the water for the week the line is inactive so fouling organisms on its interior and exterior surfaces will be killed by desiccation.

Intakes, Pumps, and Lines

The seawater intake is made of large size (ca. 10 to 12 cm) polyvinyl chloride (PVC) pipe into which multiple saw slits or drill holes are made to serve as an intake screen. One end of the pipe is capped and the other reduced to receive a smaller (ca. 5 cm) PVC fitting. The intakes are connected to flexible noncollapsible rubber intake hoses that are connected in turn by unions fitted on PVC pipes that are plumbed onto the pumps. Intakes are suspended from a frame fastened to a twin-hulled catamaran-type floating platform designed to hold the intakes suspended a selected distance below the surface (Fig. 2) or lifted out of water onto the frame to air dry. The float is moored in position with multiple anchors or pilings. A floating intake has certain advantages over a fixed intake: it rises and falls with the tides and can be suspended just below the surface where water temperature is

Figure 1. Overview of the hatchery at the Virginia Institute of Marine Science showing the larval (left) and algal (right, rear) culture vessels.

higher and natural food is more available; there are fewer problems with entrainment of resuspended silt and associated toxins; clogging with free floating macroalgae and debris is reduced; and fewer free-swimming organisms such as shrimp and small fish are entrained by the current and trapped against the intake screen.

Virtually any type of pump with adequate output (liters per minute) at a required head pressure can be used in a flow-through (non-recirculating) system. Economical 5-cm cast iron or thermoplastic centrifugal pumps are often used, but fiberglass, plastic, or resin-lined pumps are other alternatives. Submersible pumps, when used, are suspended from a float or from a cable connected to a davit so that the pump depth can be adjusted.

Dual seawater lines are made of PVC pipe, as are the dual large (ca. 15 cm) PVC drain pipes that remove water from the hatchery. The drain line not in use is closed with a commercially available plastic and rubber soil plug, which allows the water in the unused drain to become anaerobic.

Filters and Water Sterilization

Because chemical contamination of local seawater is a common problem, the water provided to eggs and embryos may be treated in the following manner. Seawater is passed through two filters connected in series. Standard pressurized fiberglass or plastic swimming pool filters or custom-built gravity flow sand filters are used for this. The first filter is filled with sand and operates like an ordinary sand filter to mechanically remove particulates. The second filter has only a base of sand, with the rest of the filter filled with activated carbon that acts as a semifluidized charcoal bed to remove chemical contaminants by adsorption. The activated carbon is replaced regularly at approximately every 10,000 L of seawater treated per kilogram of charcoal. Activated coal

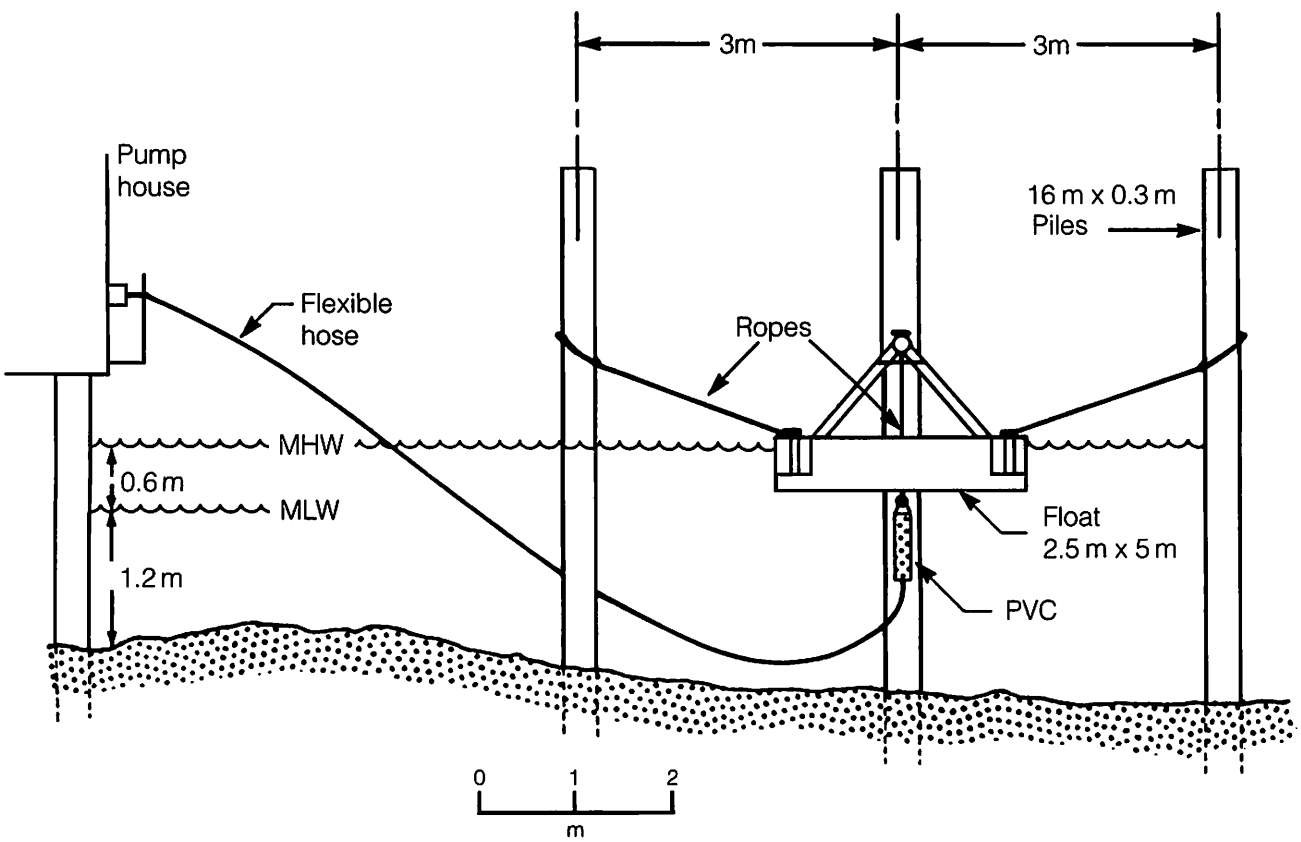

Figure 2. Diagram of intake catamaran showing location of PVC intake piping.

charcoal has proven to be efficient and inexpensive for this use.

Polypropylene bag filters of various pore sizes are also used. Bag filters (ca. 10 μm) are used either as the only filter or as a final filter when some type of prefiltering (i.e., sand filter) is used. In either case, they are normally inserted at the point at which seawater is delivered to the tank, and are replaced daily. Used filters are washed, dried, and reused.

Because bacterial contamination is often a problem in hatcheries, equipment must be washed carefully with a mild, biodegradable detergent and fresh water (Castagna and Kraeuter 1981). Oyster embryos are especially vulnerable to pathogenic bacteria, such as *Vibrio* sp. In order to reduce this hazard, after the tanks are filled with seawater that has passed through sand and carbon filters, sodium hypochlorite (NaOCl) (5.25% W:V) or a commercially available solution such as Clorox® is added at a rate of 2.5 ml per 10 L of seawater. This mixture yields 5 ppm free chlorine which chemically sterilizes the seawater, reducing the bacteria. After a minimum of 4 h, 0.75 ml of 1N sodium thiosulfate solution ($Na_2S_2O_3$) is added to dechlorinate 10 L of treated seawater. An appropriate colorimetric test, i.e. 2 drops of tolidine reagent yielding a clear color in a 10 ml dechlorinated sample (yellow indicates chlorine presence), is used to ensure that the chlorine is fully neutralized before fertilized eggs are placed in the container. After the larvae are 24 h old, the straight hinge or D stage is reached, and sand and charcoal filtered seawater is used without chemical sterilization in subsequent water changes.

Activated charcoal treatment or chemical sterilization is unnecessary in areas with good water quality. In fact, many hatcheries operate without sand filters, charcoal beds, or chemical sterilization, especially if the Brown Water or Wells-Glancy techniques are used. Head tanks or settling tanks are sometimes used to reduce sediment. The Bluepoint Hatchery in West Sayville, New York, pumps abiotic seawater from a well for use in algal culture and culture of larval stages (Butler Flower, F.M. Flower and Son, Oyster Bay, New York, pers. comm.).

Culture water can also be sterilized by exposure to an ultraviolet light of 25,000 Å. The water must not be passed through the unit faster than the flow specified by the manufacturer if a commercial unit is used. Penetration of the water column by ultraviolet is limited, so the layer of filtered water passing the light should not exceed 5 mm in depth. Custom-built units will often incorporate baffles or corrugated surfaces to mix the passing water so ultraviolet penetration is achieved.

Culture of Unicellular Algae

If seawater is passed through a sand filter and a charcoal bed before it is used for culture, most of the natural food will be removed. It thus becomes necessary to grow cultures of unicellular algae as food for the larvae. A number of flagellates and diatoms have been used, but the following three are most widely used in commercial hatcheries: the diatom, *Chaetoceros calcitrans* (Paulsen), the naked flagellate, *Isochrysis galbana* Parke (Tahitian strain, referred to as T-iso), and the diatom, *Thalassiosira pseudonana* (Hustedt) (clone 3H Hasle et Heimdal). The nutritional requirements of oysters in relation to the algal species have been reviewed by Ukeles (1971). The method described below for culturing algae is similar to that used in commercial hatcheries, such as Bluepoints Company Inc., West Sayville, New York and F. M. Flower and Son.

Pure cultures of algae kept on agar slants in test tubes stored under refrigeration (ca. 6°C) are started in 1-L glass Erlenmeyer flasks. Algae from these flasks are inoculated into 18 L clear glass water bottles that in turn serve as the inocula for 230-L Sunlite® fiberglass aquaculture tubes (45 cm diameter × 1.5 m high). Some hatcheries do not use fiberglass tubes for growing containers; rather, they employ 6, 8, or 10 mil (150, 200, or 250 μm thickness) polyethylene 61 or 91.5 cm lay-flat tubing (commonly used for food packaging), up to 180 cm in length either suspended vertically or laid horizontally on shelves. The ends of the plastic are closed by heat sealing or rolling a few turns around on PVC pipe. Large 1,600 L circular tanks (1.5 m diameter) are used in final development of mass algal cultures. Seawater passed through sand and charcoal filters and finally through a 1-μm bag filter serves as the culture medium for mass algal cultures. Seawater used for smaller cultures (Erlenmeyer

flasks and glass water bottles) is filtered sequentially through 10 μm, 1 mm, and charcoal insert cartridge filters. The enrichment medium used is Guillard's F/2 medium (Guillard 1975, 1983).

Unicellular cultures are grown in virtually bacteria-free conditions. The media, containers, siphon tubing, and aeration tubing are all sterilized. The Erlenmeyer flasks and their contents are heat sterilized at 15 psi for 15 min in a steam autoclave. The larger 18 L glass containers or the mass culture tanks, fiberglass tubes, or plastic lay-flat tubing are sterilized (including flooded tubing) with 0.5 ml sodium hypochlorite per liter of seawater (yields 10 ppm residual chlorine) for at least 4 h; they are then dechlorinated with 0.15 ml of 1/N sodium thiosulfate L^{-1} of chlorinated seawater. After the containers and media are cooled and dechlorinated, algal cells are added (inoculation). An ultraviolet light or laminar flow hood is used to sterilize the air around Erlenmeyer flasks when algae are being transferred between flasks. Algae are usually cultured in temperature-controlled rooms at 20°C to inhibit bacterial growth, but the mass algal culture tanks are usually kept at room temperature. Fluorescent lamps or metal halide lamps are used to furnish the necessary wide-spectrum light.

Aeration is furnished to all but the smallest Erlenmeyer flasks. Agitation of the culture is to prevent algal cells from clumping or settling, to ensure cells are exposed to the light, and to strip evolved O_2 from the water. Excessive oxygen and high pH are lethal to algal cells. Carbon dioxide gas (CO_2) is pulsed into the aerated containers for 15 sec about every 30 min at 1 psi above ambient pressure to maintain the pH in a range of 7.5 to 8.5. Hatcheries either do this with a system of electric timers and solenoid valves connected to a CO_2 tank or by manually purging for a few minutes twice a day.

Algal cultures can be fed directly to larvae at rates of about 10,000 to 100,000 cells ml^{-1} depending on larval density and size. The mass cultures of algae sometimes reach densities in excess of 5 million cells ml^{-1}. These cells are usually harvested by pumping the cell-rich solution through a continuous type centrifuge (Fig. 3) where the solution is spun at 15,000 rpm in a 15 cm diameter rotor (bowl) at a centrifugal force of 13,500 × g (Anonymous 1980). The centrifugal force deposits the algal cells against the wall of the bowl while the water passes through the unit. The cells can then be collected as a paste. Small portions of the paste can be resuspended as needed in filtered seawater in a blender or magnetic stirrer before being fed to larvae or spat. The paste can be stored in a 0° to 6°C refrigerator in a covered beaker for at least 30 d with little apparent decline in quality. Commercial hatcheries store algal paste under refrigeration for several months by covering the paste with a layer of filtered seawater containing antibiotics or by using various preservatives, such as brine, weak iodine solutions, or other bacterial inhibitors. Algae are seldom wasted because older cultures stored for longer periods can be safely used for older juvenile or adult oysters.

BROOD STOCK CONDITIONING AND HOLDING

Brood stock can be selected for desired characteristics such as fast growth, deep shell cup, or resistance to disease. Spawning stock is usually collected in January and February, cleaned of fouling organisms, and stored in trays placed on subtidal bottom or suspended from a pier adjacent to the hatchery. Gonads ripen naturally as ambient water temperature and food levels increase. Oysters can be conditioned out of season in the laboratory by holding them in heated sea-water (24°C) for 6 to 10 weeks with daily additions of cultured phytoplankton food (0.5 to 1.0 L algal culture per oyster per day). This can be achieved with a heated flow-through system or daily changes of warmed seawater if a static system is used. Ripe eastern oysters held in seawater at temperatures between 16° and 21°C and fed daily will not spawn or reabsorb their gametes. Seawater can be cooled by a chiller and heat exchanger or can simply be held in a cold room. If standing water is used, it must be changed three or four times a week with precooled water. Months after ripening, oysters held in this manner can be warmed to 24°C for about 72 h and then induced to spawn. These procedures greatly extend the spawning period observed in natural field populations.

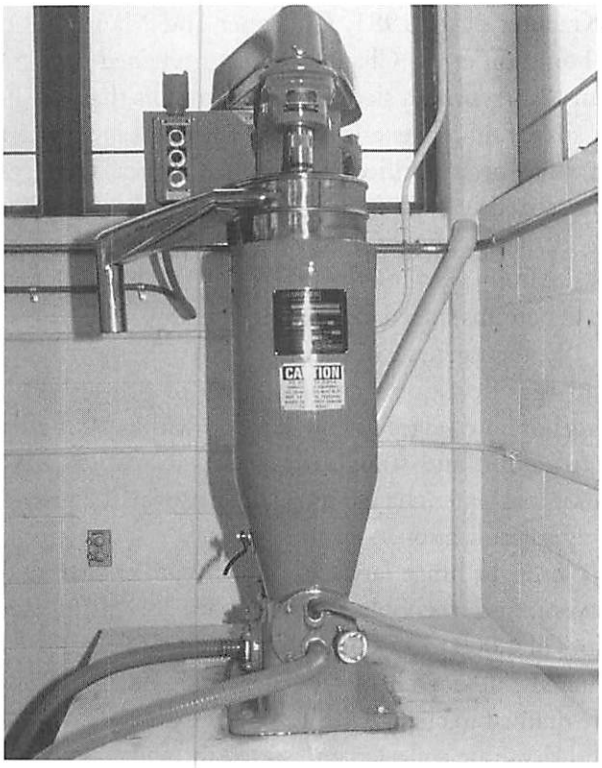

Figure 3. Sharples® centrifuge used to produce algal paste by separating algae from culture water

Spawning or Stripping Gametes

Gametes are usually obtained by inducing ripe adult oysters to spawn. Ripe oysters are placed in a fiberglass tank or trough. Filtered seawater at 20° to 22°C flows into the tank until the oysters appear to be pumping vigorously. The seawater temperature is then increased to about 28°C by the addition of seawater heated by passage through a glass, graphite, teflon, or other inert composition heat exchanger.

The oysters are held at 28°C for about 45 min. If no spawning activity is observed, the water temperature is lowered to about 24°C for 30 min and then raised again to 28° to 30°C. Temperature cycling continues until an adequate number of oysters spawn or the needed number of eggs are collected. Quite often, other stimulation is necessary to trigger spawning. The most commonly used stimulus employs gametes stripped from another oyster. That donor oyster is opened and its gametes are collected by lacerating the gonad with a scalpel and rinsing the gonadal material into a beaker of filtered seawater. The suspension of gametes is poured into the water over the oysters being stimulated to spawn or a pipet is used to release some suspension near individual oysters that are pumping strongly.

An alternate spawning protocol requires only small quantities of heated, filtered seawater. The day before spawning, ripe oysters are stored dry in a refrigerator at 6°C overnight. The next day they are placed into the spawning trough and 30°C filtered seawater is added to a depth of 5 to 6 cm. The oysters are left undisturbed for 3 h as the water cools to room temperature (ca. 20 to 24°C). The trough is drained and refilled with 30°C water. Sperm obtained from either a previous spawn and stored at 6°C or from stripping as described above is added. This process can be repeated hourly until spawning occurs.

Serotonin 5-HT (5-hydroxytryptamine creatinine sulfate complex, Sigma Chemical Company) is a susccessful spawning stimulus, especially for male oysters. Serotonin is dissolved in 1 µm filtered seawater of the same salinity as the water used in the spawning container. The concentration of 2.0 mM is reached by dissolving 7.7 mg in 10 ml of seawater. Approximately 0.4 ml of the 2.0 mM solution is injected into the oyster's adductor muscle by inserting a 23 gauge hypodermic needle through a notch filed into the edge of the shell. About 15 min after the injection a ripe oyster will spawn (Gibbons and Castagna 1984). [Serotin must be handled carefully because it can be absorbed through human skin and is a suspected teratogen.]

Once oysters start to spawn they are placed according to sex in containers of seawater to collect eggs and sperm separately. About four to six oysters can be placed in a 10 L container of seawater. After the eggs are released and spawning appears to have stopped, the adults are removed from the containers. A small volume of sperm suspension, diluted in filtered seawater to obtain sperm-to-egg ratios of < 5,000:1 (see Thompson et al., Chapter 9), is used to fertilize the suspensions of eggs (Galtsoff 1964). Fifteen minutes after fertilization, a sample of egg suspension can be

checked under a microscope to determine fertilization success by noting polar body formation. If necessary, additional sperm may be added.

If individuals or a select group of spawners are needed for a specific genetic cross, inactivated sperm or eggs of an unselected wild oyster can be used as a spawning stimulus without unwanted fertilization. Thus, an individual from the select group does not have to be killed to obtain gametes for a stimulus. The sperm or egg suspension from donor oysters is rendered inactive by freezing, pasteurizing, or microwaving. For example, a 500 ml suspension of gametes in filtered seawater is inactivated after about 90 sec in a 700 watt microwave at full power. Gamete viability should be checked under a microscope before use. Inactivated sperm will show none of the vigorous movement of living sperm. Ova will become more consistently opaque without the less opaque central area normally observed around the cell nucleus.

Oysters with well developed gonads will usually spawn when subjected to temperature cycling and stimulus such as addition of gametes. However, if spawning is not achieved either in a reasonable time or if the oysters do not have ripe gonads, gametes can be stripped from the gonad as described earlier (Castagna and Kraeuter 1981). Some commercial hatcheries simply remove the meat from a mix of male and female oysters, bisect the visceral mass just below the adductor muscle, and place the gonad portion in a blender with filtered seawater. The tissue is blended for 5 to 10 sec and the liquefied tissue poured through a 73 µm screen with the eggs collected on a 44 µm screen. The eggs are then washed with filtered seawater and a sample inspected under a microscope to check fertilization. Eggs obtained from stripping will usually produce fewer larvae than a natural spawn because immature eggs are included. Despite this drawback, a few commercial hatcheries base their entire production on stripped eggs. This method is more commonly used to obtain gametes from *C. gigas*.

Rearing Larvae

Once eggs are collected, they are graded according to size by rinsing through appropriate-sized sieves, either before or after fertilization. Larger eggs have higher lipid content and produce more viable larvae (Kraeuter et al. 1981; Gallagher and Mann 1984; Thompson et al., Chapter 9). Eggs retained on a 53 µm or larger mesh sieve give better results than smaller sizes. Embryogenesis is more successful in seawater that has been purified as described earlier. The eggs are started in culture at densities as high as 60,000 L^{-1}. Culture containers, constructed of fiberglass, range in size from a few hundred liters to 50,000 L and often have bottoms sloped to promote draining.

Embryogenesis occurs in a relatively short time, and eggs develop to embryos in 4 to 8 h at 24°C. Trochophore stages usually develop within 12 h after fertilization and straight-hinge larvae within 24 h. An initial water change is usually performed 24 to 48 h after fertilization.

Straight hinge larvae are much hardier than embryonic and trochophore stages. They are held at densities of 4,000 to 15,000 L^{-1} in seawater that has been filtered but not chemically sterilized. Rearing tanks are drained to change the water three times each week and larvae are collected and sorted through a descending size series of sieves (200 to 64 µm). Samples of larvae are counted under the microscope and inspected for evidence of disease. Different sizes are segregated when they are returned to the culture containers, and slow growers are sometimes culled.

Oyster larvae grown at 24° to 28°C develop through the larval stages to become eyed larvae (competent to settle) or pediveligers of 250 to 300 µm size between 12 to 21 days. The exact development time is dependent on culture conditions, including salinity, temperature, and food quality. Estimates of the percent of larvae that reach this stage can be made by microscopic evaluation after the larger larvae are collected on a sieve. Pediveligers and eyed larval stages will metamorphose within about 72 h, if water conditions (oxygen, salinity, temperature, clarity, purity) are adequate. Competent pediveligers will seek suitable substrate such as oyster shell, and will attach, secrete shell, and become fixed juvenile oysters (spat).

Coon et al. (1985a, b) discuss induction of settlement and metamorphosis of Pacific oysters with chemical stimulants such as L-DOPA, epinephrine, and related compounds. Settlement and metamorphosis of competent larvae can be induced by L-DOPA for both the Pacific and eastern oysters (Coon et al. 1985a). Loosanoff and Davis (1963) and Lutz

et al. (1970) used high water temperatures to induce metamorphosis in *C. virginica*. In actual practice, oyster larvae will metamorphose without additional stimulants when they are competent and their environment is near optimum or at least adequate. A reason for using chemicals or other setting stimulants is to achieve synchronous settlement and narrow the span of growth variation over time. Also, cultchless seed oysters used for off-bottom tray culture can be produced using these stimulants. Most commercial hatcheries do not use chemicals to induce setting.

Larvae are sorted on sieves of increasing mesh size at every water change. Larvae retained on screens > 202 µm mesh size are usually the eyed stage or pediveligers. These are rinsed onto and retained on a patch of 183 µm mesh nylon bolting cloth (Nitex®) (Fig. 4). The larvae are wrapped in the moist cloth, then in several layers of seawater-dampened paper towels to form a ball, placed in a covered plastic beaker, and stored in a refrigerator at about 5°C. A ball about 4 cm in diameter comprises about 2.5 million eyed larvae. The larvae can be held in a refrigerator for about 5 d in this condition. After 5 d or less in a refrigerator, the accumulated larvae can be rinsed back into filtered seawater and will metamorphose within about 72 h. If refrigerated longer than 5 d, the larvae will lose some vitality and produce a lower percentage of successfully setting larvae. This situation can be ameliorated by rinsing the ball of larvae back into filtered seawater at 4 or 5 d and allowing them to swim for about 2 h before being reconcentrated and refrigerated. They can then be held for an additional 4 or 5 d.

TREATMENT OF DISEASE

Sick larvae in a culture exhibit slow growth, poor color, and weak swimming activity (finally sinking to the bottom); they often have debris attached to the velum; and they almost always have a high number of protozoans swimming with them. Most protozoans feed on bacteria and are excellent indicators of bacterial infestations (Castagna and Kraeuter 1981). Sick larvae usually stop feeding, so a low rate of algal consumption will also indicate a problem.

If most of the larvae within a culture appear to be infected, it is often expedient to discard the entire batch. The containers and sieves can be cleaned with a biodegradable detergent and potable water followed by a wash with chlorine bleach solution (20 ml of 5.25% sodium hypochlorite per 20 L of seawater) and a thorough rinse with filtered seawater.

A decision may be made to save as many of the larvae as possible from an infected cohort. If so, the culture is drained and the larvae sorted through sieves of decreasing mesh sizes. The larvae on the smallest mesh are usually discarded on the assumption that the lack of growth is indicative of either moribund or dead larvae. The larvae to be retained are rinsed with generous amounts of filtered seawater and concentrated in about 10 L of filtered seawater. Aqueous penicillin G (10,000 to 15,000 units L^{-1}) and 0.0125 to 0.019 g L^{-1} of streptomycin are added to the concentrated larvae, which are gently stirred and allowed to stand for 45 to 60 min. Properly combined rations of commercially available veterinary grade antibiotics such as Combiotic® work equally well. Neomycin

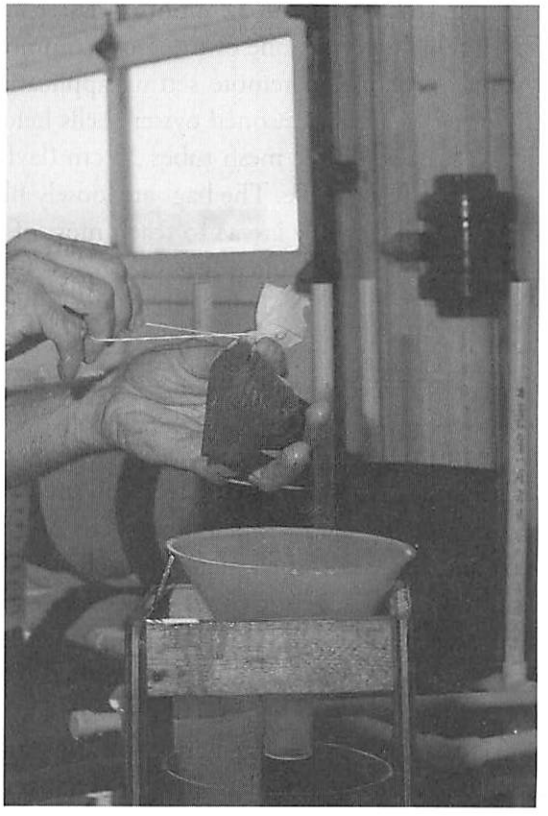

Figure 4. Eyed larvae in screening material before being packaged and refrigerated.

sulfate (1 mg L^{-1}) and streptomycin (1 mg L^{-1}) are very effective and many commercial hatcheries are now using these antibiotics. (Ford and Tripp in Chapter 17 caution against indiscriminant use of antibiotics which may lead to resistant bacteria.) The medicated larvae are again collected on an appropriately sized sieve, rinsed with generous amounts of filtered seawater to remove residual antibiotics, and placed into clean containers of filtered seawater. This treatment may be repeated daily if there is evidence of continuing bacterial infection (Castagna and Kraeuter 1981).

SETTING AND REMOTE SETTING

Cultch is the substrate to which oyster pediveligers attach when they metamorphose. Virtually any firm non-toxic surface will serve as cultch. It should be clean and placed in easily handled non-toxic containers. Perhaps the best, and certainly the "basic" cultch that others are compared to, is clean, seasoned (air dried for about 12 months) oyster shells (Crisp 1967). Therefore, one of the most commonly used surfaces for use in remote setting applications (or any other) is clean, seasoned oyster shells held in plastic mesh bags. Plastic mesh tubes 33 cm (layflat) are commercially available. The bags are loosely filled with oyster shell to allow larvae to reach most of the shell surfaces. The filled cultch bags are soaked in filtered seawater at least 12 h before use so that a bacterial film forms on the shell surfaces (Weiner and Colwell 1982). Drawbacks to using shell bags are the amount of handling necessary and the amount (bulk) of shell required.

Many other types of cultch have been used with varying degrees of success, including shells of other bivalves, and some non-shell materials such as grooved plastic PVC tubes, plastic cones, lime-coated strips of wood veneer, rubber tire chips, tiles, and marble or limestone chips. However, whole and crushed oyster shell is the most commonly used cultch material.

Most east coast oyster hatcheries use mini-cultch or cultchless setting techniques. These individual oyster spat are suitable for off-bottom culture in trays that afford protection from predation. The most popular of the two is mini-cultch, a clean finely crushed clam or oyster shell spread evenly over the bottom of a trough or placed in sieves or mesh bottom trap (called downwellers). Filtered seawater covers the shells to about 15 cm depth, and the water is gently aerated. Eyed larvae and pediveligers are placed in the trough or downwellers for 72 to 96 h and algal food added. Troughs are usually in well-lighted areas so the negative phototrophic behavior of the larvae will encourage them to settle among the shell chips. A thin film of petroleum jelly is sometimes spread on the exposed tray to discourage setting except on the shells. The cultchless technique is much the same except the substrate used is mylar, plastic, or a firm flat surface like polished marble or plexiglass from which the recently-set oysters can be removed (Dupuy and Rivkin 1972; Hidu et al. 1975). Chemical induction of metamorphosis with L-DOPA produces "cultchless" spat that are not attached to a substrate.

Remote setting has revolutionized the production of Pacific oysters on the west coast of the U.S. and Canada (Jones and Jones 1983, 1988). In this process, eyed larvae are set by oyster growers in locations remote from the hatchery that produced the larvae. This method has proven cost effective due to reliable hatchery production of eyed larvae and reduced handling and transport of cultch (Jones and Jones 1983, 1988). The remote setting technique can also be applied to eyed larvae of *C. virginica* (Castagna 1987).

To apply the technique, eyed or pediveliger larvae are concentrated onto a piece of dampened Nitex® cloth (Fig. 4)and the resulting larval ball is wrapped in paper towels wet with water of ambient salinity as described on page 683. Larval balls may be shipped from the hatchery in an insulated shipping container packed with a coolant, e.g., refrigerant gel pack or container of ice (a plastic bottle or bag prevents melted ice water from damaging the ball of concentrated larvae). An algal paste made of the diatoms *T. pseudonana* or *C. calcitrans* may be shipped in the same container for the culturist to feed the larvae during the setting period (Jones and Jones 1983, 1988).

The recipient of the larval balls prepares a setting tank filled with warm (ca. 24°C) filtered water of ap-

propriate salinity and quality and with clean, conditioned cultch such as oyster shells or trays of oyster shell grit. The tank should be drained and refilled with warm filtered seawater just before use as a setting tank. The ball of larvae is stirred into a container of filtered seawater and then poured over the surface of the water in the tank. The water is gently aerated either continuously or at 15 to 30 min intervals for 72 to 96 h depending on temperature, larval condition, etc., after which most of the larvae should have set. If suitable algae or an algal paste is available, the larvae are fed daily to enhance setting. After 48 h the water is partially drained through a 202 µm sieve. If a relatively high number of pediveligers is collected on the sieve, the tank should be refilled and setting should continue for an additional 48 h for a total of 96 h. If a low number of pediveligers is collected, an additional 24 h (total = 72 h) are required.

NURSERY

Larvae of *C. virginica* metamorphose and set at about 300 µm in size, whereas *C. gigas* larvae metamorphose at 300 to 340 µm. The resultant spat are vulnerable to smothering, competitors, and a host of predators. Thus, a nursery system is necessary to ensure good survival of the spat until a large enough size is reached to withstand some competition and the smothering effects of silt (Matthiessen 1989). Juvenile oysters larger than 2 to 2.5 cm can withstand some of the more common predators such as crabs. Spat size, nursery site, type of nursery, and the time of planting are important factors to consider in selection of a nursery system.

Several nursery systems have been used successfully. They may be land-based or located in an estuary. Land-based nursery systems (Fig. 5) consist of flow-through troughs or "upwellers" (Bayes 1981). Upweller systems consist of screen-bottomed containers (silos) of oysters held inside a larger container in such a manner that seawater enters through the screen bottom, flows upward past the oysters to produce a semi-fluidized bed of oysters, and discharges usually through a side exit pipe (Bayes 1981; Spencer et al. 1986). The passage of plankton-rich seawater through the oyster assemblage allows oysters to be held in large numbers, yet permits rapid growth while discouraging fouling and clumping of spat. There are a number of different configurations for upwellers. Some are vertical pipes or cylinders (ca. 15 to 20 cm diam.) individually plumbed so water can flow from the bottom and out the top with oysters packed loosely within the column. In addition to tanks or troughs on shore, upweller systems may be designed on rafts or floats to be deployed in bays or tidal streams (Bayes 1981; Mook and Johnson 1988; Baldwin et al. 1995). Juvenile oysters held in flow-through seawater troughs or upwellers must be routinely cleaned of fouling and biodeposits and their numbers thinned as they grow to reduce competition for food.

Nursery systems may also be located in the intertidal or subtidal zones. A variety of suspended and on-bottom methods may be used, depending on whether cultched or single spat are grown. Shell bags, coated steel wire mesh, or plastic containers such as trays, and milk crates or chicken cages containing spatted cultch may be placed on pallets or poles for on-bottom rearing or may be suspended from docks, bulkheads, rafts, and buoyed longlines for off-bottom rearing (Matthiessen 1989).

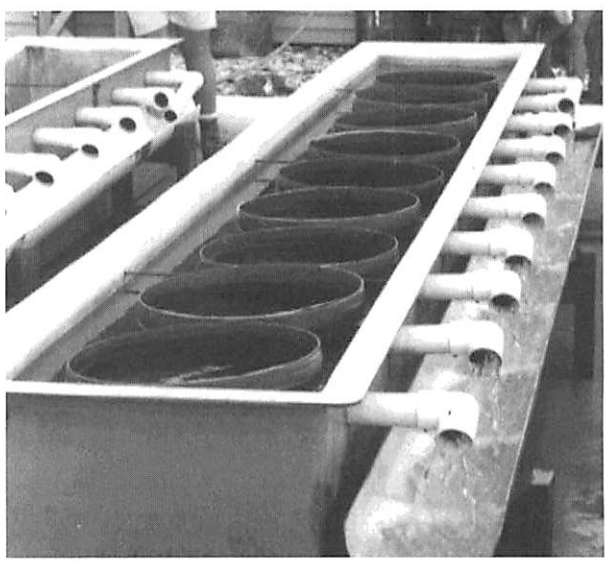

Figure 5. Flow-through troughs ("upwellers") used in land-based nursery systems.

One excellent nursery method places up to four layers deep of single oyster spat in plastic mesh trays which are then submerged in protected bays. Experiments have shown that faster growth and increased survival occur when oysters are grown off the bottom (Shaw 1962a,b, 1963, 1966, 1969, 1971; Baab et al. 1973; Aprill and Maurer 1976; Anderson 1979; Matthiessen 1989). This can be achieved by suspending trays from floats, rafts, longlines, docks, and bulkheads or by constructing the trays with supports or legs. Horizontal supports can be fastened under docks to hold trays at the appropriate level. In shallow areas, trestle and tray systems are successful. Here the trays can be situated so they are intertidal for a period of each low tide. This process of intertidal exposure, referred to as "hardening" in the oyster industry, reduces competition, fouling, and predation and improves spat survival. Oysters are normally held in a nursery system until they grow to a size of about 2.5 to 3.0 cm height, by which time they are less vulnerable to predators.

COMPETITORS, PREDATORS, AND PARASITES

On the Atlantic coast, fouling organisms such as barnacles, gastropods, encrusting bryozoans, bivalves, and ascidians settle on, overgrow, and eventually suffocate oysters (Engle and Chapman 1952; Hancock 1960; Galtsoff 1964; Barnes et al. 1973; MacKenzie 1977, 1981; Kennedy 1980). In addition, boring invertebrates weaken oyster shells by their destructive tunneling (Lunz 1941; Warburton 1958; Hancock 1960; Galtsoff 1964).

Predation greatly influences the success of a nursery. Predators tend to consume greater numbers of smaller thinner-shelled oysters. Cultchless or minicultched oysters are more vulnerable to predation than spat growing on oyster shell where the smaller oysters are protected by the larger shell of the cultch. Of the invertebrate predators, crabs can be the most destructive due to their high predation rates, mobility, and abundance. Oyster drills prey on oysters by burrowing through the shell (Carriker 1955; Hancock 1960; Galtsoff 1964; MacKenzie 1981; Butler 1985). In contrast, species of whelks penetrate oysters by chipping or wedging apart the shells (Colton 1908; Carriker 1951; Menzel and Nichy 1958). A variety of other gastropod species also prey on oysters (Gunter and Menzel 1957; Menzel and Nichy 1958; Wells 1958a, b). Starfish can consume oysters up to 67% of their own diameter (MacKenzie 1981). The flatworm, *Stylochus ellipticus,* preys primarily on oyster spat but can kill oysters as large as 61 mm shell height (Loosanoff 1956, 1965; Landers and Rhodes 1970). Very small *S. ellipticus* prey on newly set spat of the eastern oyster (Newell and Kennedy 1992). Finally, vertebrates, including some birds (Tomkins 1947) and fish (Merriner and Smith 1979; Cave and Cake 1980), can crush adult oysters.

Predators may be controlled by a variety of mechanical, chemical, biological, and exclusion techniques. These measures are costly and labor intensive. Starfish can be controlled through application of quicklime (CaO) (Loosanoff 1961, 1965).

Oysters may harbor commensals such as the pea crab, *Pinnotheres ostreum* (Christensen and McDermott 1958). The ectoparasitic snail, *Boonea impressa,* may locate along the edges of the shell and penetrate the oyster mantle with its proboscis, causing a reduction in feeding and growth and often spreading the disease dermo (White et al. 1984). Finally, parasitic copepods and trematodes reduce the marketability of oysters (Galtsoff 1964). This topic of predators, pests, and competitors is considered in greater detail by White and Wilson in Chapter 16.

GROW OUT

After the oysters in the nursery system grow large enough to be less vulnerable to predators, they are usually moved to a grow-out area, which has greater water exchange and thus more phytoplankton than the nursery areas. Some grow-out is successfully carried out in tray systems after the number of oysters in each tray has been adjusted to reduce competition for food. Although tray culture is labor intensive and more costly than traditional on-bottom culture, these added costs are offset by increased survival, increased growth rate, and ease of harvesting. Market size can be attained in 13 to 24 months at about 7.6 cm in shell height in trays, compared with 15 to 36 months on bottom.

Survival of oysters in the grow-out phase, as in the nursery phase of culture, is influenced by siltation, overgrowth by fouling organisms, competitors, predation, parasitism, and disease. Some of these mortality factors can be reduced through selection of appropriate sites and suitable grow-out techniques.

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Chapter 20

Transfers and World-wide Introductions

JAMES T. CARLTON AND ROGER MANN

Crassostrea virginica occurs naturally in the western North Atlantic Ocean from the Gulf of St. Lawrence, Canada in the north to the Gulf of Mexico, Panama, and the Caribbean Islands in the south (Yonge 1960; Abbott 1974; also Carriker and Gaffney, Chapter 1). The history of human-assisted movements of *C. virginica* within its natural range and around the world has never been reviewed. We provide here an outline of this history, along with remarks on what these movements imply for population genetics and for the accidental transportation of disease organisms, and associated animals and plants. We briefly discuss the general failure of *C. virginica* to establish self-reproducing populations after introductions to other regions, in marked contrast to the more widespread establishment of intentional introductions of the Pacific oyster, *Crassostrea gigas*. We follow the International Council for the Exploration of the Seas (ICES 1984) definitions in referring to movements within the natural range of the oyster as "transfers," and movements outside its range as "introductions."

INTRODUCTIONS TO EUROPE

France: 1860s to 1870s

The earliest recorded movements of the eastern oyster outside its natural range of which we have a record were introductions in the 1860s to the European mainland (Table 1). Shipments were made to Arcachon Basin in southern France, along the Bay of Biscay in 1861 and 1863 (Fischer 1864, 1865). Another shipment of oysters to Le Havre in 1861 (de Broca 1865), which may have been planted, was followed in 1862 by four or five more shipments (the total quantity is not recorded) from New York and Delaware. Most of these oysters were apparently planted (along with perhaps ten thousand hard clams, *Mercenaria mercenaria*, and hundreds of the softshell clam, *Mya arenaria*) at Saint-Vaast-la-Hogue, on the Cotentin peninsula, in Normandy (de Broca 1865). Bouchon-Brandely (1878) observed that the eastern oyster "has prospered well in the basin of Arcachon." Sometime in the mid-1870s, a French oyster company (MM. Venot & Co.) also introduced two "lots of spawn" (actual quantity not stated) that were "placed in the parcs of Crastorbe" in Normandy (Bouchon-Brandely 1877). "The American oyster has been little appreciated in our country," Bouchon-Brandely wrote in 1877, and thus "Its rearing has been abandoned." Edwards (1976) provides further references to these mid-century attempts to naturalize the eastern oyster in France.

England: 1870s and Later

These early movements to France followed a much longer era in the early 19th Century when "For many years the captains and passengers of steamers sailing from New York to Liverpool [were] accustomed to take with them a barrel or two of oysters in the shell, to be eaten on the voyage...and occasionally an American living in England would have them sent over to him as a treat" (Ingersoll 1881).

Inspired by the success of shipping large numbers of living oysters to France in the early 1860s, the merchant George H. Shaffer, of the famous Fulton Fish

Table 1. Successes and failures in establishing populations of the eastern oyster *Crassostrea virginica* from introductions around the world.

Introduced to	Years	Results
Europe		
England: Essex and Kent	1871 to 1939	Not established
Wales: Menai Straits	pre-1896	Not established
Ireland	pre-1939	Not established
France: Arcachon	1860s to 1870s	Not established
Netherlands: Oostende	1939 to 1940	Not established
Denmark: Aro	1880, 1884	Not established
North America: Pacific Coast		
British Columbia	1880s to 1930s	Established: see text
Washington	1874 to 1940s	Population now extinct
Oregon	1870s; 1896 to 1940	Reproductive but not established; private holdings continue
California	1869 to 1940	Reproductive but not established; trial introductions continue
Mexico (Baja California)	Planted, but no official records	Not established
Hawaii		
Oahu: Pearl Harbor	1866; 1883 to 1949 and perhaps later years	Established: see text

Market of New York City, sent "over a dozen barrels as an experiment" to the English market in about 1871. Ingersoll (1881) records the results: "They retained their freshness, were landed in good condition, and speedily sold. The agent telegraphed Mr. Shaffer to forward a larger consignment, which also was sold advantageously, and a regular trade was established."

In a short time a number of suppliers were shipping eastern oysters to Europe — "almost at a bound the exportation of oysters reached its full strength as a profitable business" (Ingersoll 1881). Edwards (1976) quotes Stevenson (1899) as stating that the English trade began in 1861, but we suspect a misquotation and that Stevenson was referring to the earlier French introductions described by de Broca (1865).

Although it thus appears that the initial inspiration for what was to become an important non-indigenous oyster fishery came from American entrepreneurship rather than from British solicitation, the reception afforded these oysters in England was no doubt in large part due to the rapidly declining supply of native European flat oysters, *Ostrea edulis*. A significant commercial trade existed in England for the European flat oyster during the 19th Century. By the 1860s to 1870s, the oyster fishery of the south of England was considered "nearly exhausted" and "pri-

vate breeding beds were an actual necessity" (Yonge 1960). Essex fishermen were working as far afield as the west coast of Scotland and the Netherlands to find adult and brood oysters. By the early 1870s it was necessary to import *O. edulis* from the Netherlands (Murie 1911) to maintain the fishery.

The decreasing abundance of European flat oysters provided the necessary motivation for the regular importation and relaying of *C. virginica* in England (Utting and Spencer 1992). Whether there was a more fundamental goal to establish the eastern oyster in Britain is not clear, but it would be difficult to believe that this was not in the minds of at least some of the principals involved. Walford and Wicklund (1973) note that the importations were "primarily to supplement the larger summer market demand."

When the first large shipments were relaid (outplanted) in open waters is not clear. Spencer and Utting (1992) have noted that "the Conway Oyster Company was importing one million seed American oysters per week in the early 1870s, of which an unknown proportion were for relaying." By at least 1881, however, Ingersoll was able to record the following

> To provide against loss [before the next shipment arrived], the largest dealers own spaces of sea-bottom, where the surplusage is thrown overboard to keep in good condition and be drawn upon as required. Some thousands of barrels are sent annually, which are intended to lie and grow there from one to three years. American oysters laid down thus in foreign waters have never been known to spawn, so far as I could learn, but the conditions have never been favorable; and no experiment, that I am aware of, has been tried, to ascertain whether seed-oysters from the United States, properly planted, would not grow into good health, emit spawn, and establish their race upon the European coasts. I see no reason why such an experiment should not prove entirely successful. It is said that the English beds [of *O. edulis*] are becoming so depopulated as practically to have become worthless.

Murie (1911) notes that about 1880 or a few years earlier, an agency was established at Brightlingsea, Essex for the regular importation of eastern oysters in quantity for sale and distribution among merchants and oyster growers for relaying. The Great International Fisheries Exhibition in London in 1883 further "gave a stimulus to the consignment of American oysters for replanting..." (Murie 1911). Thus, commencing in the 1870s and 1880s, adult and eventually seed oysters were transported, largely from Long Island Sound, to estuaries in Suffolk (River Orford), Essex (Brightingsea in the River Colne, River Blackwater, River Crouch), and Kent (Whitstable on the south side of the Thames estuary), localities important for their proximity to the London markets (Philpots 1891; Bulstrode 1896; Cole 1956; Utting and Spencer 1992). By 1896, 100,000 barrels of oysters were being shipped annually to England (Kochiss 1974 and citations therein).

Ease of importation throughout these and subsequent decades resulted in a continued failure to address problems relating to poor spat settlement and degeneration of the native flat oyster fishery. Yonge (1960) records that eastern oysters were regularly relaid on the beds at Brightingsea and West Mersea in southeast England until 1939, when importation was terminated, presumably because of wartime hostilities in Europe.

The "European Export Trade"

By 1879 the "European export-trade" was blossoming, with regular shipments not only to England but also to Germany (Hamburg, Bremen) and France (Le Havre) (Ingersoll 1881). How many of these oysters were for direct market consumption, and how many found their way to oyster beds in open water, cannot now be known. It is difficult to imagine, however, that outplantings were not made in Germany (where we have not yet located planting records) at least as regularly as has been recorded in France. In 1880 and again in 1884, *C. virginica* from Canada (Gulf of St. Lawrence) were relaid on the west coast of Denmark at the island of Aro in Little Belt (Mobius 1883, 1885). Oysters from the 1880 planting were alive three years later, but there was no evidence of reproduction (Mobius 1883). We have little doubt that other such trials were attempted throughout western Europe between the 1880s and the 1930s. There are

further records, for example, of eastern oyster introductions to Wales (Bulstrode 1896) and Counties Louth and Dublin in Ireland (Went 1962). At the close of this period, oysters were also introduced from Long Island to the North Sea at Oostende (Ostend), Netherlands, in 1939 to 1940 together with the accidental introduction to Europe of the hydroid, *Gonionemus murbachi* (Leloup 1948).

The full record of exports commencing in the 1860s of living eastern oysters from the east coast of the United States, in terms of destinations and numbers exported, may be available but remains to be examined in old oyster house, freight broker, and custom records, many of which are extant. Ingersoll (1881) notes large quantities of exports from the United States commencing in 1864, although apparently most of these earlier (pre-1871) shipments were to Canada. Small quantities were exported (before 1879) to Mexico and the "East Indies" (the Malay Archipelago), but whether these were received alive and relaid we have not determined.

Recent Trends

Although the eastern oyster failed to produce self-sustaining populations in Britain (discussed below), the continuing development of oyster hatchery technology in post-World War II years at the Conwy (formerly Conway) laboratory in North Wales lead to renewed serious consideration to supplement the limited British oyster industry through the use of a non-indigenous species. Hatchery technology provides a method of controlled introduction while essentially eliminating associated introductions of pests, parasites, and pathogens. After examination of several candidate species, efforts were focused on *Crassostrea gigas.* The complete account of this introduction, effected in compliance with guidelines that were the precursor to the original ICES "Code of Practice" (ICES 1984), is given in Walne and Helm (1979).

The success of *C. gigas* as a hatchery-supplied product for subsequent grow out stimulated consideration of other introductions, and in 1984 — only slightly more than 100 years after the first eastern oysters were brought to Britain — *C. virginica* from the Maryland portion of Chesapeake Bay were placed in quarantine at Conwy (Spencer 1987). *C. virginica* was considered a potentially valuable alternative to stocks of native *O. edulis* affected by the sporozoan, *Bonamia ostreae,* from 1982 onwards. Elston et al. (1987) provide further details of the spread of this disease-causing sporozoan.

Sustained *C. virginica* production in the United Kingdom will depend upon a hatchery supply of juvenile (seed) oysters. In accordance with ICES guidelines (ICES 1984), only F_1 progeny from the 1984 importation were released into selected experimental sites in United Kingdom waters, and then only after an 8-month quarantine period accompanied by regular histological examination of animals to confirm the absence of disease-related pathological abnormalities. Growth trials comparing these *C. virginica* to the now widely cultured *C. gigas* were made in 1986 and 1987 at six sites on the Welsh coast and along the southern coast of England (B.E. Spencer, Conwy, pers. comm.). Although growth rates were lower than those of *C. gigas* (Utting and Spencer 1992), survival was very good (74 to 99% in predator-exclusion, off-bottom cages). The future development of the *C. virginica* fishery appears promising because F_1 progeny have been offered to commercial hatcheries, and growth enhancement studies using triploidy induction offer prospects of improved product quality to the commercial market.

INTRODUCTIONS TO THE PACIFIC COAST OF NORTH AMERICA

The history of the introduction of eastern oysters to the Pacific coast of North America is given in detail in Carlton (1979a). *Crassostrea virginica* is one of seven species of exotic oysters imported to the Pacific coast since the late 19th Century. Here we briefly review the records of oyster plantings by province and state, and summarize the evidence for reproduction and establishment.

British Columbia

Crassostrea virginica was introduced to British Columbia (on the mainland side of Vancouver Island,

and on the mainland itself) from the early 1880s to about 1940; reviews include those of Stafford (1913), Sherwood (1931), Elsey (1933), and Quayle (1964, 1969). Although most literature suggests that the first introductions were made in 1903 (Bourne 1979) or 1906 (Elsey 1933), the first plantings actually occurred about 1883 in the Victoria Arm of Vancouver Island (Carlton 1979a). Although Elsey (1933) stated that the first records of reproduction were in 1917 and 1918, Taylor (1895) had reported the discovery of naturally-set specimens in 1893 in Victoria Arm of Vancouver Island, and Stafford (1913) reported planktonic eastern oyster larvae at Ladysmith Harbor, Vancouver Island, in 1911. Reproduction, at times relatively extensive, occurred in a number of areas in Boundary Bay. A small population now remains in only one locality, the Nicomekl River (see p. 697).

Washington

Townsend (1896), Doane (1901), Galtsoff (1929), Chapman and Esveldt (1943), Kincaid (1951), and Sayce (1976) have reviewed the eastern oyster industry in the state of Washington. Importation of eastern oysters into Washington supported a small industry between the late 1890s and the 1930s. Although plantings were attempted in the 1870s, the larger native oyster industry based upon *Ostreola conchaphila*[1] (= *Ostrea lurida*), which lasted until the 1890s, and the lack of a transcontinental railroad line until 1883 discouraged large-scale importations of eastern oysters.

Eastern oysters were first planted in Willapa Bay, a coastal arm of the sea, in 1874, but none were planted again until 1894, when 80 barrels arrived from Long Island Sound and Chesapeake Bay (Carlton 1979a). Starting about 1897, many carloads of eastern oysters were shipped to Willapa Bay. The industry ceased after World War I and in 1919 a "red tide" killed most of the remaining adult eastern oyster population in Willapa Bay (Chapman and Esveldt 1943; Sayce 1976).

In Grays Harbor, situated to the north of Willapa Bay, eastern oysters were planted starting about 1900 (Doane 1905) and these plantings continued sporadically until at least the early 1940s (Chapman and Esveldt 1943). In Puget Sound and in waters north to Bellingham, which is close to the Canadian border, an early attempt in the 1870s or early 1880s of planting two "sacks" (the actual quantity is not known) preceded more extensive experimental plantings in 1899 and 1900 that yielded promising results (Townsend 1893, 1896; Doane 1901).

By the late 1920s the eastern oyster industry on the west coast had declined markedly (Kincaid 1928; Galtsoff 1929), soon to be replaced by the Pacific oyster industry. "A few spat" were said to be "found occasionally" as the result of natural settlement (Galtsoff 1929).

Oregon

Washburn (1896), Fasten (1931), and Hubbs and Miller (1965) provide historical reviews of Oregon's commercial eastern oyster industry. Few records of these importations into Oregon were kept and details are obscure.

The only official records of plantings in Oregon are for the sheltered estuary of Yaquina Bay; it may be assumed, however, that plantings were also made elsewhere. The first plantings were made about 1872, when "a large number" were brought from San Francisco Bay (Washburn 1896); a second attempt was made over two decades later, when 25 barrels of oysters were planted in 1896. Plantings continued sporadically thereafter, with commercial plantings ceasing in the 1930s when Pacific oysters became more readily available (Hubbs and Miller 1965). Private aquaculture companies now hold *C. virginica* for grow-out purposes in open water in Oregon (Carlton, pers. obs. 1989).

Washburn (1899, 1901a) and Sweetser (1905, 1907, 1909) gave detailed accounts of attempts to induce reproduction in eastern oysters in Yaquina Bay. Reproduction and settlement in Yaquina Bay occurred on occasion, but more rarely and in less quantity than in California. Washburn raised eastern oyster larvae in the laboratory, and released them "by

[1] The authors originally identified this species as *Ostrea lurida*. The editors have renamed it following the convention of Carriker and Gaffney (Chapter 1).

thousands" in Yaquina Bay — perhaps the earliest attempt at open-system marine invertebrate culture on the Pacific coast.

California

The California oyster industry was reviewed by Ingersoll (1881), Collins (1892), Townsend (1893), Bonnot (1935), Skinner (1962), and Barrett (1963). The first eastern oysters were imported to San Francisco Bay in 1869 with the completion of the transcontinental railroad (Barrett 1963). Shipments to California were of varying quantities until the 1890s, when the industry grew and flourished in San Francisco Bay, only to taper off sharply after 1900 (see below). The last seed shipments to San Francisco occurred about 1910; shipments of adult oysters for holding before sale continued to be received until the 1930s. Experimental plantings have continued irregularly since then in San Francisco, Tomales, and Drakes Bay in central California. Andrews (1980) noted that "regular importation and planting of market-sized oysters from Long Island" continued for the restaurant ("rawbar") trade into the 1970s.

Accurate details of the history of the oyster industry in California are difficult to obtain, a situation that Skinner (1962) has commented upon relative to oyster production figures. For many years data were either not reported or were reported incompletely or inconsistently for the same year. Oyster shipments and production were reported variously in pounds, gallons, shells, number of seed oysters, shucked meat, bushels, barrels, cases, sacks, (railroad) carloads, dollars, and, later, shiploads and truckloads. Carlton (1979a) reviews in detail the oyster importation data.

A consequence of numerous ambiguities and incomplete records in oyster production figures is uncertainty in the literature concerning the timing of the decline of the eastern oyster industry in San Francisco Bay, a decline heralded in terms of the deterioration of the quality of oysters produced in the bay. Growth became slower and the meats became "thin and watery" (Scofield 1928; McMillin and Bonnot 1931; Bonnot 1935; Barrett 1963). This situation occurred "about 1900" (Bonnot 1935), "beginning sometime after 1905" (Barrett 1963), by 1908 (Skinner 1962) and by about 1917 (Scofield 1928). Seed oyster shipments were discontinued as oysters raised from seed were no longer fit for market.

The cause or causes of the lowered quality of the oysters (and thus the demise of the industry) has been speculated upon by numerous authors, and these are reviewed at length by Carlton (1979a). It appears that the oyster industry may have failed because of the combined effects of altered freshwater supply to San Francisco Bay (successive years of low water runoff and increasing amounts of freshwater drawn off for irrigation purposes). Such reductions in flow reduce flushing of the lower bay and thus allow a build-up of poorer-quality south bay waters. Greatly increased sewage and industrial pollution of the bay after the turn of the century must have compounded these adverse effects.

On occasion *C. virginica* reproduced and set in large numbers in San Francisco Bay, particularly in the late 1880s and early 1890s, although never in quantities so extensive as to support a sustained commercial fishery. Natural settlement was observed on native Olympic oyster shells before 1873, and again in the 1880s on posts, piles, and rocks (Ingersoll 1881; Townsend 1893; Cooper 1894); young oysters were said to be at times "numerous." "An immense catch" (natural spatfall) occurred in the summer of 1890 in the south portion of the bay (Washburn 1901b). *Crassostrea virginica* was also said to grow faster in San Francisco Bay than on the Atlantic coast (Davidson 1871; Ingersoll 1881; Cooper 1894; Wilcox 1895, 1898). Oystermen failed, however, to encourage settlement by placing out cultch.

Eastern oysters were also planted along the California coast in Humboldt Bay (1896 to the 1930s), Tomales Bay (1875 to date, in small numbers), Drakes Estero (1949 to date, in small numbers), and Elkhorn Slough (1920s to 1930s); occasional plantings were made in Morro Bay (1938), Mugu Lagoon (1930s?), Ballona Bay (near Santa Monica; a few plantings between 1880s and 1891), Anaheim Bay (1932), Newport Bay (1930s?) and San Diego Bay (1880s) (Carlton 1979a). These latter attempts and holdings were too few and too sparse to assess the ability of *C. virginica* to establish in the warm bays of southern California.

Baja California

There appears to be no official documentation of the plantings of eastern oysters in bays or lagoons of the outer coast of northern Baja California, Mexico. Radwin and Hemingway (1976) noted that eastern oysters were planted in Estero de Punta Banda, but give no information as to date.

Establishment of Reproducing Populations on the Pacific Coast

One self-sustaining population of *C. virginica* is currently established on the Pacific coast. A natural set occurred in the Nicomekl River in Boundary Bay, British Columbia, as early as 1917 (Elsey 1933; Freudenberg 1934; Quayle 1964, 1969) and the population is still viable (Bourne 1979; Ketchen et al. 1983). *Crassostrea virginica* and *C. gigas* "have coexisted on intertidal bars in this river for many years" (Bourne 1979), perhaps the only such co-existing populations in the world.

Under what may have been similar environmental conditions, a reproducing population of *C. virginica* was established for many years in the Naselle River and in adjacent portions of southern Willapa Bay, Washington (Edmondson 1922; Cobb 1929; Galtsoff 1929). Reproduction first occurred there in 1914. The population appears to have died out about 1938 (Kathleen Sayce, Nahcotta, Washington, pers. comm.; based upon information provided by Clyde Sayce, Dennis Tufts, and Harlan Herrold, a Chinook oysterman and fisherman, who reported the 1938 date). Post-1940 reports of this population (for example Hedgpeth [1968] and Kozloff [1973]) appear to be repetitions of earlier records.

INTRODUCTIONS TO HAWAII

Crassostrea virginica was transported to Hawaii as early as 1866 (Kay 1979), an event that would presumably have required transport of living oysters by ship around Cape Horn. Accurate records of importations begin in 1883 (Coleman 1923; Brock 1952, 1960), with irregular plantings occurring until 1949 (Brock 1952) and perhaps later (Kay 1979). Releases have occurred throughout the islands.

As a result of plantings in 1893 and 1895 at Manana, Ewa, in Pearl Harbor on Oahu, *C. virginica* became established in Hawaii. A large population exists today in West Lock (Loch), Pearl Harbor (Brock 1960; Sparks 1963; Sakuda 1966; Preston 1971; Kay 1979), although it suffered extensive mortality by the protistan parasite, *Perkinsus marinus,* in the summer of 1972 (Kern et al. 1973) presumably as a result of the undocumented importation of infected oysters. There has been increased aquaculture interest in *C. virginica* in Hawaii (Lam and Wang 1990).

TRANSFERS OF EASTERN OYSTERS ON THE NORTH AMERICAN EAST COAST

There is no documentation of the transfers of eastern oysters within or between the Atlantic and Gulf of Mexico coasts of the United States, despite a substantial history that begins in the early decades of the 19th Century. Our brief review here outlines what we tentatively define as three major phases of this history, each period reaching ever further south because of dwindling oyster populations in the north:

- Phase I, the movement of Chesapeake Bay, Delaware, and New Jersey oysters to more northern waters, commenced in 1808
- Phase II, the movement of "south Atlantic" (South Carolina and North Carolina) oysters to Chesapeake Bay and seaside Virginia, commenced in the 1940s, and the movement of seaside Virginia oysters to Delaware Bay commenced in the early 1950s
- Phase III, the movement of Gulf of Mexico oysters to the Atlantic coast commenced in the late 1950s

Phase I: The "Southern Trade"

The presence of extensive shell middens along the Atlantic coast demonstrates widespread exploitation of *C. virginica* resources by native American Indians

before European settlement. Indeed, Ingersoll (1881) reviews evidence that Indians were transferring oysters within Maine in prehistoric times. Early settlers continued and greatly accelerated this exploitation. By the mid-18th Century, stock depletion in some locations was considered a sufficiently major problem that many laws restricting both the harvesting methods and quantities were enacted (Ingersoll 1881).

Stock depletion occurred on a north-to-south basis, with aboriginal oyster populations being rendered essentially extinct sequentially from Maine south to Cape Cod, and then from Cape Cod into Long Island Sound. By the early 19th Century, transport of oyster stock from more southern locations (originally New Jersey and Delaware, and then eventually Chesapeake Bay) had commenced (Ingersoll 1881, 1887; Hall 1894; Stevenson 1894; Sweet 1951; Kochiss 1974; and Galpin 1989). Oysters were shipped from Chesapeake Bay to Long Island Sound as early as 1808 (Stevenson 1894) and 1823 (Sanford 1897; Kochiss 1974), although intensive transfers apparently did not commence until after 1830 (Galpin 1989).

Oysters from "southern" waters eventually were transported from many mid-Atlantic bays to Maine, New Hampshire, Massachusetts, Rhode Island, Connecticut, New York, New Jersey, and Delaware (Ingersoll 1881, 1887; Kochiss 1974; Carlton and Scanlon 1985). This activity has continued to the present day, largely in the form of oysters officially intended for direct consumption (but see remarks under Population Genetics, p. 700). The accidental introduction of the nonindigenous green alga, *Codium fragile tomentosoides*, from Long Island Sound to Cape Cod and to Maine in the 1960s is believed to have resulted in part from the transport of commercial oysters from New York to these more northern waters (Carlton and Scanlon 1985).

Phase II: The "South Atlantic Trade" and the "Seaside Trade"

The movement of oysters from the Carolinas to Chesapeake Bay is poorly documented in the published oyster literature. These movements are referred to by Andrews and McHugh (1957), Andrews and Wood (1967), and Hargis and Haven (1988).

Andrews and McHugh (1957) note that "prior to 1947 considerable quantities of Pamlico Sound (North Carolina) seed oysters were used in Chesapeake Bay and particularly on the seaside of Eastern shore." Andrews (1979) implied that *Perkinsus marinus* (Dermo disease) may have been introduced to Chesapeake Bay "about 1940" from South Carolina, and later stated (Andrews 1980) that "it is suspected that [*Perkinsus*] was introduced with seed oysters from South Carolina or the Gulf of Mexico prior to 1940." Andrews and Hewatt (1957) is cited as the source of that suspicion, but no such statement appears in that reference. Andrews and Hewatt (1957) conclude that "There is no proof that the disease was present in Chesapeake Bay prior to 1949. Until there is evidence of recent introduction, however, we must assume that it has been present for many years."

We are informed that in general, however, oysters from the Carolinas were transported to Chesapeake Bay only in small quantities (Andrews, Virginia Institute of Marine Science, pers. comm. 1991). In contrast, beginning about 1951, "large quantities" of oysters from seaside Virginia were transplanted to Delaware Bay (Andrews and Wood 1967; Andrews 1980). This latter activity, which extended into the late 1950s, led to the importation of MSX into Delaware Bay (Andrews 1979b). We have little doubt that the movement of oysters back-and-forth within the mid-Atlantic continues on a regular basis.

Phase III: The "Gulf Trade"

Even more poorly documented is the importation of market oysters from the Gulf of Mexico. This period is considered to have begun "about 1960" (Van Engel et al. 1966) or about 1962 (Andrews 1980; Andrews, pers. comm. 1991). Andrews (1980) implies that Gulf oysters may also have been brought to Chesapeake Bay "prior to 1940." Andrews (1970) and Hargis and Haven (1988) refer to the Gulf of Mexico trade without details. We consider the timing of the commencement of this traffic important relative to understanding whether the appearance(s) of species native to the Gulf of Mexico in Chesapeake Bay at about this time are linked to this phase of the oyster industry.

According to Van Engel et al. (1966) and Andrews (1980, 1991, pers. comm.) the motivation for turning to the Gulf of Mexico for oyster stock was the decline of oyster resources in Chesapeake Bay because of MSX disease (caused by the sporozoan *Haplosporidium nelsoni*) and by increased harvest of surviving stocks for the soup industry. Market demand and price were high, making it profitable to truck live oysters from Florida, Louisiana, and Texas (Andrews 1980). This practice continues to this day. Andrews (1980) further implies that there was a potential for the introduction of the oysters themselves (and the Gulf genomes), and oyster pathogens, parasites, and epizootics, by noting that the oysters were shucked "at waterside plants where shells and wastes were discarded near native oyster beds." Such disposal practices inevitably result in disposal of unshucked oysters in the same waters.

FAILURES AND SUCCESSES OF INTRODUCED POPULATIONS OF *CRASSOSTREA VIRGINICA*

Crassostrea virginica maintains reproducing populations in only two localities outside its natural range (Table 1): in a small river in British Columbia, and in a small basin in Hawaii. At the other locations where oysters were introduced and did not reproduce on a sustained basis (marked "not established" in Table 1), it remains possible that *C. virginica* could have been successful if oysters had been introduced in sufficient quantities over long enough periods of time. Indeed, a great many nonindigenous species fail to become established after many inoculations, only to succeed on the "n^{th}" release or invasion.

Although individual *C. virginica* can spawn at temperatures as low as 15°C (Galtsoff 1964), successful spawning typically begins at 20°C (Andrews 1979a). Mass spawning "is more likely to take place in warm water above the 22° to 23°C level" (Galtsoff 1964). It is thus probable that *C. virginica* introduced to the maritime climates of Britain (Orton 1937) and the Pacific coast of Canada and the United States rarely experienced temperatures warm enough even in summer to induce spawning. Ingersoll (1881) and Keep (1881) thus suggested that San Francisco Bay water temperatures were too cold to permit reproduction of the eastern oyster. However, Townsend (1893), aware of the successful set of the summer of 1890, concluded that the water was not too cold, but that the intertidal nature of the oyster beds that exposed spat to desiccation at low tide, plus a lack of suitable substrate, and disinterest by local oysterman in attempting cultivation were the major reasons why no large successful sets had occurred.

The general failure of *C. virginica* to develop self-sustaining populations in central California (Tomales Bay) was further investigated by Berg (1969, 1971). Although *C. virginica* undergoes gametogenesis and spawns in this region, no evidence of recruitment to the benthos is seen. Berg (1971) attributed this lack of recruitment to excessive turbidity, lack of proper food, presence of toxic dinoflagellate blooms, and, to a lesser extent, prolonged pelagic life of the larvae because of suboptimum temperatures and the absence of suitable settling surfaces.

The establishment of a reproducing population of *C. virginica* in the Nicomekl River, British Columbia (and at one time in the Naselle River, Washington), must be due in part to these regions reaching sufficiently warm temperatures, at least in some years, to permit successful gametogenesis and spawning. It can be assumed also that the factors noted by Berg (1971) are at least relaxed or absent at these localities on occasion. Shallow rivers and estuaries at otherwise colder temperate latitudes are well-known to reach high summer temperatures for short periods of time (Carlton 1979a; Carlton and Scanlon 1985). Bourne (1979) noted that "it is only in above-normal temperature years that water temperatures [in the Nicomekl River] are conducive to [*C. virginica*] larval survival."

The population in Oahu, Hawaii, is presumably successful not only because of sufficiently warm temperatures but also because of other environmental factors prevailing in the West Loch. *Crassostrea virginica* has apparently not spread from the Pearl Harbor region despite its presence there for almost 100 years.

As with all invasive species, one factor (such as temperature) rarely regulates successful establishment. The general failure of *C. virginica* to establish on the Pacific coast of North America must be only partially

due to low temperatures. The Pacific oyster, *C. gigas,* appears to require even warmer temperatures than *C. virginica* for reproduction (Cahn 1950; Loosanoff and Davis 1963; Quayle 1969) and yet it has become an abundant "naturalized" species in Washington and British Columbia.

POPULATION GENETICS AND MOVEMENTS OF *CRASSOSTREA VIRGINICA*

Mixing of Populations

The genetic structure of *C. virginica* along the Atlantic and Gulf coasts of North America has been examined by electrophoretic allozyme techniques by Buroker et al. (1979), Groue and Lester (1982), Buroker (1983), and Hedgecock and Okazaki (1984), and by mitochondrial DNA (mtDNA) analyses by Reeb and Avise (1990). (For a review of studies of oyster genetics see Gaffney, Chapter 11.) Buroker (1983) estimated genetic similarities for contiguous populations from Cape Cod, Massachusetts to Corpus Christi, Texas, as 96.2 to 99.7%, and suggested that these high genetic values are attributable to the lengthy planktonic larval life and consequent dispersal by coastal currents. Earlier, Buroker et al. (1979) estimated 82% similarity between Nova Scotia and West Florida populations. Based upon these and other biochemical analyses, four genetic "races" of *C. virginica* are believed to be found in North America: Canadian, U. S. Atlantic, northern Gulf of Mexico, and southern Gulf of Mexico (Bay of Campeche) stocks (see Hedgecock and Okazaki 1984 for review). Physiological races, based upon differences in growth, reproduction, morphology, disease resistance, and other factors, have been widely noted in the literature, and may or may not correspond to those geographic populations identified by genetic studies.

Reeb and Avise (1990) have used mtDNA analysis to show that there are primarily two different genetic stocks of the eastern oyster, represented by Atlantic coast populations and Gulf coast populations; these have been isolated for at least 1.2 million years. If Gulf coast oysters have been introduced to and become established in Chesapeake Bay in the past 30 or more years, they should be detectable through mt-DNA analysis (D. Hedgecock, Bodega Marine Laboratory, pers. comm. 1990).

These published accounts of the genetic structure of modern day *C. virginica* populations do not mention the history of the movements of this oyster along the Atlantic coast of North America nor between the Gulf and Atlantic coasts. Although it would be of interest to examine mtDNA from tissues of the oldest preserved museum material in the U.S. and in Europe of *C. virginica,* it is probable that no museum specimens predate the very old history of oyster movements, at least in central and north Atlantic states. It may thus be difficult to determine if oyster populations in certain regions (such as pre-Phase I populations north of Cape Cod, or pre-Phase II trade Chesapeake oysters) were genetically more distinct before human-mediated population homogenization commenced in the early 19th Century. Genetic mixing may have been suppressed in part, however, by the reduced ability of southern oysters to spawn in more northern waters (Andrews 1980; see also Thompson et al., Chapter 9).

Genetic Analysis of Long-isolated Populations

There are two populations of *C. virginica* that presumably have been isolated from parental Atlantic stocks for a considerable length of time. These are the populations in the Nicomekl River, British Columbia (established before 1917), and in Pearl Harbor, Oahu (established around 1893 to 1895). The population genetic structure of these populations, isolated from parental populations since the 1930s and 1940s, respectively (although there is evidence for later importations in Hawaii), would be of considerable interest in terms of the effects of bottlenecks (if such occurred) and inbreeding on population fitness and other population-level parameters. These peripheral populations would further serve as useful comparative stocks relative to assumptions about the role of gene flow in eastern oyster populations from the mainland Atlantic, Gulf coast, and Caribbean islands (Buroker 1984) in maintaining observed levels of genetic heterozygosity.

Accidental Transport of Associated Organisms

We cannot conclude this survey without referring to the most important consequence of the introductions and transfers of eastern oysters: the indiscriminate movement of oysters throughout most of the history outlined here led to the ironically successful introduction of scores of other organisms transported in the animals and on their shells (Carlton 1992).

The introduction of many Atlantic coast estuarine invertebrates as a result of the planting of eastern oysters on the Pacific coast of North America has been discussed by Carlton (1979a, b, 1987). Particularly conspicuous are the common Atlantic molluscs now established in many Pacific coast bays (Hanna 1966; Carlton 1979b; Andrews 1980), including the bivalves *Mya arenaria, Geukensia demissa, Gemma gemma,* and *Petricola pholadiformis* and the gastropods, *Urosalpinx cinerea, Crepidula convexa, Crepidula plana, Crepidula fornicata, Ilyanassa obsoleta,* and *Busycotypus canaliculatus.* All of these introductions are directly related to oyster transplantations; no other commercial shellfish were introduced in sufficient quantities to account for these unintentional releases. Other western Atlantic oyster-associated taxa now well-established in the eastern Pacific include sponges, coelenterates, polychaete worms, bryozoans, ostracods, amphipods, a crab, ascidians, and algae. Many other species have, of course, been introduced by other means (such as ballast water). In San Francisco Bay today, many of the most predominant macroinvertebrates would be familiar to a biologist trained solely in Long Island Sound.

Kornicker (1975) and Zibrowius and Thorp (1989) have summarized some of the introductions of U.S. Atlantic coastal invertebrates to southern Britain as a result of oyster movements. The most famous of these include the oyster drill, *Urosalpinx cinerea,* and the slipper limpet, *Crepidula fornicata,* the latter now having spread widely through northwestern Europe. The oyster drill is a serious predator on native and introduced oysters and the slipper limpet fouls oyster beds with its feces and pseudofeces. We have found no summary of the invertebrates introduced with oysters into Hawaii, although several Atlantic taxa (such as the nereid polychaete *Neanthes succinea*) now occur in Oahu. It is possible that a number of southern species of coelenterates, molluscs, and crustaceans currently living as far north as Massachusetts were transported accidentially as a result of the extensive transfers of eastern oysters along the Atlantic coast of North America throughout the late 19th century.

Equally critical have been the movements of diseased oysters, and thus the spread of parasites (such as the sacculinid parasite, *Loxothylacus panopaei,* of the mud crab; Van Engel et al. 1966) and pathogens along the Atlantic and from the Gulf coasts. In addition to sustained fishing pressure, the continuing 20th Century decline of the eastern oyster fishery from eastern Canada to the Gulf of Mexico has been assisted by significant disease losses (see Ford and Tripp, Chapter 17). These diseases include Malpeque Bay disease in eastern Canada, *Perkinsus marinus,* ranging from Delaware Bay to the Gulf of Mexico (Andrews and Hewatt 1957; Andrews, 1988), the sporozoan, *Haplosporidium nelsoni,* ranging from Maine to Florida (Kern 1989), and *H. costale* from Maine to Virginia (Rosenfield and Kern 1979). There is now little doubt that *P. marinus* and *Haplosporidium* spp. have been extensively moved about the Atlantic coast by oyster transfers (Andrews and Wood 1967; Andrews 1979b; Andrews and Hewatt 1957; Rosenfield and Kern 1979; Sindermann 1990). This is especially true for *H. nelsoni,* which was spread from the Delaware and Chesapeake Bays to New England with the spread of infected adult oysters.

Interstate commerce in oysters for transplantation throughout the eastern U. S. is now regulated by the Interstate Shellfish Sanitation Commission of the United States Food and Drug Administration in an attempt to prevent further deterioration of an already critical situation. In 1989 the Atlantic States Marine Fisheries Commission issued "A procedural plan to control interjurisdictional transfers and introductions of shellfish" (Krantz 1989). In this plan, the movements of oysters intended for planting in open waters would be subject to a measure of control. Interstate transport of oysters for shucking and processing, however, continues as a major commercial venture to support processing facilities affected by short-term

fluctuations in local production because of disease or public health-related closures of fisheries. How many of these oysters intended solely for "shucking and processing" find their way, accidentally or intentionally, into open waters is not known, nor does the fate of oysters said to be transported for such purposes appear to be monitored by shellfish authorities.

EPILOGUE

Much remains to be learned about the history of movements of eastern oysters, both within North America and to other parts of the world. Buried in the published and unpublished reports of government agencies and private companies are the records that would help us more clearly interpret the modern-day distribution and history of the spread of many of the species' parasites and disease organisms, as well as provide a clearer understanding of the potential that these movements had for the introduction of exotic species of invertebrates, algae, and perhaps fish.

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Chapter 21

Management of Natural Populations

CLYDE L. MACKENZIE, JR.

INTRODUCTION

From Canada's Maritime Provinces to Texas, nearly all eastern oysters, *Crassostrea virginica*, are produced from natural populations (Table 1). Before the oyster beds in eastern North America were depleted largely by overfishing, most were covered with dense populations of eastern oysters. They provided an environment for a variety of invertebrates, fish, and benthic algae; biological diversity and productivity on beds was high. Oysters also removed a considerable quantity of phytoplankton and silt from the water, and probably kept it clearer than it is now. The contrast with currently depleted beds is sharp. On these, oysters are relatively scarce, silt covers most oyster shells, and fewer associated animals inhabit the beds. Moreover, phytoplankton populations are often so dense that the increased turbidity results in reduction or absence of submerged aquatic vegetation and increased hypoxic events. Newell (1988) suggested that certain algae blooms in Chesapeake Bay are a recent phenomenon and result in part from the scarcity of oysters. He suggested that hypoxia in the deeper areas of Chesapeake Bay is related to this excess of phytoplankton and speculated that when oysters were abundant in the 1800s, they cropped most of the phytoplankton in a short period of time.

During the past 30 years, and especially since 1980, overall production of oysters from Maine to eastern Florida has declined, while their annual landed value (inflation-corrected) has remained about level. Comparable data for the Gulf of Mexico show variable production to be essentially level over the long-term; annual landed values have risen slightly (Fig. 1). The decline along the Atlantic coast was due mainly to disease, particularly *Haplosporidium nelsoni* (MSX), and *Perkinsus marinus* (Dermo), which killed most oysters in Chesapeake Bay before they could be marketed; MSX has devastated the Delaware Bay stocks. On the other hand, Prince Edward Island and Connecticut have increased their oyster production, while Florida, Louisiana, and Texas have maintained their production.

The principal goal of managing oyster populations is to optimize production to the benefit of harvesters, packers, fishing communities, and consumers. Secondarily, proper management can also benefit estuarine environments because oyster beds increase habitat structure and faunal diversity, and may reduce turbidity and hypoxia by reducing suspended silt and phytoplankton populations. Natural oyster populations can be maintained or increased by sustaining or improving the environments of oysters. Improvements involve enhancing sites for setting oyster larvae, controlling mortalities of oysters, and transplanting growing oysters to beds where conditions are more conducive for growth. This chapter focuses on processes necessary to increase supplies of seed oysters, the most pressing need in oyster management. In the future, oyster beds will require more cultivation than is common at present, and to do this efficiently, more details about bed environments will have to be understood. Once sufficient quantities of seed are produced, managing them to market size will be relatively straight-

forward in most estuaries. In those regions where MSX and Dermo are present in high incidence, care must be taken to minimize exposures to these diseases.

ELEMENTS OF OYSTER MANAGEMENT

Management-related studies should be made on a broad front, i.e., examining several possible limiting factors, rather than only one. The advantage of doing such studies is that some factors may be impractical to control, whereas one or more of the others may be controlled easily and at low cost. Moreover, the advantages of addressing multiple effects may result in an "abundance takeoff" of the oysters as was achieved in Long Island Sound in the late 1960s and 1970s (MacKenzie 1981).

Effective management must focus on understanding the factors that control local oyster abundances. Requirements to produce an abundance of seed include: (1) an adequate spawning stock size, (2) a suitable aquatic environment for larvae, (3) a suitable substrate environment for settling larvae, and (4) a suitable environment for spat and seed to survive and grow.

Adequate Size of Spawning Stock

The minimum size of spawning stock needed to produce an optimal set of oysters is unknown. In the James River, Virginia, since the outbreak of MSX disease during the late 1950s, most of the oysters have died on leased beds near the river mouth where salinities exceed 15 ppt. Consequently, the spawning stock in the river may have fallen below the minimum necessary, because annual oyster sets on seed beds gener-

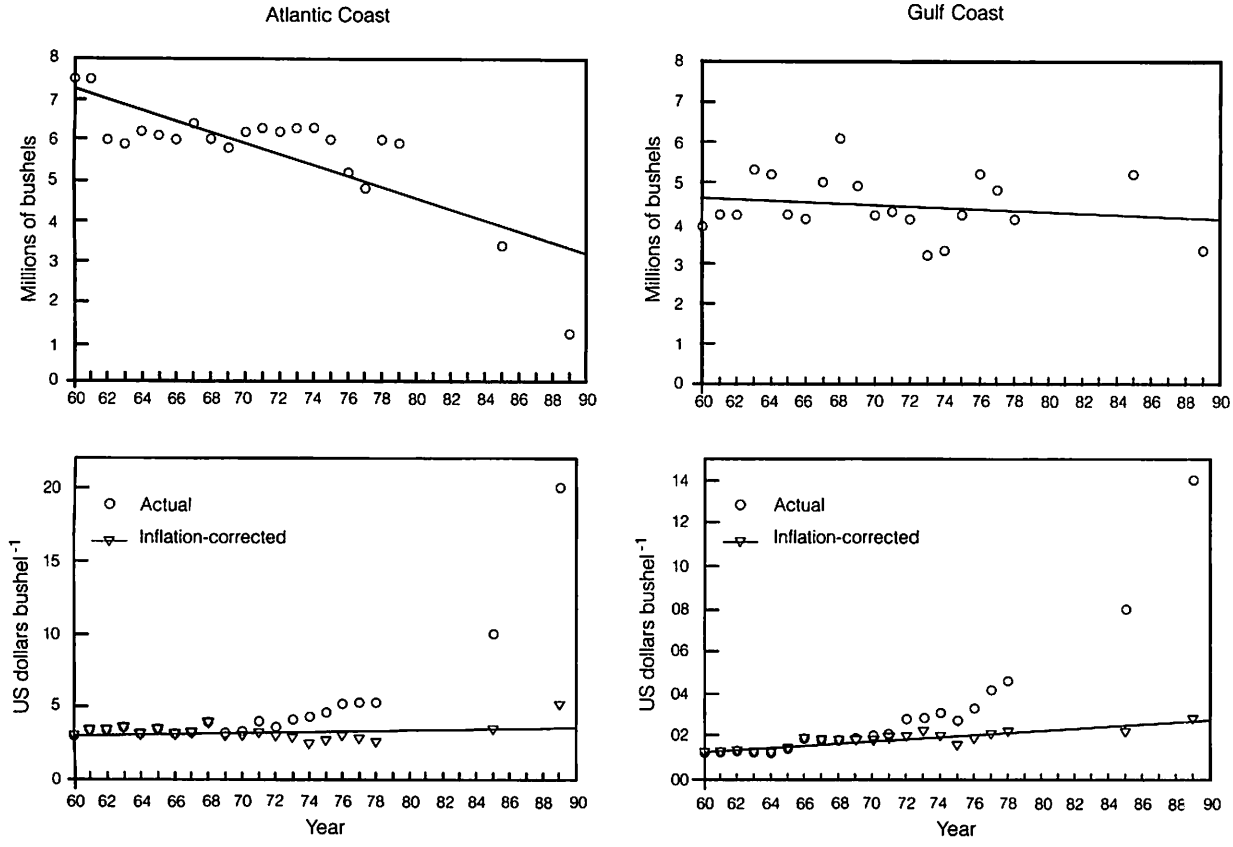


Figure 1. U.S. oyster landings and value, Atlantic and Gulf coasts, 1960 to 1989. Regression lines fitted using least squares method. One bushel = 0.036 m^3.

ally have been lower than those before the disease (Haven and Fritz 1985). Some scientists believe, however, that effects of the presence of chlorine and other pollutants may have also contributed to the reduced setting (Hargis and Haven 1988).

Given an adequate spawning stock population, no known correlation exists between spawning stock size and setting success. Long-term surveys have shown that the numbers of oyster spat may vary substantially among years even when the numbers of adults are about constant, and dense spat sets can occur even if numbers of adults are reduced. Conversely, sparse sets may result even when adults are abundant (Loosanoff 1966; Kennedy and Breisch 1981).

Suggested Management

Making the environment more conducive for larval settlement will lead to higher abundances of seed oysters and subsequently more adult oysters that will, in turn, serve as broodstock and be available for harvest. If disease-resistant oysters can be perfected (see Ford and Tripp, Chapter 17), they may be reared in hatcheries for use as spawners to produce disease-resistant populations for growing in areas where diseases are enzootic. The size of spawning beds may not have to be larger than about 20 m^3 (1 m^3 equals 27.8 U.S. bushels) of mature oysters, but no data exist to show that this is sufficient. Several such beds, at sites dispersed about a kilometer apart, could be established in estuaries where setting is poor.

Future Research Needs

The establishment of spawning beds where diseases are present requires knowledge of whether disease-resistant stocks would breed resistance into disease-susceptible natural stocks. We do not know if the ages of brood oysters should be mixed, and what the optimum age mixture should be (Kennedy and Breisch 1981). In a protandric species, such as *C. virginia*, most of the youngest oysters are males and the oldest are females. It would seem that oysters of mixed ages would produce the most fertilized eggs.

Table 1. Production of meats (kg) and value of eastern oysters havests in 1989. (Sources: Canadian Department of Fisheries and Oceans, Charlottetown, Prince Edward Island; Statistics Branch, National Marine Fisheries Service, NOAA, Washington, D.C.). Oysters not produced from natural sets were produced in hatcheries.

Locality	Thousands of kilograms	Thousands of U.S. dollars	Percentage from natural sets
Canadian Maritimes	127	4,296[a]	100
Maine	20	227	5
Massachusetts	18	512	50
Connecticut	878	14,000	100
New York	154	1,956	5
Maryland	982	7,760	100
Virginia	898	6,019	100
North Carolina	260	1,640	100
South Carolina	169	1,094	100
Georgia	21	95	100
Florida	686	3,788	100
Alabama	5	26	100
Mississippi	45	346	100
Louisiana	5,179	32,316	100
Texas	925	5,037	100
Totals (Kg and U.S. Dollars)	10,367	74,816	

[a] Canadian dollars.

A Suitable Aquatic Environment for Larval Development

Abundance of oyster larvae in the plankton has been regularly monitored at several sites over a period of years, and, at times, broods of partially-developed oyster larvae have disappeared before attaining setting size (Loosanoff 1966). Loosanoff (1966) speculated that certain phytoplankton blooms could produce external metabolites detrimental to the larvae, or that blooms of some nanoplanktonic algae (the food of larvae) disappeared and the larvae starved. Additionally, the benthic stage of the scyphozoan, *Chrysaora quinquecirrha,* and the pelagic ctenophore, *Mnemiopsis leidyi,* may prey on oyster larvae (Purcell et al. 1991), larval broods may not be coincident in time and space with adequate food, temperature may be too cool to allow for successful development, or currents might sweep larvae off the beds before they settle.

Suggested Management

If algal blooms promoted by eutrophication of estuarine waters indeed hinder the development of oyster larvae, continuing efforts to reduce nutrient loading in estuaries should be made. Similarly, pollutants such as metals (see Roesijadi, Chapter 14) and organic contaminants (see Capuzzo, Chapter 15) that are directly toxic to vulnerable juvenile life stages must be controlled.

Future Research Needs

The following questions, posed by Kennedy and Breisch (1981) regarding Chesapeake Bay, can be asked of any oyster-producing area: What are the principal causes of larval mortality and why do broods sometimes disappear before they attain setting size? Has there been a change or decline in phytoplankton species, similar to that of submerged aquatic vegetation populations? Have conditions favored less nutritious (or otherwise less desirable) algal species at the expense of "good" algal species? What influence do predators, such as planktivorous fish and gelatinous zooplankton, have on survival of oyster larvae?

A Suitable Substrate Environment for Larval Setting

A major reason for poor oyster sets and limited supplies of seed is a lack of suitable substrate for larval metamorphosis. Larvae require clean, hard, stable surfaces, preferably oyster shells, for setting (Fig. 2). Productivity on oyster beds is much higher where shells are abundant and silt deposits (Fig. 3A) and fouling organisms on shells are scarce (MacKenzie 1983). On the other hand, bay anemones, *Diadumene leucolena,* predators of oyster larvae, are commonly abundant on seed beds in Delaware and Chesapeake Bays (Fig. 3B) and, in concert with other predators of larvae, may be responsible for reduced sets (MacKenzie 1977a; Steinberg and Kennedy 1979).

In Mississippi and perhaps other states bordering the Gulf of Mexico, some oyster beds are partially covered with a layer, several centimeters thick, of a grit of oyster shell fragments less than 2.5 centimeters long (MacKenzie 1977b; Gunter 1979). The grit is in constant motion during windy periods and does not collect sets of oysters, probably because the larvae setting on the grit are killed by abrasion.

Suggested Management

Several suggestions can be made to increase setting sites for oyster larvae. One is to increase shell plantings; clam shells from shucking plants and limestone may be useful alternatives if oyster shells are scarce. Another is to increase the efficiency of planted shell by placing it only in areas of moderate-to-high setting and at the most favorable time (Hargis and Haven 1988). Yet another is to tow boards to scour silt from shell bottoms, an effective technique where there is abundant larval settlement (Fig. 4). Its use should be expanded to other oyster-growing localities where silt is an impediment to larval settlement. Hargis and Haven (1988) recommend that an efficient technology be developed to re-expose slightly buried shells before the setting time of larvae. In some areas, a weighted, rigid-tine agricultural cultivator towed over the beds might be effective in doing this.

Figure 2. Connecticut bed prepared with clean shells immediately before the setting season and in ideal condition for oyster larval settlement. From MacKenzie (1983).

Future Research Needs

The following questions need answering: At what thickness of silt accumulation on shell does larval settlement become impaired? What effect do anemones and other benthic suspension feeders have on larval settlement? When do larval anemones set and how old are they before they and other sessile suspension feeders become predators of oyster larvae? What are the annual fluctuations in abundances of sessile predators? Can the sessile predators be controlled with quicklime, salt dips, or other means non-toxic to commercial shellfish (MacKenzie 1977c; Hargis and Haven 1988)? What is the best density of shell planting for different areas? How effective is the technique of "hilling" (planting shells in lumps), which is meant to increase the setting surface area to larvae-bearing water and reduce siltation of shells (Hargis and Haven 1988)?

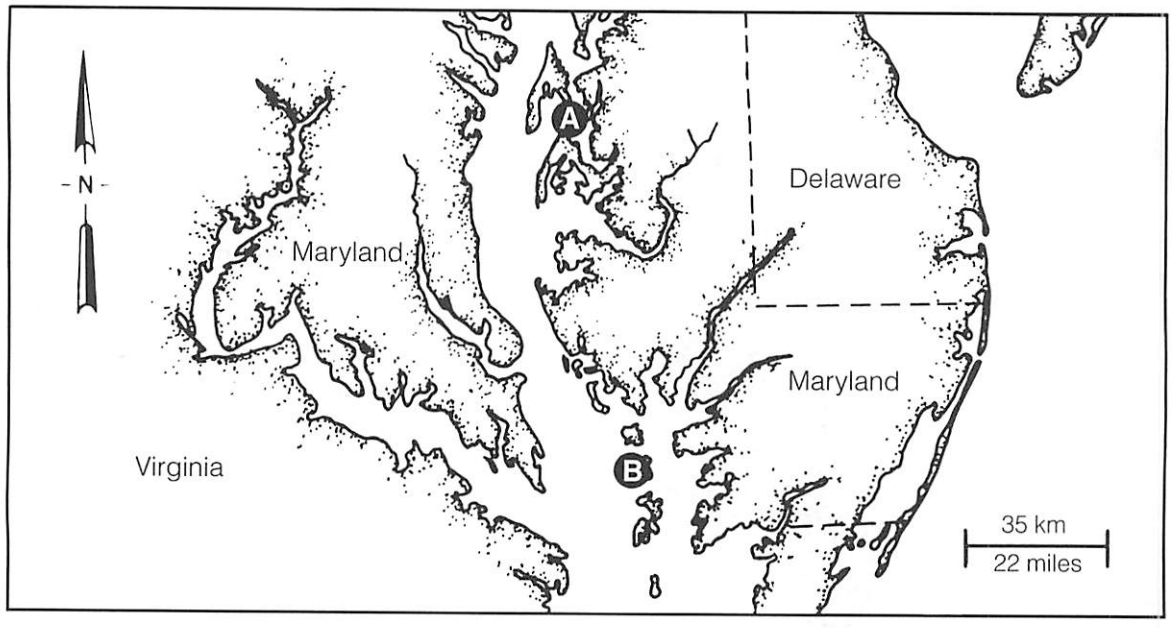
A Suitable Environment for Spat and Seed

Substantial numbers of spat are killed by predators. In Chesapeake Bay and possibly in more southern estuaries, small flatworms, *Stylochus ellipticus*, are major predators of spat 0.5 to 2.0 mm long (Newell and Kennedy 1991). Mud crabs, Xanthidae, and blue crabs, Portunidae, may be the most important predators of larger spat in salinities from 7 to 15 ppt (Luntz 1947; Menzel and Hopkins 1956; McDermott 1960; Krantz and Chamberlin 1978; MacKenzie 1981). Adult mud crabs can be abundant, numbering at least as many as 60 m^{-2}. Usually, annual mortalities of spat from mud crab predation are less than 50%, but in 1987 I observed the complete destruction of a year class of oysters apparently caused by mud crabs on beds in Delaware Bay, New Jersey. In August, three beds had good sets of spat, i.e., every oyster shell or cluster of two or three oysters (about 4 to 5 cm long) had 2 to 4 spat (4 to 5 mm long) attached. By October, however, every spat had been killed before they attained 10 mm, presumably by numerous mud crabs, the only visible predator on the beds.

In salinities above 15 ppt, besides crabs, boring gastropods, *Urosalpinx cinerea*, *Eupleura caudata*, *Thais haemostoma*, and *Melongena corona*, and starfish, *Asterias forbesi*, and *Asterias vulgaris*, kill substantial numbers of seed oysters, sometimes eliminating the oysters (see White and Wilson, Chapter 16).

Suggested Management

Probably, flatworms are difficult to control. The same is true for mud crabs because they have a small size and a cryptic habitat. Besides their role as spat predators, little is known about the role of mud crabs on oyster beds. They may keep silt from accumulating on beds by their movements and may consume a variety of oyster competitors and predators. Boring gastropods have been controlled with suction dredges where practical and starfish have been controlled



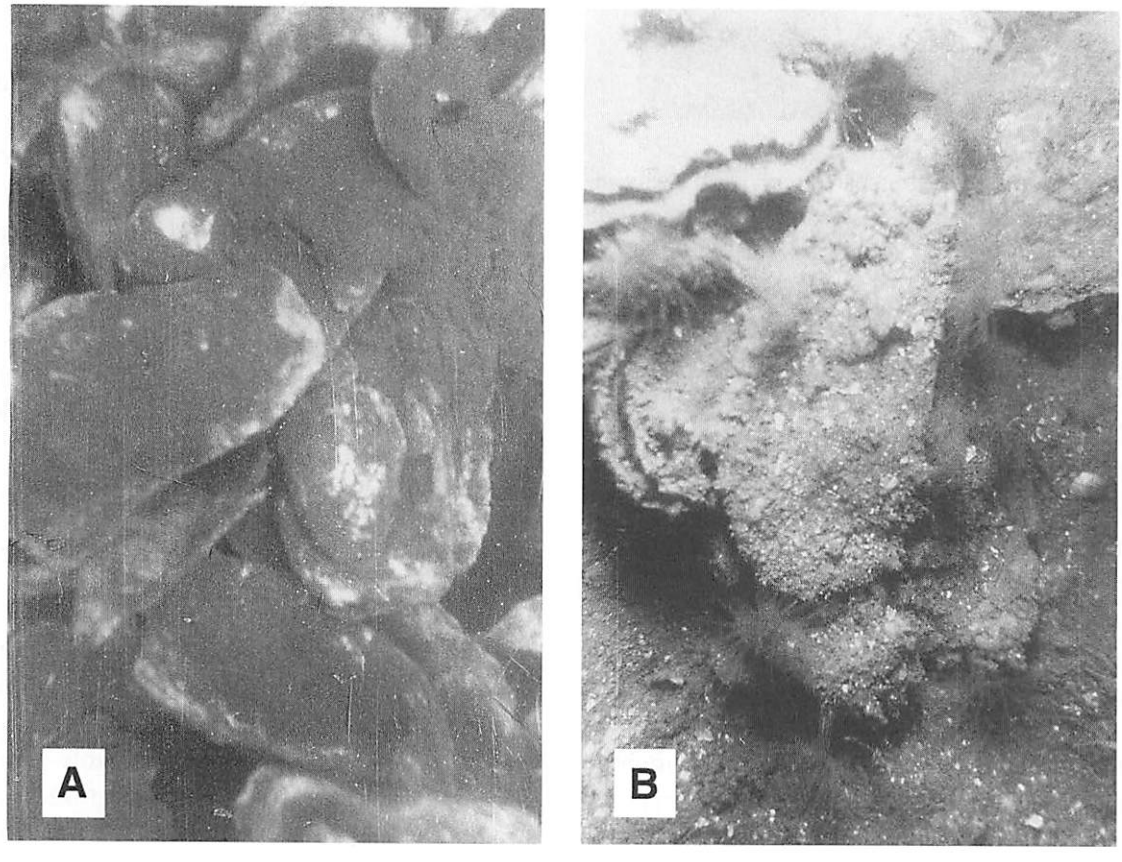


Figure 3. The condition of two beds in the Maryland portion of Chesapeake Bay. (A) Shells with silt near Parson's Island, Maryland. (B) Shells with bay anemones near Holland Strait, Maryland. From MacKenzie (1981).

with mops and quicklime (see White and Wilson, Chapter 16).

Future Research Needs

Can the suction dredging technique used to control oyster drills in Long Island Sound be used effectively in other localities (Hargis and Haven 1988)? Can additional physical methods be developed to remove predators from oyster beds? At what densities do predators cause substantial mortalities? What "buffer prey" do various predators consume? How would oyster bed ecosystems be affected if predators were eliminated?

Growing Seed to Market Size

Management of seed oysters varies by locality. For instance, in Prince Edward Island, parts of Chesapeake Bay, Florida, and Texas, oysters usually remain on the beds where they set; harvesters are allowed to gather them when they attain a length of 7.6 cm, but must leave smaller oysters and shells in the beds. Private companies in Connecticut, Delaware Bay, Virginia's portion of Chesapeake Bay, and Louisiana, usually transplant seed from beds where it has set to growing beds from which it is marketed.

From the Maritime Provinces of Canada through Long Island Sound particularly, a substantial proportion of oysters is sold for the half-shell trade. Customers prefer well-shaped oysters. For example, in 1991, in eastern Canada, the well-shaped choice grade oysters were sold to buyers for three times the price of more poorly-shaped commercial grade oysters. In the Maritime Provinces, oysters grow in clusters that consist of a few generations of oysters. Harvesters break up the clusters when they remove the market-size oysters. In the past 18 years, however, some government efforts to cultivate beds have produced commercial and standard grade oysters because clusters of the first three or four year classes have not been handled by the harvesters before they attain market size. In the future, such growing oysters should be transplanted at least a couple of times to break up the clusters.

Connecticut companies have transplanted most of their seed oysters once every year. Besides spreading the growing oysters, the practice breaks up their clusters and produces single or double oysters that are well-shaped and less expensive to cull when they attain market size. If not transplanted, the oysters would remain in clusters and assume an undesirable long, narrow shape and also be expensive to cull and pack.

Whenever Connecticut companies have had an excess of oysters, as was true in the first half of this century and again in the early 1990s, they have stored them on offshore beds at depths of 10 to 15 m to prevent them from growing too large for market acceptance. One disadvantage of the procedure is that the oyster meats become thin. To provide a supply for the market, companies transplant needed quantities to beds in depths of 3 to 6 m in the spring. By the fall marketing season, the oysters have fattened.

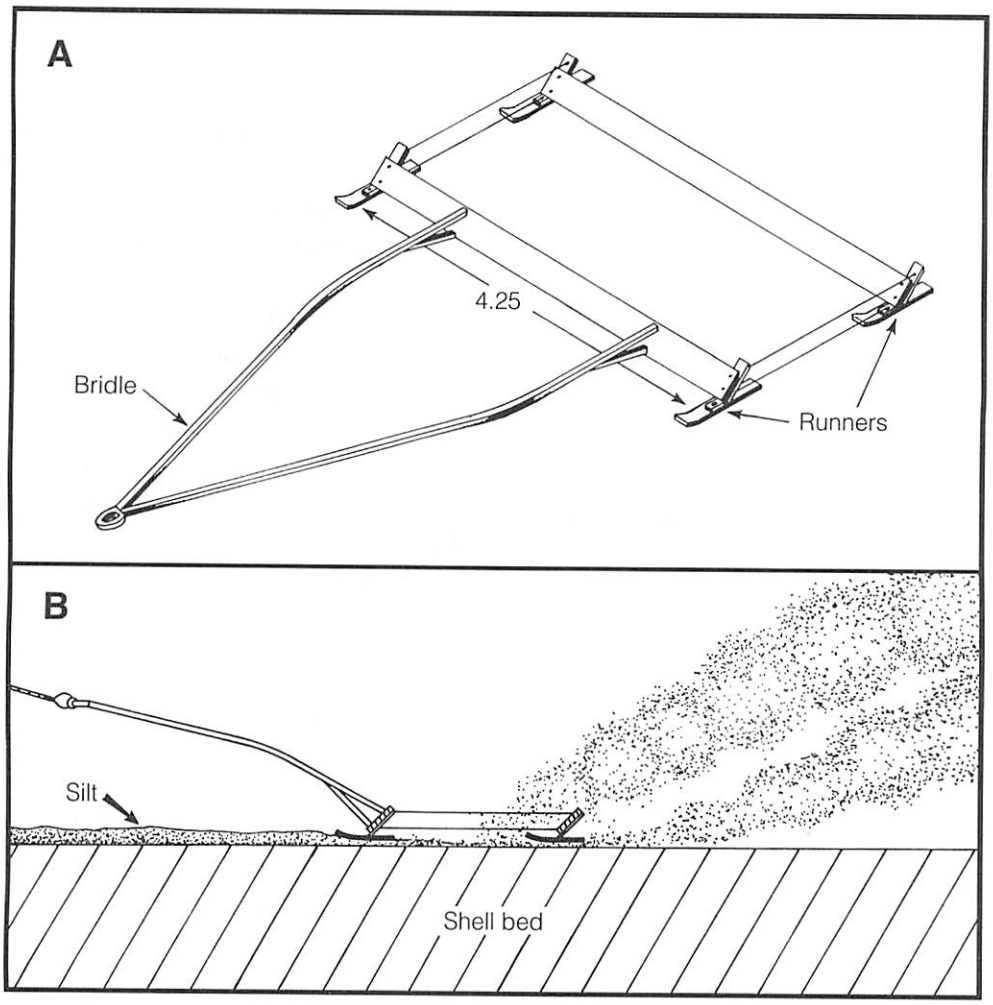
Planting densities of seed on growing beds vary from about 45 m^3 to 70 m^3 per hectare, or 2.5 acres. Oysters grow more slowly and have thinner meats at densities much above these rates, probably as a result of competition for food.

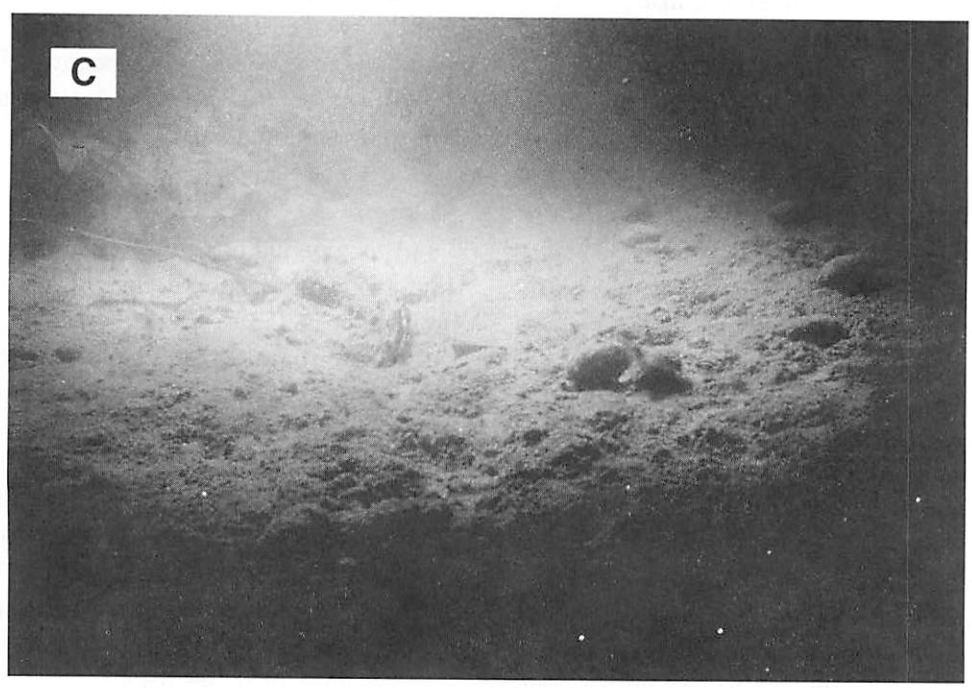
RECENT SUCCESSES IN INCREASING OYSTER PRODUCTION

A common feature in the areas that have maintained or increased oyster production is the ready availability of clean shells necessary for settlement of oyster larvae. The following examples illustrate how bed cultivation, particularly using available shells, enhances oyster abundance.

Prince Edward Island

After a period of declining landings from 1954 to 1972, when production declined by two thirds, oyster production in Prince Edward Island increased about three-fold from 1972 to 1989. Before 1972, harvesters had gathered oysters from beds that had never been cultivated. The increased production after 1972 resulted from transplanting oysters with dredge boats from a river channel and an intertidal flat, along with quantities of shells, to beds where oysters were harvested for market (MacKenzie 1975, 1989). In 1988 and 1989, the province directed government boats to tow pressure boards (MacKenzie 1983) over 24 hectares of dormant oyster beds to remove 5 to 8 mm-thick silt deposits. Subsequently, about half of the


A
4.25
Bridle
Runners
B
Silt
Shell bed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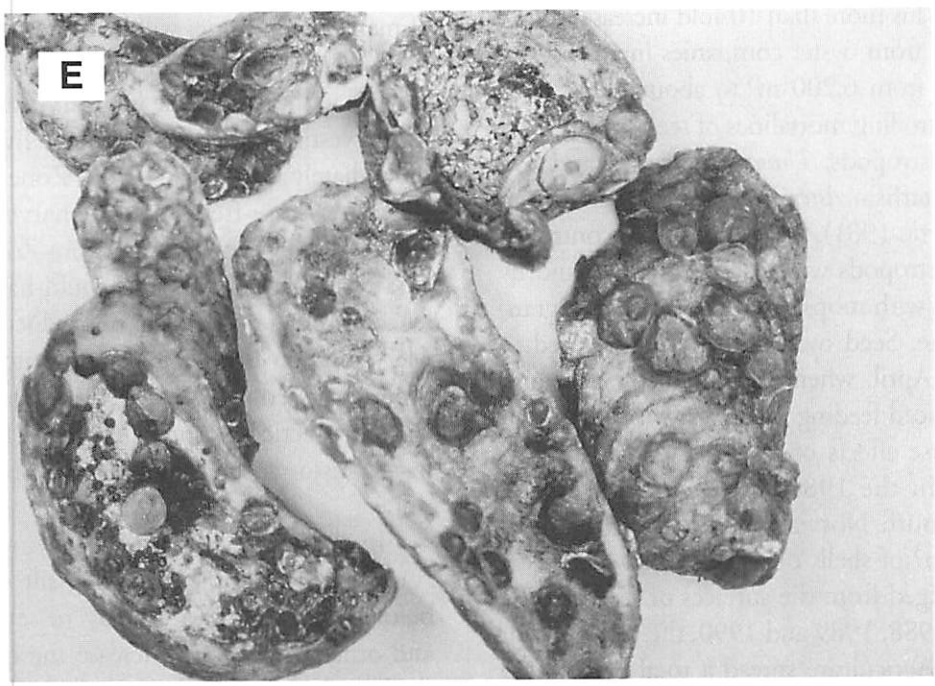

Figure 4 (opposite page and above). Cultivating shell beds to remove silt and collect an oyster set in Prince Edward Island, 1990. (A) and (B) Double board 3 m wide, which is towed over beds to scour silt; (C) View of dormant bed with deposit of silt, 5 to 8 mm thick, on shells; (D) View of bed after silt was removed; (E) Oyster spat on shells from cultivated bed.

beds received good sets, some as high as 8 to 10 spat on a shell (Fig. 4E); the remaining beds received only light sets. This desilting procedure was most successful in locations where oyster setting densities were above average. Removal of silt from shell beds is one of the least expensive means to increase oyster seed production. Another inexpensive procedure used by the province was to dredge seed oysters from the surface of a 57-hectare bed of shells that was a few meters thick and transplant them to other beds. The transplanting has been done during May and June for several consecutive years. Afterward, the remaining exposed shells were clean and received dense spat sets in July and August.

Long Island Sound

Oyster production in Long Island Sound rose from only about 85,000 kg of meats during the late 1960s to about 1,000,000 kg of meats in 1975 (MacKenzie 1989). This more than 10-fold increase in production resulted from oyster companies increasing the shelling of beds from 6,200 m^3 to about 8,000 m^3 a year and by controlling mortalities of seed oysters from predation by gastropods, *Urosalpinx cinerea* and *Eupleura caudata*, starfish, *Asterias forbesi*, and suffocation by silt (MacKenzie 1981). Mortalities were controlled by removing gastropods with suction dredges and by catching starfish with mops or killing them with granulated quicklime. Seed oysters were transplanted in March to early April. when they were still dormant. The oysters reduced feeding activity at this time minimized the adverse effects of resuspended silt in clogging the gills. In the 1980s the Tallmadge Oyster Company of South Norwalk, Connecticut, spread nearly 35,000 m^3 of shells on its seed beds each year using shells dredged from the surfaces of Connecticut oyster beds. In 1988, 1989 and 1990, the Connecticut Department of Agriculture spread a total of 100,000 m^3 of fossil shells purchased from Maryland over a 1,200-hectare public oyster bed. Since the mid-1980s, oyster sets have been widespread, but varied in density from low to high each year on the spread shells. Thus, in 1993, Connecticut beds had a stock of oysters totalling above 200,000 m^3, the largest quantity since the 1940s, and production of market oysters totalled about 3,000,000 kg of meats (J. Volk, Connecticut Department of Agriculture, Aquaculture Division, pers. comm.).

Chesapeake Bay, Maryland and Virginia

Since the late 1940s especially, many hundreds of thousands of cubic meters of shells have been spread on the oyster beds of Maryland (Kennedy 1989) and Virginia (Haven et al. 1978). The results were that oyster production increased substantially in Maryland and, to a lesser extent in Virginia. In recent years, disease mortalities of the oysters have cancelled the positive effects of the shelling.

In an attempt to restore oyster production in Maryland, various local groups concerned with oysters, including fishermen, scientists, and state administrators, prepared an enhancement plan in the early 1990s (Maryland Department of National Resources 1993). The plan calls for grounds in low salinity waters of bay tributaries to be planted with seed oysters. Three types of management zones will be established. Zone A with the lowest suitable salinity for rearing oysters will be planted with disease-free hatchery-produced seed, and no harvesting will be allowed for five years. Zone B immediately downstream from Zone A will be planted with disease-free seed, but harvesting will be allowed. Zone C downstream from Zone B will receive seed from natural bars and could have a high prevalence of disease. As hatchery production of oysters in Maryland now is negligible, more hatchery capacity for producing seed oysters will have to be developed. The current practice of spreading shells on grounds that have a history of fairly regular sets of oysters will continue.

In the James River, Virginia, oyster abundance could be increased by flushing silt off grounds just before oyster larvae are ready to set. Such desilting and other methods to increase the quantity of clean shell available on the bottom for oyster larvae would increase abundance of harvestable oysters on many low-salinity grounds in the bay.

Apalachicola Bay, Florida

Florida oyster landings in 1984 were 2,800,000 kg of meats (Berrigan 1990) with 92% coming from Apalachicola Bay (Berrigan 1988). In 1985, however,

Hurricane Elena severely damaged the bay's oyster reefs by removing and burying shells and oysters and leaving the reefs with too few oysters to harvest (Berrigan 1988). The state had developed programs to construct and rehabilitate oyster reefs by spreading shells on them over a 40-year period (Whitfield and Beaumariage 1977; Futch 1983), but the extent of damage by Hurricane Elena necessitated expanding the scope of restoration (Berrigan 1988). In 1986 to 1987, about 156 hectares were restored with a planting of 73,578 m^3 of wedge clam, *Rangia cuneata*, shells (Berrigan 1990). Oyster production increased after the planting, but not as much as anticipated. Drought conditions had produced relatively high salinities which permitted crabs, *Menippe mercenaria* and *Menippe adina*, boring snails, *Thais haemastoma* and *Melongena corona*, and protozoan disease, *Perkinsus marinus*, to spread into the beds and kill many oysters before they could reach a harvestable size (Berrigan, pers. comm.) In the late 1980s and early 1990s, a wet weather cycle returned, salinities fell, and with continued large plantings of shells, oysters became abundant again in the bay. Oysters have also become abundant in Alabama, Mississippi, Louisiana, and Texas (Dugas et al. In press).

Louisiana

During the early 1990s, Louisiana was the largest producer of eastern oysters in the U.S. with production level stable at nearly 5.8×10^6 kg of meats a year. Since 1926 the state has maintained production by spreading about 7.65×10^5 of *Rangia cuneata* shells from Lake Pontchartrain and oyster shells from reefs and oyster-processing plants over its public grounds. *Rangia cuneata* shells are preferred because they are relatively small and only a few oysters can grow on them, thus requiring minimal culling (Dugas 1988; Perret and Chatry 1988). Their low cost ($11.50 m^{-3}) in the 1980s allowed extensive areas to be shelled. In 1989, however, environmental groups caused the mining of these fossil shells to be halted. In the future, the Louisiana Department of Wildlife and Fisheries may substitute broken oyster shells or limestone, both of which are effective, though they are more expensive than *R. cuneata* shells.

Texas

From 1977 to 1988, the Texas oyster industry had a variable annual production ranging from 4.55×10^5 to 3.636×10^6 kg of meats. It is the only state that has maintained oyster production without much shell planting. From 1947 to 1982 the state created 41 oyster reefs totalling 152 hectares by the spreading of 41,255 m^3 of shells, and, in 1981 and 1989, spread a total of 6,000 m^3 of shells to collect spat (Marwitz and Bryan 1990). Dredgers gather seed oysters from public beds which lie on shell deposits at least 10 m thick. The harvesters may retain oysters of 7.6 cm or larger and must return shells and smaller oysters to the oyster beds. Apparently, the exposed shells and live oysters on the beds remain sufficiently clean to allow spat to set without cultivation.

DEVELOPING A MANAGEMENT PROGRAM

Oyster populations on public beds could be managed by shellfish production specialists, a concept used by the author off Prince Edward Island and in Long Island Sound (MacKenzie 1989). Their specific role would be to design programs to sustain and increase oyster production. By examining beds and oyster fishery operations, they could recommend to public agencies and politicians efficient methods to maintain and improve the beds. They could advise private growers who hold leased ground as well. Specialists should be first-class "field" biologists who can make proper recommendations.

Shellfish production specialists should use visual observations of the bottom to determine the potential for improving the condition of oyster setting beds. Such observations should be sufficient, but, if need be, confirmation of which factors are limiting can be obtained from several test plots on the beds immediately before the setting season. The plots should contain clean shells or oysters. The plots and surrounding areas should be examined on a regular basis for factors, such as silt or sessile predators, likely to reduce setting densities. After the setting season, seed densities and survival in the plots and surrounding areas can be analyzed.

Techniques for Examining Beds

One method for observing the bottom involves the use of SCUBA. Another is underwater video. A number of different systems may be deployed, including television, micro-TV, video recorders, and camcorders. Any of these systems can be wired to the surface for recording purposes or real time observations, or they can be deployed and operated by a diver. Video cameras can be mounted on remotely operated vehicles (ROVs). The advantages of video over still or movie photography are: instant results, longer recording times, continuous recording, and excellent stop-frame resolution. Compared with still photography, the disadvantage of video is poor resolution, so that organisms smaller than about 1 cm are difficult to identify in a video image in an area larger than 0.25 m^2. However, recent developments make it possible to digitize the video image for computer enhancement and analysis (Maney et al. 1990).

A video-equipped ROV allows an overview of oyster beds without the use of SCUBA. Low-light-level cameras are the most useful. The ROV is towed slowly over the bottom and images viewed on a monitor can be recorded. Oysters and some smaller invertebrates can be observed continuously, identified, and their behavior noted. Probes can be mounted on the ROVs to measure dissolved oxygen, salinity, and temperature.

Environmental Concerns about Cultivating Oyster Beds

Specialists may have a problem trying to restore the productivity of oyster beds because environmentalists and environmental review agencies might object to proposed actions. To better respond to questions and concerns, specialists could test a proposed method on a small scale to determine the side-effects on the environment.

One chronic problem that might raise environmentally-related questions will be the removal of silt from shell beds. Nearly all silt initially originates from runoff, especially from agricultural and urban habitats. The silt settles on oyster beds and lowers productivity by preventing oyster larvae from setting on shells. If silt was scoured from the beds just before the oyster setting season, it would resettle downstream and contribute little to what is already there because oyster beds usually constitute less than a tenth of total estuarine areas. Another manipulative operation would be to raise shells buried within the sediments to the sediment surface so they would be accessible to a set of oyster larvae. Such an operation would produce little environmental damage.

The use of quicklime to control biological fouling, anemones, and starfish is, at most, only temporarily and selectively harmful to organisms on beds. Quicklime kills only animal and plant tissues that it contacts directly. It does not harm organisms protected by shells or scales, or exposed tissues of animals such as bryozoa or sponges that it does not contact or cover if they live on the underside of oysters (MacKenzie 1977c; Shumway et al. 1988). Productivity of oysters would be increased on beds after fouling organisms, including anemones, are controlled by applying lime. Oysters would then set in larger quantity and the associated animals that settle on oysters' shells would increase in number, as is true when shells are made available by spreading them on beds or removing silt.

Manipulations might also include mechanical removal of predators such as starfish by mops and oyster drills by suction dredges. These devices would be used only for brief periods.

Because the presence of oyster populations enhances the production of the benthos and health of estuaries, their enhancement should be encouraged by environmentalists. Moreover, the presence of commercially-harvestable populations of a shellfish species offers environmentalists another justification for preserving habitats threatened by developers (MacKenzie 1991).

Formulating a Program

An eastern oyster management program should include the following elements:

1. Accumulation of data on negative physical and biological features of the beds.

2. Evaluation of available and relevant technologies and methods needed to increase oyster abundance, and anticipation of potential objections of environmentalists.

3. A plan to accomplish the objectives within a certain time period, through use of the best strategies with available resources.

Various combinations of available methods may be integrated to form a workable, productive program. A specialist lays out a tentative plan, revises certain aspects, and adds new ideas and information. If it is not possible to list a precise and definitive set of recommendations, alternative proposals with an analysis of the consequences of each is then the best approach. A few iterations of the plan will bring it into sharper focus, teach people more about it, and provide for better critiques, while enhancing communication.

Decisions to cultivate will be based on the best estimates of bed conditions made from extensive field observations in addition to consultations with harvesters, other biologists and specialists, environmentalists, administrators, and politicians. Scientific evidence documenting the environmental status of beds is desirable, but may be too time-consuming and costly to gather, and perhaps may never be available. The best available information should be used.

Careful selection of beds is important because returns will diminish rapidly if attempts are made to increase oyster abundances on beds with major deficiencies, i.e., those having soft bottoms, high abundances of predators, or being in locations susceptible to storm damage. The costs of creating favorable environments on poor beds may be excessive. Specialists should aim for a program design that will provide substantial increases in oyster abundances. The program should be conducted on as large a scale as possible, including many hectares of beds.

Years of experience have demonstrated that natural oyster populations can be enhanced by cultivation. Oyster production can theoretically be increased in every estuary although, in reality, oyster diseases are a severe impediment to restoration activities in many locations. The incentive for restoration must be based on economics: cultivation of seed beds must be economically feasible for public agencies and leaseholders. The challenge is to develop cost-effective and environmentally sound methods.

Harvesters will invest neither time nor money to cultivate public beds, and use of public funds to do this has met with some resistance. Similarly, proposals to lease public grounds to individuals so they can be cultivated privately usually have been met with strong resistance by harvesters and politicians who fear that leasing will lead to less employment on the water and reduced local income (Kennedy and Breisch 1981). However, if specialists could develop inexpensive bed-specific ways to obtain substantial increases in oyster abundances, it would then be more worthwhile to spend public funds for cultivation of public beds. If gainful employment on public beds were increased, harvesters and politicians might be willing to accept a package in which some public beds would be leased to individuals who would carry the cost for cultivating the methods and hiring the extra labor to work the beds, again leading to increased employment. Increases in oyster production would promote employment in packing houses and benefit fishing communities and consumers. With an increased stock of oysters, ancillary improvements to the environment would include increased diversity and abundance of biota and perhaps a reduction of turbidity, and the adverse consequences of eutrophication (Newell 1988).

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General Index

Species Index

This index includes all scientific names of animals other than *Crassostrea virginica* used in Chapters 2 to 21. Chapter 1 is a catalog of many species names of oysters, not all of which are used in the subsequent chapters.