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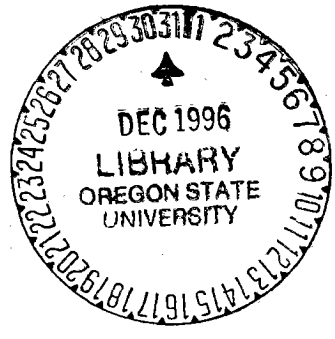
Genetic Improvement of Cultured Pacific Oysters by Selection

1996 Coastal Marine Experiment Station Annual Report



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1996 Coastal Marine Experiment Station Annual Report

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by Selection

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I. INTRODUCTION

A. The Molluscan Broodstock Program

The Molluscan Broodstock Program (MBP) is an inter-university project, funded through CSREES, United States Department of Agriculture (USDA). The program is based at the Hatfield Marine Science Center (HMSC), Oregon State University, Newport, Oregon, USA. The overall objective of MBP is to benefit commercial industry through conservation, genetic improvement and the wise management of the genetic resources of molluscan shellfish. The program received its first funds in July 1995 and additional funds were received in 1996 to continue research to December 1997. It is anticipated that MBP will be federally funded for at least 5 years after which time MBP should be mainly supported by private industry.

During the first period of funding, MBP has supported the development of a tetraploid Pacific oyster broodstock through a contract with Dr. Standish Allen, Rutgers University. A contract with Dr. Dennis Hedgecock, University of California, Davis, has provided funds to genetically screen putative Kumamoto oysters in order to establish a pure Kumamoto broodstock.

At Oregon State University, the main focus of MBP is to undertake a genetic selection program to begin the domestication and improvement of the Pacific oyster. The selection program will depend on the excellent facilities at HMSC and the long experience of researchers in oyster culture. Facilities include quarantine culture rooms, seawater supply of reliably high salinity and quality, temperature-controlled rooms for algal, larval and spat culture and a floating dock for suspended culture.

B. The Western Regional Coordinating Committee (WRCC-99)

In 1994, the Directors of the Western Regional Agricultural Experiment Stations approved the first-ever regional coordinating committee on a marine topic, WRCC-99, "Broodstock Management, Genetics, and Breeding Programs for Molluscan Shellfish." WRCC-99 has held three meetings, the first in April 1994 at Charleston, SC, the second in January 1995 at San Diego, CA, and the third in November 1995 at Newport, OR.

WRCC-99 has played an important role in the development of MBP. At its January 1995 meeting, the committee made the recommendation that MBP focus primarily on the genetic improvement of Pacific oysters by selective breeding and only secondarily on serving as a repository of stocks. The committee felt that this shift in focus would be more likely to produce tangible results relevant to industry within the expected lifetime of federal funding for MBP. Besides, long-term conservation of commercially important shellfish genetic resources would probably best be achieved by teaching industry how to manage broodstock so as to avoid inbreeding.

The main purpose of the November 1995 meeting in Newport was to design a genetic improvement program for Pacific oysters that could be achieved with MBP's facilities and funding. This document is largely the outcome of that meeting and represents the consensus of participants, which included, besides members of WRCC-99, the Executive Board of MBP and industry representatives (Appendix A).

C. Rationale for Selection of Cultured Pacific Oysters

Farming of the Pacific oyster along the U.S. West coast annually produces approximately $35 million worth of product (Toba and Chew 1995). At present, markets are expanding particularly in Asia (T. Smith, Executive Director, Pacific Coast Oyster Growers Association, pers. comm.), but the available oyster-growing grounds are shrinking on the West coast, primarily because of urban development and increasing pollution. Increases in production will have to be achieved by increases in production efficiency. Genetic improvement by selective breeding is one obvious means of increasing production efficiency.

Another rationale for undertaking a selection program is that farming of the Pacific oyster along the U.S. West coast depends almost exclusively on hatchery-propagated seed. An

exotic species imported from Japan to the U.S. West coast beginning in the 1920s, the Pacific oyster cannot reproduce along much of the West coast due to low summer water temperatures and has naturalized in only a few bays, where conditions permit successful spawning, larval development and retention, and setting (Mann 1979; Chew 1984). Thus, closed populations of Pacific oysters can be established in many bays and estuaries without the risks of genetic contamination from unselected wild stocks.

Commercial shellfish hatcheries generally propagate oysters by mass spawnings and large-scale, mixed-family larval cultures. Such methods of propagating a species with enormous fecundity and high early mortality can lead to severe restrictions in the effective population sizes of hatchery stocks (Hedgecock and Sly 1990; Gaffney et al. 1992; Hedgecock et al. 1992) and preclude controlled matings and pedigree information that would greatly facilitate the development of a selective breeding program. Facilities for rearing a large number of families do not exist in most universities either, so lack of appropriate hatchery facilities to spawn and rear families has represented a major constraint on the development of genetic improvement programs. Funding for MBP at HMSC, with its facilities for spawning and rearing a large number of families, has now removed this constraint.

MBP is thus in a position to emulate the highly successful genetic improvement program for the Norwegian salmon (Gjedrem et al. 1989, and pers. comm. at the January 1995 meeting of WRCC-99). This program, which was initiated with government funding in 1972, has achieved gains of 10-15 percent per generation in growth rate and has dramatically improved resistance to specific pathogens. The genetic gains evident by the third generation induced the Norwegian fish farmers to build their own breeding center in 1986.

D. Related Projects and Funding

MBP and the Pacific coast oyster farming industry have a unique opportunity to leverage the substantial support that the U.S. Department of Agriculture has provided in recent years for scientific research on the genetics of Pacific oysters. Besides WRCC-99 and MBP itself, the USDA has funded two projects on the development of Pacific oyster broodstocks through the Western Regional Aquaculture Consortium (WRAC; investigators Hedgecock, Hershberger, Langdon, Robinson, Manahan, and Cooper) and a project funded by the National Research Initiative Competitive Grants Program (NRICGP) on the genetic and physiological bases of hybrid vigor in the Pacific oyster (investigators Hedgecock,

Manahan, Bayne, and Cooper), which has just been renewed for another three years. This unprecedented level of support for research on Pacific oysters permits MBP to act as a conduit for the direct transfer of research on genetics to the selection program and, through industry participation in MBP, to the improvement of commercial stocks.

II. GOALS OF SELECTION

A. Industry Priorities

Prior to the November meeting of WRCC-99, Mr. Bill Dewey, Taylor United, conducted an informal survey of 16 shellfish companies to solicit their ideas about what traits should be selected. Of the companies surveyed, eight were located in Willapa Bay/Grays Harbor, six in Puget Sound, and one each in Oregon and California. Most of the companies were producers of shucked meats, but some were producers of half-shell product and one was a hatchery. Respondents were asked to rate thirteen characteristics as having low, moderate, or high priority for genetic improvement. The traits with the highest proportions of high priority ratings were meat-yield (9/10 responses) and fast growth (11/16 responses; Table 1). Longer shelf-life, which is important to producers for the half-shell market, received high priority ratings from 9/16 respondents. The remaining traits did not receive a majority of high priority ratings, although disease resistance was of high priority to growers in areas affected by summer mortality.

TABLE 1.
MOST IMPORTANT OYSTER CHARACTERISTICS FOR SELECTIVE BREEDING OF PACIFIC OYSTERS ON THE WEST COAST, U.S.*
*Data collected by Mr. Bill Dewey, Taylor United, 10/95

OVERALL CHARACTERISTIC	PRIORITY LOW	MODERATE	HIGH	% SCORE
Meat yield		1	9	**93**
Growth (faster)	3	2	11	**60**
Better shelf life (live)	2	5	9	**59**
Shell shape - depth	2	7	7	**56**
Resistance to summer mort.	7	3	6	**56**
Spawn at warmer temp.	5	4	7	**52**
Shell shape - L x W	3	10	3	**48**
Disease resistance	7	3	6	**47**
Mantle color	7	7	2	**41**
Meat color	5	8	3	**41**
Spawn at cooler temp.	10	3	3	**38**
Hinge characteristics	8	5	3	**36**
Growth (slower)	13	2	1	**30**

B. Scientific and Practical Constraint on Goals

Only traits that can be measured, that are variable, and that have a heritable component of variation can be improved through selection. Determining the heritability of traits, however, involves large mating experiments that are difficult and time-consuming to execute for oysters. Although information on heritability is lacking for most traits included in the industry survey, some of the traits are not likely to be highly heritable. Shell-shape and shelf-life, for example, are strongly determined by environment or culture conditions (Galtsoff 1964). Fortunately, previous WRAC research has shown that the traits of interest to industry, growth and meat-yield, can be measured in mating experiments and may well have heritable variation. Individual wet meat-weight at harvest, which was studied in the first WRAC project, appears to have a heritability of about 0.2 (Hedgecock et al. 1991, 1993a), although this estimate lacks precision primarily because of the small number of sires used in these early WRAC mating experiments.

Practical constraints also limit the number and kind of traits that can be considered in a selection program for oysters. Evaluation of traits must be highly simplified so that a limited labor force can make observations and measurements on a large number of oysters. For this reason, reproductive traits like age at maturation, fecundity, and spawning season are very difficult to measure and will not be considered initially in the MBP selection program. Traits associated with size and growth are more tractable to measure, so MBP selection will focus primarily on the improvement of growth and meat-yield, noting survival, sex-ratio, mantle-color, and hatchery performance as covariates, or auxiliary traits.

C. Survival, Growth, and Meat Yield at Harvest

Survival of families of oysters is easily estimated at various stages in the grow-out period, as detailed in section V.E. Growth is estimated by measurements of size or weight measured at two or more times, so that gain over a specified interval of time can be estimated. We will determine growth as the increment in live weight in the second year, from approximately 8 months of age to market size, estimated to be at approximately 18-22 months of age depending on grow-out conditions. At market size, destructive sub-sampling of families will be carried out to estimate sex-ratios, wet meat-weights, and meat volumes to provide data for estimation of meat-yields. In previous WRAC experiments, meat-weight was found to depend on sex, females being 16 percent heavier than males

when measured at the peak of sexual maturity (Hedgecock, unpubl.). It is not known whether this sexual difference in weight is due to differences in volume or meat density; thus, the growth performance and meat-yield of a family should be corrected for sex-ratio to avoid selection for all-female families.

D. Interaction of Genotype and Environment

Another important issue in genetic improvement programs is what geneticists call "genotype by environment interaction" or more simply, "GxE", from the statistical notation for this interaction term in an analysis of variance. A GxE interaction component of variation in yield arises when the rank order of family performances changes depending upon grow-out site. A large, significant GxE component was reported, for example, in the juvenile growth of hard clam families in five different locations along the Atlantic Coast (Rawson and Hilbish 1991). Changes in the relative performance of families across environments could impede selection for a stock that was superior for most sites on the West coast.

Proper evaluation of GxE interaction, however, demands large experiments, across many environments. MBP cannot hope to conduct experiments of this size in the near future; however, MBP families will be tested in three or four high-productivity environments. Some impression certainly will be obtained over the first several years of whether GxE is large when families are reared on what the industry regards as good oyster ground. In this way, MBP may also effectively minimize GxE interaction that arises by comparing performances of families in good and marginal habitats. In marginal habitats, genetic components of variation in yield are often significantly reduced or modified (Rawson and Hilbish 1991), giving rise to a significant GxE term. Yet, this source of GxE interaction is of little commercial significance and should be ignored in genetic improvement programs by testing only at productive sites in the first place (T. Gjedrem, pers. comm.).

III. SOURCE POPULATIONS

A. Geographic Races or Sub-populations of the Pacific Oyster

Four major geographic races of Pacific oysters were recognized in Japan by early taxonomists and biologists (e.g., Amemiya 1928; Imai and Sakai 1964; Numachi 1974): Hokkaido, Miyagi, Hiroshima, and Kumamoto. The Kumamoto race is now recognized to

comprise two species, a local race of the Pacific oyster *C. gigas* (Thunberg), and the Kumamoto oyster *C. sikamea* (Amemiya) (Banks et al. 1994). Although Imai and Sakai (1964) showed that the Hokkaido race outperformed the Miyagi oysters in Gig Harbor, the Miyagi race has become the predominant stock propagated by the West coast oyster industry. Kumamoto oysters, which may be extinct in Japan, are propagated in small numbers by the West coast oyster industry, but broodstocks are threatened by hybridization with the Pacific oyster and loss of genetic diversity through random genetic drift in small populations (Hedgecock et al. 1993b). Identification of pure Kumamoto oysters by molecular markers is an objective of MBP in its first year of funding.

B. Industry Stocks and Sources

Oyster farmers on the West coast have used primarily the Miyagi strain for production since the first major importation's of Pacific oysters in the 1920-30s (Bourne 1979; Chew 1979). The Hokkaido and Hiroshima races were tested by some farmers at various times but were never adopted (L. Weigardt, pers. comm.). A consensus decision by the WRCC-99 committee was that MBP ought to focus on the improvement of the Miyagi-derived Pacific oyster presently being cultured by the West coast industry rather than undertake extensive testing of other races that the industry has already discarded. In future years, once the improvement program is well underway, MBP could undertake some well controlled comparative tests of the Hokkaido and Hiroshima races to verify industry's perceptions.

Up until the 1970s, except during World War II, the oyster industry relied heavily on the importation of wild oyster-seed from Japan (Chew 1979, 1984). During the 1970s, however, domestic seed production became increasingly available from naturalized oyster populations in British Columbia and Washington, while the supply of seed from Japan was decreasing, and its cost was increasing. Yet, unpredictable failures of domestic seed production, such as occurred in 1969 and 1976, set the stage for the development of oyster hatcheries (Breese and Malouf 1975). After the spread of methods for remote-setting of hatchery-produced eyed-larvae (Jones and Jones 1983), the industry came to rely almost exclusively on hatchery seed. This reliance on hatchery seed has in turn set the stage for genetic improvement of broodstocks. Nevertheless, closed commercial hatchery stocks of the Pacific oyster have small effective population sizes (Hedgecock and Sly 1990; Hedgecock et al. 1992), which makes them undesirable source populations for a genetic improvement program.

The committee therefore recommended that the naturalized, self-recruiting populations of Pacific oyster on the West coast should serve as sources of broodstock for MBP. Although these populations may naturally undergo some genetic drift (Hedgecock 1994), they have relatively large effective population sizes (the N_e of Dabob Bay is estimated to be 400; op. cit.) and levels of genetic diversity not significantly different from those of native Japanese populations (cf. allozyme frequencies given by Buroker et al. 1979, Ozaki and Fujio 1985, Hedgecock 1994).

C. Initial Focus on Dabob and Willapa Bays

Various locations along the Straits of Georgia, British Columbia, particularly Pendrell Sound, have natural spat-falls of Pacific oysters and serve as seed sources (Quayle 1988). In the United States only two locations, Dabob and Willapa Bays, in the state of Washington have large natural seed-sets. Both locations serve as sources of natural seed or broodstock for the industry, but oyster farmers believe that there is a difference in the quality of seed from the two sites.

Except for a quantitative genetic study of these two stocks, which did find evidence of genetic differentiation (Pongtahna 1987), there has been little genetic characterization of the Willapa Bay population. The growth of progeny from Willapa Bay, Dabob Bay, and hatchery broodstocks were not noticeably different in the early WRAC crosses, although these were not designed as comparative experiments (Hedgecock, Cooper, Hershberger, and Guo, unpubl.). On the other hand, as noted above, the population genetics of the Dabob Bay stock has been fairly well studied. Also, most of the inbred lines under investigation in the current WRAC and USDA NRICGP projects were derived from this Dabob Bay population.

The committee recommended that broodstock be selected from Willapa and Dabob Bays for the initial crosses in 1996, and that four hundred adults from each bay should be collected and moved to good fattening areas in December of 1995 to improve their condition for spawning in 1996.

IV. MATING DESIGN FOR SELECTION

A. Breeding Design for Improving Yield

Overall design:

During spring and summer of 1996, 100 full-sib families will be reared in the MBP hatchery through larval stage and metamorphosis (sequentially in two groups of 50 families). Spat from each family will be placed in replicate mesh bags for grow-out to market size at each of three or four commercial sites. Families will be ranked by average growth and survival to market size. Individuals from the five best-performing families in each cohort of 50 (i.e. 10 best families) will be crossed to found the next generation, and so on in subsequent generations. Oysters mature in approximately 18 months so selection will be on a two year cycle. Consequently, another two sets of 50 families (source populations chosen as discussed above) will be used to start an alternate-year selection line beginning in spring of 1997.

Family-level vs individual-level selection: The standard approach for breeding livestock is to raise individuals of known pedigree, and then to select based on an index of performance for each individual, which is a weighted combination of the individual's performance and the performance of his relatives. Unfortunately, oysters must be grown in groups, and they compete for resources, so they cannot be considered independent data points for this type of selection. Therefore, selective breeding of oysters has more in common with plant breeding, where individuals are grown in competition, the unit of replication is not individuals but plots, and the unit of selection is yield per plot (Hayward et al. 1993). Consequently, we will grow oysters at a standard density in bags, and family performance will be measured as the mean performance of replicate bags in each family. Here performance equals total weight of oyster meat per bag at harvest, which will, in turn, be dependent on both survival and tissue growth.

Full-sib selection vs. more complicated designs: Of the breeding designs that do not involve inbred lines, selection based on the performance of paternal half-sib families is the most commonly used design (usually a nested design in which each sire is mated to a different set of females; Pirchner, 1983; Falconer, 1989). The expected variance among paternal half-sib averages, $V_{hs,} = 1/4(V_a)$, where V_a = the additive component of total genetic variance. In contrast, the variance among full-sib families, $V_{fs,} = 1/2(V_a) + 1/4(V_d) + V_m$ + small fractions of the various interaction variances, where V_d = genetic variance

Additive variance controls the response to selection, so full-sib family selection could be led astray if large dominance, interaction, or maternal variances cause some families to appear better than they ought to, based on their true breeding value (which is the additive component of deviation from the population mean). Nevertheless, full-sib selection is more appropriate for our purposes for the following reasons:

1) It allows us to start with the largest sample of the underlying genetic diversity in the source population. For example, compare 99 full-sib families vs. 99 families created by crossing each of 33 sires to 3 different dams. In the first case you sample 198 individuals (396 alleles per locus from the source population) while in the second case you sample 132 individuals (264 alleles). This is a very important concern in a breeding program like ours where practical constraints limit us to a relatively small number of families each generation (50-100 families).

2) Full-sib families make it easier to control the rate of inbreeding in later generations because we begin with a larger number of independent founders.

3) Creating full-sib families is much less complicated for the hatchery, entails less risk of cross-contamination of gametes, and gives a design that does not become unbalanced if some families die out in culture.

4) One needs a large number of dams per sire (usually 15-20 or more) to estimate sire breeding values with any precision (Osborne 1957; Falconer 1989). Given MBP is limited to raising 50-100 total families per generation, we could only use at most 3 or 4 dams per sire. When heritability is greater than about 0.05, the rate of response to selection among full-sibships is expected to be much greater than the rate for selection among paternal half-sibships when such a small number of dams are used (assuming only additive effects; Osborne 1957).

5) While maternal effects may be pervasive for early larval traits (Lannan 1980a) they can probably be safely ignored for adult growth. Although non-additive genetic effects may be particularly large in bivalves (Pogson and Zouros 1994; Hedgecock et al. 1995), non-additive components of variance are usually much smaller than the additive components (Falconer 1989), and the coefficient for the V_a term (1/2) is twice that of the largest coefficient for any of the non-additive effects (1/4 V_d). So even in the face of large non-

additive effects we will still see response to selection. It will just be slower than predicted from the among-family variance in performance under an assumption of strictly additive gene action.

The nature of the gene action underlying the dramatic heterosis evident in crosses among inbred lines of Pacific oysters (Hedgecock et al. 1995) is the focus of the continuing NRICGP project. In addition, we anticipate that a continuing WRAC project will be able to look specifically at the non-additive genetic components of yield variation among MBP families. Should response to full-sib family selection prove disappointing in the early generations, these ongoing USDA-funded research projects will enable MBP to evaluate non-additive genetic components of variation in yield and, if necessary, to incorporate crossbreeding as a strategy for improvement.

6) Plant breeders routinely see substantial gains under full-sib selection (Moreno-Gonzalez and Cubero 1993) so the main argument in favor of the full-sib family selection approach is that it works.

B. Numbers of Families and Bags

Optimum number of families and replicates per family: The phenotypic variance among mean values for family bag scores can be summarized by the intra-class correlation, t, whose variance is minimized as the number of bags per family, n, and the number of families, N, goes to infinity: $t = \sigma^2_b / \sigma^2_p$, where σ^2_b = the between family component of variance, and σ^2_p = the total phenotypic variance (again, among bag scores). Interestingly, when the product nN is constrained, the variance of t is minimized when $n = 1/t$ (Falconer 1989). Unfortunately, we will not know t until we have raised the first generation of families. Nevertheless, we can make a best guess at the optimum N and n if we assume additive effects and guess at the heritability of the trait (in this case heritability is for phenotypic variance among bag scores, not among individuals). Assuming additive effects only, σ^2_b = the covariance among full-sibs $=1/2(V_a)$, so $t = 1/2\ (V_a)/\ \sigma^2_p$ = one half the heritability, h^2.

Therefore, the optimum number of replicates per family for various possible heritabilities is as follows:

h^2	n
0.05	40
0.10	20
0.20	10
0.50	4

Attempts to estimate the heritability of growth rate (among individuals) in oysters have yielded values ranging from 0 to 0.2 (Hedgecock, pers. comm.), which is in line with heritability values estimated for growth rates in most livestock species (Pirchner 1983; Falconer, 1989). Therefore, we should use at least 10 bags per family during the first generation of the project. Once we have an empirical estimate of t, we can better optimize the number of families and replicates per family in subsequent generations. Given few commercial growers have the resources to care for more than 500 bags at a time, we will begin with 50 families and 10 replicate bags per family at each grow-out site.

Is 50 families enough? Although 50 families (100 parents) is not a very large sample of the genetic variation in the source population, our experimental design allows us to make the most of this sample. Firstly, by practicing pair-wise matings and raising the same number of bags per family we are keeping the variance in family size = 0, so the variance effective size of the selected population is actually twice the census size each generation (Hartl and Clark 1989). As a rule of thumb, one should keep the effective size of a selected population above 20-30 individuals in order to prevent chance fixation of unfavorable alleles owing to genetic drift (Rawlings 1980), so drift will not be a problem for us. Secondly, pair-wise matings and a complete pedigree on every individual in the experiment (to be additionally verified using microsatellite markers and allozymes), allow us to precisely control the rate of inbreeding in later generations. Also, we will be crossing between the two cohorts of 50 families within each year class, and can ultimately cross between even/odd year classes in later generations when the inbreeding and effective size of our broodstock populations become unacceptably small.

C. Second and Subsequent Generations

Second generation crosses: Family mean performance will be estimated after adjusting for block effects within site, and then overall performance across sites estimated as discussed above. The top 5 best-performing families will be chosen from each cohort of 50 raised in 1996 (which gives a selection intensity, i, of 1.7 within each cohort). Representatives from each chosen family will be crossed with representatives of each of the nine other families (hereafter "primary lineages"). Thus, we have a 10 x 10 factorial cross in which the rows and columns are 1° lineages (no crosses along the diagonal, of course). Note that individual crosses are strictly pairwise. Different individuals from each lineage are used in each cross involving that lineage. Crossing 1° lineages factorially has the benefit of ensuring that each of the original top performing families is represented equally in the second generation. Again, equalizing founder representation slows the rate of genetic drift and makes it easier to avoid inbreeding later on. Note also that this approach generates 90 families, which can be created in two sets of 45 to run through the hatchery as two independent cohorts.

In following generations we'll again select the top 5 families per cohort (i =1.6), which generates 90 families per generation, and so on in each following generation (given constraints described below).

Third generation and later crosses: From this point onward, crosses will be made among top performing families given the following constraints, in order of importance: (1) keep the rate of inbreeding below one percent per generation, which is the maximum rate below which livestock breeders typically observe notable inbreeding depression (Franklin 1980). (2) Keep the variance effective population size above 30. (3) Keep the selection intensity above 1.5 per generation. Note also that we will have two selection lines going in parallel, the even and odd year classes. At some point in the future, when it becomes difficult to satisfy the above criteria, we can cross between year classes.

The expected response to selection (gain) under family-level selection will be:

$$R_f = ih^2_f\sigma_f$$

where σ_f = standard deviation of family means, and h^2_f = heritability of family means.

Assuming i = 1.7 per generation and $h^2_f = 0.10$, then $R_f = 0.17\sigma_f$. So, for example, if

family mean wet weight = 10 g and $\sigma_f = 2.6$ (from unpub . data by Lannan for oysters grown on long lines, and ignoring an obvious need for log transformation), then we would expect a gain of 0.44 g in the next generation (4.4 percent gain). If $h^2_f = 0.20$, we'd see a gain of 0.88 g.

After the first generation of crosses, when we will have real data to estimate h^2_f, we will be able to better predict the expected rate of gain.

D. Control Populations

Evaluating the progress of any selection program necessitates comparison to an appropriate, unselected, control population (Fredeen 1986). Devising control populations for selected lines of Pacific oysters is made difficult by the large phenotypic and genetic variation in oyster growth (Hedgecock et al. 1991, 1993a), by the impossibility of identifying and rearing individuals independently, by the danger of genetic drift and inbreeding effects in closed, finite populations (Hedgecock and Sly 1990), and by the constraints on MBP hatchery and nursery capacity for rearing families separately. The committee decided that the most practical control would be to spawn, in each generation, individuals obtained from the same natural population from which the selected line was originally derived (i.e. Dabob Bay or Willapa Bay), and to make 10 full-sib families by pair-wise matings. These control families would be stocked in a minimum of 10 bags, just like the families in the selected lines, and included in the growth trials on commercial testing sites.

Another opportunity for comparing MBP stocks to a control population will come when selected MBP broodstocks are transferred to commercial hatcheries for multiplication and distribution (see Section VI.C.). The industry multipliers will be asked to culture, in parallel, both mass spawns of MBP broodstock and mass spawns of typical commercial broodstock. If oysters from these mass spawns are tracked from the hatchery to the shucking house or market, MBP will be able to compare the yield of their select stock to existing, unselected, industry stocks, on a very large scale of production.

V. CULTURE METHODS

A. Broodstock

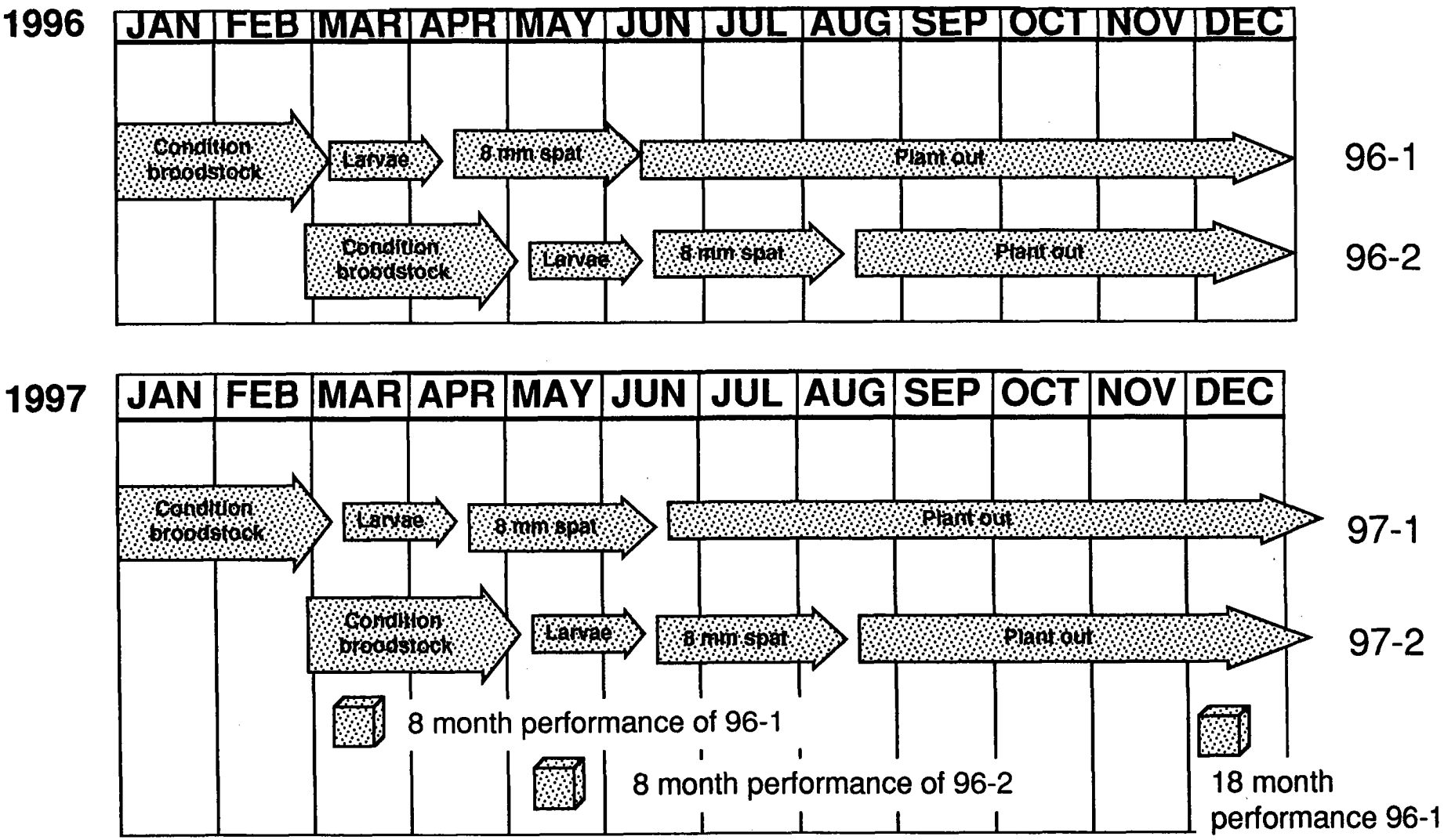
This report was written in the summer of 1996 and includes culture methods recommended by the WRCC-99 committee for producing full-sib families as well as some reports on the application of these culture methods. An outline of the sequence of tasks is given in Figure 1.

Two sets of broodstock will be managed for spawnings in 1996 (see section V.G.), one from naturalized beds in Willapa Bay and one from Dabob Bay. In December 1995, approximately 400 naturalized broodstock oysters were collected from inter-tidal grounds adjacent to the Willapa Bay Wildlife Refuge, south Willapa Bay. A similar number of Dabob oysters were collected in December 1995 from Dabob Bay from inter-tidal grounds of Mr. Dick Steel adjacent to Dabob. The two groups of oysters were transferred to Dahman Oyster Co., Totten Inlet, south Puget Sound for "fattening".

In February 1996, examination of the Willapa Bay broodstock held at Totten Inlet indicated that they possessed low glycogen reserves and were not in good "condition"; therefore, an additional 400 animals were kindly collected by Dr. Brett Dumbald and colleagues (Washington State Department of Fisheries) from sub-tidal grounds in southern Willapa Bay and transferred to Totten Inlet. It was anticipated that sub-tidal oysters would be in better condition than oysters collected from crowded inter-tidal grounds. Unfortunately, even the sub-tidal Willapa oysters were not in sufficiently good condition to be used for production of the first set of families and, therefore, they were kept at Totten Inlet for production of the second set of families in 1996.

In March, Dabob Bay oysters were collected from Totten Inlet for conditioning in temperature-controlled (20 °C) systems at HMSC. However, these oysters appeared to be in poorer condition than when first collected from Dabob Bay, perhaps because of the adverse effects of disturbance on their condition; therefore, an additional 400 animals were collected from Dabob Bay in mid-March and transferred directly to a conditioning system at HMSC. The two groups of Dabob Bay broodstock oysters were conditioned in flowing seawater at 20 °C and fed on algal supplements for about one month before being stripped spawned.

Figure 1. Schedule of MBP



In future years broodstock will be collected directly from the place of origin for conditioning at HMSC as transfer to "fattening" grounds late in the year does not appear to be effective in improving condition. An alternative approach would be to condition broodstock over winter on more productive grounds, such as the commercial facility based at the OTEC site, Hawaii. This annual cycle of winter conditioning, spring and early summer spawnings, which is slightly accelerated, compared with the natural cycle, to allow for good growth of seed in the first summer, is likely to be maintained by MBP for at least the first two generations.

B. Spawning and Larval Culture

Variation in conditioning response and larval performance appears to have a large genetic component (Lannan 1980a, Lannan et al. 1980b, 1980c, Muranaka and Lannan 1984) and could adversely effect spat production. In order to compensate for these possible effects, more broodstock (60 pairs) will be crossed at one time than the number of full-sib families required for planting (50 pairs).

For each spawning period, oysters will be picked at random from conditioned broodstock. Broodstock oysters will be strip-spawned and pair-crossed to generate 60 full-sib families within two days. Methods for spawning and fertilizing eggs are described by Breese and Malouf (1975). The carcasses of the spawned broodstock will be put in labeled plastic bags and frozen at -70 °C for subsequent typing of allozyme (Buroker et al. 1975; Hedgecock and Sly 1990; Hedgecock et al. 1993b; Hedgecock 1994), microsatellite and other DNA markers (McGoldrick and Hedgecock 1995; Gaffney, unpublished).

From each female, from 5 to 10 million oocytes will be taken, counted, re-suspended at approximately 1,000 eggs ml^{-1} in seawater at 30-35 ppt, 25-27 °C, fertilized with the sperm of one male, and stocked immediately into one 20 l bucket at a density not to exceed 200 eggs ml^{-1}. Constrained hatchery space and algal supply do not permit replication of family larval cultures, thus traits related to larval performance and nursery production cannot be meaningfully measured in the selection program.

Stocking 4 million fertilized eggs is expected, conservatively, to yield 1 to 2 million D-hinge larvae at 24 h, at which time the larvae will be transferred to 100 l culture tanks at densities adjusted to 5 ml^{-1} (yielding 0.5 million larvae per family). After one week, the density of larvae retained on a 80 micron screen will be adjusted to 3 larvae ml^{-1} (300,000

larvae per tank) in each culture to reduce competition among larvae for food.

Screening and changing water of each 100 l larval culture will be carried out every 2-3 days, using a 37 micron screen during the first week, a 80 micron screen in the second week, and a 180 micron screen when more than ten percent of the larvae are larger than 240 micron. Care will be taken to ensure against cross-contamination of full-sib larval cultures by thoroughly cleaning screens before re-use by immersion in hot tap water.

The larvae will be fed on a 1:1 algal mixture (by cell number) of the diatom *Chaetocerous gracile*, and either *Isochrysis* aff. *galbana* (Tahitian strain) or *Pseudoisochrysis paradoxa* (strain VA-12). The larvae will be fed on a standard schedule (Breese and Malouf 1975), beginning with densities of $3x10^4$ cells ml^{-1} and increasing to $8x10^4$ ml^{-1} cells at setting. Only minimal husbandry data will be collected during the larval stage, although detailed hatchery log sheets should be kept for future reference.

When about ten percent of larvae in a culture reach the late pediveliger stage ("eyed-larva") and can be retained on a 240 micron screen, setting-size larvae will be harvested on 3-4 successive days from the larval culture. Larvae from the first days will be kept moist and refrigerated (Carlson 1982; Henderson 1983; Joe 1984) until collection of eyed-larvae is complete. Larvae will then be treated with $2x10^{-4}$ M (-)epinephrine for 2 hours (Coon et al. 1985, 1986) in order to induce metamorphosis without attachment ("cultchless" spat).

The treated oysters will be rinsed with seawater and placed into mini-upwelling tubes. Each upwelling tube will be 6 cm in diameter and 60 cm long with a 230 micron-screened base. The outlet of each upwelling tube will be screened with a 230 micron screen to capture non-metamorphosed larvae that are washed out of the upwellers. These larvae will be returned to the appropriate larval culture for further growth.

C. Nursery systems

Spat in the mini-upwelling system will be fed on the same algal mixture as larval cultures and maintained at 25 °C and at a salinity of 30 to 35 ppt. Algal rations will be adjusted to provide the spat with cell concentrations of 30 to 80,000 cells ml^{-1}. The flow through the upwellers will be adjusted so that the spat are gently suspended in the flow in order to prevent them from adhering to the sides of the upwelling tubes.

When the spat in the mini-upweller system reach 750 microns in size, spat from each of 60 families will be transferred to a large-scale, upweller nursery system. The number of families will be reduced to 50, either by dropping families with less than the minimum number of seed or by random culling of families if more than 50 families yield substantially more than the minimum number of seed. The number of spat of each family will be reduced to 10,000 when spat reach 2 mm in size in the upweller system.

The large-scale, upweller system will consist of 60 upweller tubes 26 cm in diameter and 40 cm deep. The bottom of the upwellers will be covered with a 750 micron mesh screen. Seawater in the large-scale upweller system will be maintained at 20 to 25°C and will be partially recycled to conserve heat and algal food. During the first year of MBP, spat will be grown to about 2 mm in size before being transferred from the nursery to lantern nets suspended in Yaquina Bay where they will be grown to 8 mm in size. However, after the first year of MBP, a greenhouse will be constructed to produce large quantities of algae for raising spat to at least 5 mm in size in the large-scale nursery system before transfer to lantern nets (see details in the next section). The use of the large-scale nursery for production of 5 mm sized spat will result in less dependence on natural production for rearing spat to 8 mm size, allowing spat production to occur in the early spring when algal abundance in Yaquina Bay may be growth limiting.

D. Algal Culture

Success of larval and seed culture is critically dependent on the availability of adequate supplies of algal foods. Algae will be cultured following standard batch culture methods (Breese and Malouf 1975). Algae will be cultured in 20 and 200 l tanks continuously illuminated with fluorescent light at 18 °C. It is estimated that sixty 100 l cultures of larvae will require a maximum of 180 l of algal culture (algal concentration 3×10^6 cells ml^{-1}) per day. The algal culture room at MBP has the capacity to produce about 800 l of algae (at 3×10^6 cells ml^{-1}) per day.

Consumption of algae by spat increases greatly with spat size. Assuming a live weight of 20 mg for spat 5 mm in shell length (Spencer 1990) and an algal dry weight of 2.0×10^{-8} mg $cell^{-1}$, it can be estimated that a single 5 mm spat will consume about 19 ml of algal culture (at 3×10^6 cells ml^{-1}) per day (Utting and Spencer 1991). A total of 285,000 spat [(135 x 10 replicates x 4 sites x 50 families) + 300 for MBP repository] of this size will,

therefore, require 5,415 l (1,354 gallons) of algal culture per day.

In order to supply the spat with sufficient algal food, algae will be cultured in circular tanks 8 ft in diameter and 4 ft high (capacity of 1,500 gallons or 6,000 l) in a greenhouse (air temperature 18 °C). The tanks will be filled with heated, sand-filtered, UV-irradiated seawater at 18 °C. If contamination of cultures with blue-green algae becomes a problem, the seawater will be sterilized by addition of chlorine solution for 24 h, and neutralized with sodium bisulfite. After inoculation with algae, the tanks will be aerated with CO_2-enriched air (0.25 percent v/v CO_2). Algae cultured under these conditions are expected to double every 24 h. If a 6,000 l tank was inoculated with 600 l of algal culture at 3×10^6 algae ml^{-1}, a cell concentration of 2.7×10^6 cells ml^{-1} would be expected in the tanks after 4 days of culture. A total of eight 6,000 l algal culture tanks will be installed during the 1995-1996 and 1996-1997 funding periods. One to two tanks will be inoculated each day.

E. Grow-out

Grow-out for evaluation of meat yield will take place at three to four testing sites in California, Oregon, and Washington. Spat will be planted in either on-bottom bags or in suspended lantern nets. However, MBP will also retain about 300 seed per family as broodstock. These will be held in lantern nets at the MBP repository in Yaquina Bay.

When spat reach 8 mm in size, each family will be stocked into 1/8 in (3 mm mesh) mesh bags or 1/4 in (6 mm) lantern nets. Each family in the set of 50 full-sib families produced by one MBP hatchery/nursery cycle will be planted in 10 bags at each of four testing sites and a sub-sample of 300 oysters per family will also be held at the MBP broodstock repository in Yaquina Bay. Each site, therefore, will support 500 bags/lantern net compartments of oysters plus, in later generations, at least 100 bags/lantern net compartments with control oysters from unselected source populations. The families will be arranged in a randomized block design at each site to take into account the effects of tidal height and current direction.

At about 3 months of age and an approximate shell-height of 2 cm, oysters will be transferred to 1/4 in (6 mm) mehs bags or 5/8" (15 mm) lantern nets. The grower will be responsible for maintaining the bags or lantern nets. Normally bags of oysters will be turned and lantern nets inverted every month and brushed clean of fouling organisms.

In the first year of the breeding program, 175 spat from each family will be added to each mesh bag. Of the 175 spat, 30 spat will be sampled after 8 months of growth, and 100 of the remaining oysters will be put back in each bag for final grow-out. In subsequent funding periods, 5 mm mesh bags will be initially stocked with 135 spat per bag and the number reduced to 100 per bag at the time of transfer to 10 or 15 mm bags. The slight excess of oysters initially added to the 5 mm mesh bags will allow for mortality of oysters during the grow-out period.

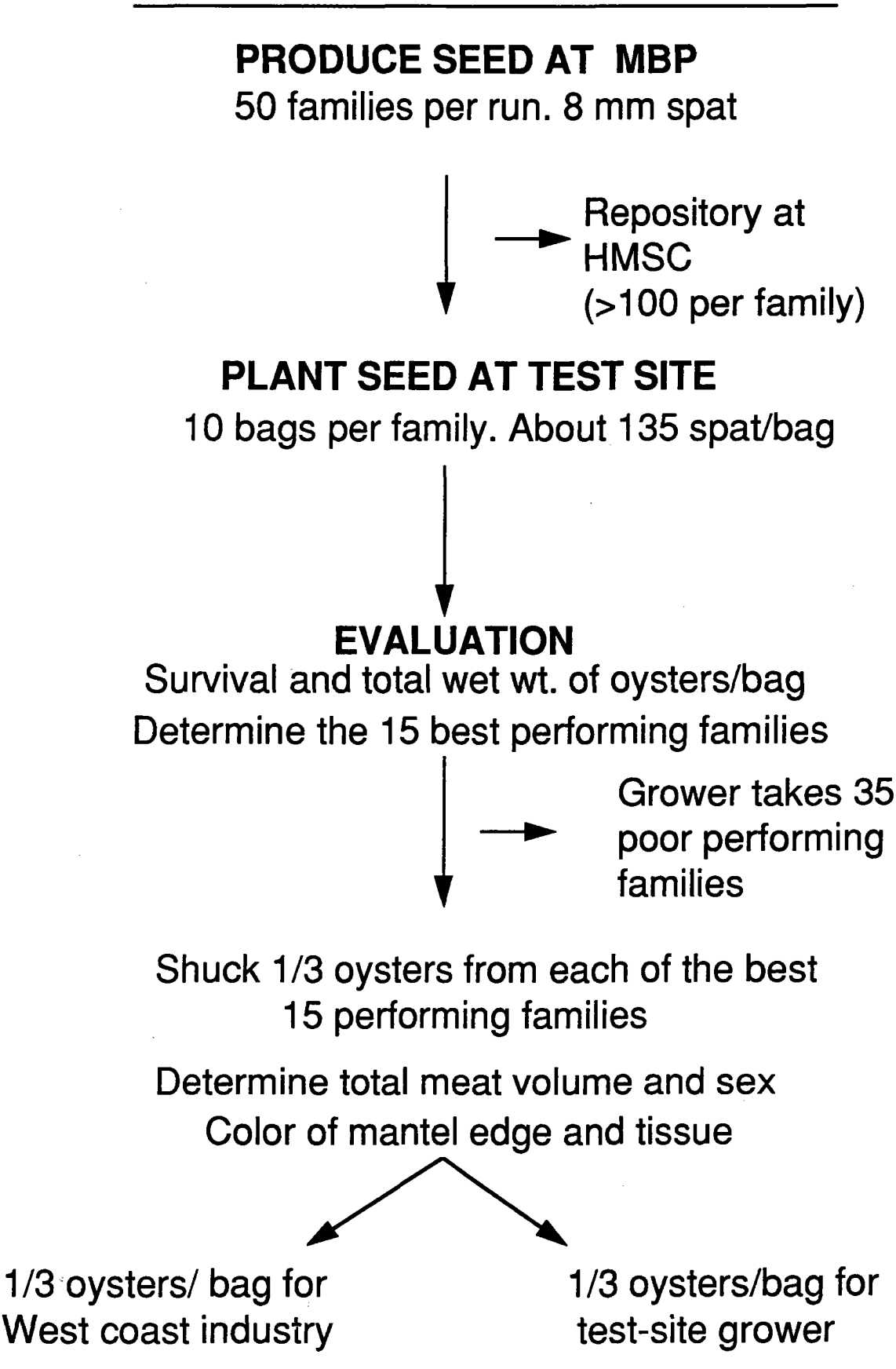
Bags will be harvested at market size (after approximately 18-22 months of grow-out). Oysters will be harvested before the peak of the spawning season at sites where oysters are known to spawn naturally in order to avoid the effects of gamete loss on meat weights and yields.

F. Evaluation and Selection

As described in section II.C.and shown in Figure 2, families will be evaluated for average survival, growth, and meat yield per bag. Total live weight and number of oysters for each of the 500 bags of oysters will be determined at 8 months of age and at final harvest (approximately 18-22 months of age). Survival and growth can thus be calculated over two intervals, from seed stocking to the 8 month evaluation, and from then until final harvest. Weight gain per bag will be determined as final (18-22 month) minus initial (0 or 8 month) total live-weight; daily growth rate can be calculated by dividing overall growth by the number of days in the interval and specific growth rates will be determined by expressing growth logarithmically. Furthermore, because of the dependence of growth on temperature, growth will also be expressed in terms of day-degrees. A temperature logger will be placed at each site to record temperature over an annual cycle. These are cheap (less than $100) and data can be automatically transferred to spreadsheets for analysis.

Meat yield per bag, per family, requires measures of meat weight and volume. At final harvest, a random sub-sample of 30 oysters will be taken from each bag (10 bags per family) for the 15 families with the greatest average total live weight per bag. This choice of a sample size of 15 families is based on the expectation that these top 15 families will include the top ten percent (5) families that have the highest average meat yields, based on existing live weight to meat-weight regressions derived from a previous USDA-supported WRAC project (Hedgecock et al. 1993a).

Figure 2. Production and evaluation of MBP oyster seed



The 30 sub-sampled oysters per bag, per family, will be placed in 150 (15 families x 10 bags) buckets labeled by family and replicate number. Oysters in the labeled buckets will be shucked with the help of industry-supplied shuckers. The total meat volume for each of the 150 buckets of oysters will be measured in a graduated cylinder after gravity-compression of the meats with a perforated, weighted plunger. The meats will then be transferred to a colander to drain and the meats weighed. A qualitative score for color of the mantle-edge will be noted for each family (Hedgecock et al. 1993a), as marketability of Pacific oysters in certain areas can be affected by this trait.

The sex of each oyster in each bucket will be determined in order to estimate family sex-ratio. The correlation between sex (male, female, hermaphrodite, or undetermined) and individual meat weight and volume will be determined for a sub-sample of 5 oysters per bucket (750 in total), so that a sex-corrected total meat yield can be estimated for each family.

Selection of broodstock for the next generation will be based on average meat-yield per bag (the product of meat yield per individual and survival). The yield data from each site will first be examined and analyzed by ANOVA using a randomized block model. After allowance for block effect and sex-ratio, the families will be ranked according to total wet weight, survival, and meat yield.

The effect of site on the ranking of family meat yields will be determined by ANOVA and an overall rank order of families by meat yield over all test environments will be calculated. Broodstock for production of the next generation of MBP selected oysters (which will be held at HMSC) will be taken from the top ten percent of families with the highest overall meat yields across all test sites (5 families from each group of 50 tested families).

G. Number of Spawns per Year

Two hatchery-production cycles, generating two sets of 50 full-sib families for evaluation, can be completed each year in MBP facilities at HMSC. These spawns will be labeled by year and number, i.e. 96-1 and 96-2 for the two sets of crosses in 1996. Willapa and Dabob broodstocks will contribute equally to crosses in 1996.

H. Odd/Even Year Classes

Production of families will commence in 1996 and continue each year thereafter. If final harvest is at 18-22 months, selected families can be identified and spawned at 2 years of age. This harvest schedule results in odd and even year-classes within each generation of the selection program. This will enable a larger effective population size to be maintained in MBP selected stock, particularly if matings between year classes can be accomplished (see next section).

I. Acceleration to an Annual Cycle

The rate of genetic improvement of Pacific oysters could be accelerated if it were possible to select broodstock on the basis of their performance at 8 months of age. Therefore, in the first few years of the program, correlation between meat yield at 8 months and at final harvest will be determined. If the correlation is significant, then selection and breeding of 8-month old oysters could be undertaken to reduce the generation time and increase the rate of gain in meat yield at harvest. Should this prove to be the case, MBP would seek to hold broodstock for each family in a site favoring rapid growth and sexual maturation at one year of age. Some candidate sites are Totten Inlet, Puget Sound, WA, Humboldt and Tomales Bay, CA, and Hawaii, where commercial nursery facilities for Pacific oyster seed are being established at the OTEC site.

The feasibility of using growth-accelerated broodstock will be tested in the first year. Fifty oysters from each of 50 families will be grown out at a site that accelerates growth. These growth-accelerated oysters will be examined periodically to assess sexual maturity and sex ratio. These oysters will not be spawned in the second year because at that time the correlation between meat yield at 8 months and final meat yield will not have been ascertained. In the second year, 50 oysters from each of 50 families will once again be relayed to a site for accelerated growth and may be used as broodstock in the third season if they are sexually mature and families with superior meat yields at harvest size can be reliably identified based on their performance at 8 months.

VI. INDUSTRY PARTNERS

A. General Considerations

For practical and scientific reasons, MBP will require partnerships of several kinds with the West coast oyster industry, the primary intended beneficiary of this USDA special project. An immediate practical reason, for example, is that MBP's budget is not sufficient to cover the costs of growing families to maturity and evaluating their performance. Industry partners are thus required, beginning in 1996, to serve as testing stations for evaluating MBP families. A second kind of partnership will be required, beginning in 1998, when the first generation of MBP select broodstock will be available to industry for hatchery propagation and distribution to growers. This partnership would be analogous to the multiplying stations in the Norwegian salmon breeding program (Gjedrem, pers. comm.). These various industry partnerships are discussed below.

As a federally funded program, MBP must make its products available to all of the industry, not just to its immediate partners. Both WRCC-99 and the Executive Committee of MBP are anxious to have the genetic improvement program embraced by the industry as a whole. In the following descriptions of partnerships, we show how the structure of the MBP allows not only for general improvement of broodstock for the whole industry, but also for specific adaptation of MBP select stocks to local environments or particular markets. Thus, industry-wide and proprietary interests can be served at the same time by MBP. Establishing this new cooperative venture, however, will require opening and maintaining as many lines of communication between MBP and the industry as possible.

B. Testing Stations

MBP families of oysters need to be evaluated for yield on West coast oyster farms capable of managing separate families of oysters by either bag culture or lantern net culture. Three or four testing sites are needed for each seed production cycle, and to date four companies have expressed an interest: 1) Coast Oyster Co. at its site in Willapa Bay, WA, 2)Taylor United at a site in south Puget Sound, WA, 3) Hog Island Oyster Co. in Tomales Bay, CA, and 4) Oregon Oyster Company, Yaquina Bay, OR.

Each testing station will be responsible, with MBP help at critical points, for stocking, care, transfer and harvest of 500 labeled bags of oysters. MBP will work closely with the

grower and field hands to ensure that the integrity of bag labels and the layout of bags on each test plot is maintained. Details of the responsibilities of the testing stations and of MBP are given in a draft Memorandum of Understanding that will be signed by both parties (Appendix B). Some testing stations may receive more than one set of families, others may only receive one set.

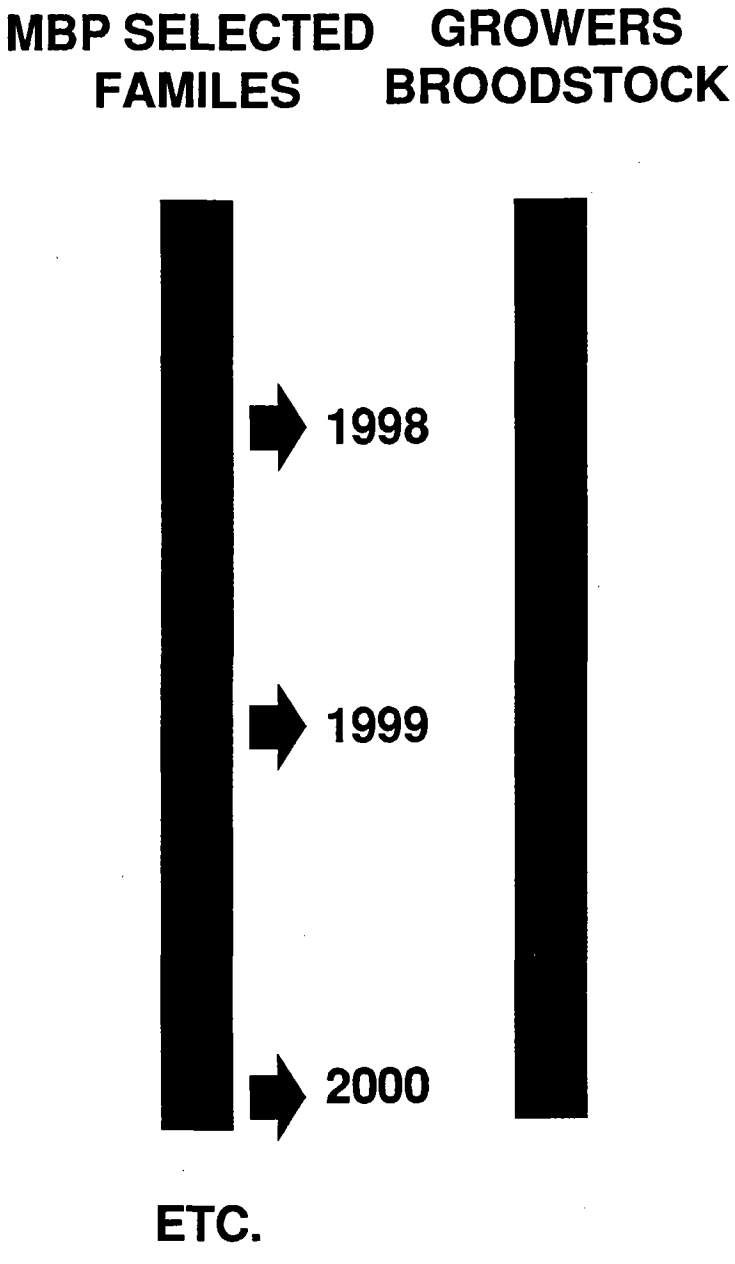
There is opportunity for testing stations to recoup some costs by the sale of oysters not needed for analysis or breeding. Approximately 50,000 oysters will be stocked at each site for final grow-out at 8 months of age. After removal of oysters required for meat yield analysis at harvest (4,500 oysters), and distribution to the West coast oyster industry of half of the oysters in the five, top meat-yielding families (1,750 oysters), the remainder of about 44,000 oysters (assuming zero percent mortality) would be the property of the grower hosting the test site.

C. Multiplying Stations

In the Norwegian salmon breeding program, multiplying stations are vertically integrated commercial producers, who propagate select broodstock, rear and sell eggs, fry, fingerlings, or smolts, and practice mass selection for further improvement and adaptation of the stock to local conditions. Multiplication is a critical phase for any genetic improvement program (Harris et al. 1984) because it spreads genetic gains throughout the industry, yielding in turn an economic gain that justifies the initial investment in the breeding program.

The Pacific oyster industry on the West coast has several vertically integrated companies that can apply directly the Norwegian model of a multiplying station. The oyster industry also has companies that specialize either in seed production or grow-out to market size. However, functional multiplying stations could readily be formed by joint agreements between independent hatcheries and the growers that normally buy seed from them. There could be significant advantages for small growers in particular regions, like Tomales Bay, CA, for example, to form associations for the purpose of improving not only meat-yield but resistance to summer mortality. MBP can serve all of the industry by sharing performance data with growers and hatcheries and making available broodstock from the best performing families at each test site.

Figure 3. Transfer of broodstock from MBP to industry



Commercial hatcheries can multiply fecund Pacific oysters very easily by mass spawning. Indeed, the design and practices of these hatcheries maximize seed production at the expense of genetic diversity (Hedgecock and Sly 1990; Hedgecock et al. 1992; Gaffney et al. 1992), making them unsuitable as primary breeding stations (Hedgecock 1996). As multiplying stations, however, these hatcheries should excel at spreading improved seed quickly throughout the industry, without changing existing designs or practices. Except in the first generation, which is discussed below, commercial hatcheries would propagate select MBP broodstock by mass spawnings and communal rearing of larvae in existing production tanks. There will be no need for the multiplying stations to keep families separate or to maintain pedigree information on broodstock. A timeline for MBP and the multiplying stations is presented in Figure 3.

A large-scale control for the performance of MBP improved stocks will be carried out by MBP's industry partners. Multiplying stations will produce seed from mass spawns of industry broodstock in parallel with a mass spawn of MBP select stock. The multiplying station or grower receiving the two groups of seed will then be responsible for tracking the two groups through the production system to market so that information on yield and economic gain can be obtained. Production of the select and standard stocks would ideally be evaluated in more than one grow-out system (on-bottom, long-line, etc.), not just the bag and lantern net method used in MBP's own evaluation of families planted at various test sites. In this way, MBP will acquire information about the correlation in performance across culture methods as well as across sites.

At harvest, multiplying stations would practice mass selection, by determining the distribution of live-weights and meat-yields for a sub-sample of individuals in the select group and then picking the largest, meatiest oysters for broodstock. Selection of individuals at the large industrial scale can be very intense. Automated methods for size-grading commercial product by image analysis are being used by industry (K. Cooper, Taylor United, Inc., pers. comm.) and would greatly facilitate this selection. Select broodstock would be then returned to the hatchery for spawning. At this point, broodstock (or possibly cryopreserved sperm) from MBP's next generation of select stock would also be available for spawning together with the commercial, mass-selected stock. Gene flow from the pedigreed MBP population to the multiplying station's broodstock would retard the rate of inbreeding; of course, it might also retard response to selection for local conditions or local markets.

In the first generation, the number of selected families will be quite small, perhaps only five for each set of families evaluated. Mass spawning of broodstock from only five families would result in full-sib mating in about 1/5 of fertilizations if these were at random. Thus, MBP will have to work closely with the hatcheries to ensure that mating of full-sibs is avoided in the first generation. MBP staff will be on hand to help hatcheries spawn first-generation select broodstock individually and only to unrelated individuals. In later generations, the probability of sib-mating could be reduced by mating individuals from different sets of families or by using cryopreserved sperm from the current generation of MBP select stock to fertilize females from the commercial select stock.

D. Other Industry Contributions

MBP will depend on a variety of other industry contributions to be successful. Already mentioned is the need, at harvest, for professional shuckers to open and extract meats from 4,500 oysters per set of families, per site. It may be helpful also for industry to share materials like racks and bags with a test site owner and to coordinate the transfer of those materials to test sites. Likewise, it may be necessary for smaller companies to pool human resources for tending bags at a test site.

VII. SCHEDULE

See Figure 1.

VIII. MATERIALS FLOW

A. Constraints on Interstate Movement of Oysters

An important issue for MBP and its industry partners is interstate movement of live oysters, which is presently strictly regulated and restricted by states out of consideration for the potential transfer of pathogens and parasites. Appropriate permits will be obtained for the transfer of spat from MBP to out-of-state test sites. Dr. Carolyn Friedman, California Department of Fish and Game will histologically analyze 60 adult MBP broodstock and >100 spat (2 to 4 mm; produced at MBP and held in the Yaquina for several months) before issuing a permit for importation of spat into California. The Washington Department of Fisheries will use Dr. Friedman's findings for issuing an import permit for MBP spat

into that state. If there are problems with disease sources in Yaquina Bay, MBP has the capability of producing 5 mm spat in sand-filtered, UV-treated seawater once the greenhouse for large-scale algal production is completed.

MBP will retain representatives of all families in Yaquina Bay so that selected broodstock will not have to be transferred from test sites in other states to MBP for spawning.

Movement of broodstock from test sites to a commercial multiplying station or hatchery may require permits for interstate movement. MBP will work with industry partners to obtain whatever certifications and permits are required in these situations.

B. Propagation of Selected Stock by Industry

MBP will make the first sets of families in 1996. Select broodstock from these families will thus be available to multiplying stations in 1998. Pair-wise spawning of these broodstocks at commercial hatcheries will be carried out with the help of MBP staff, as described in section VI.B. The offspring of these oysters will reach the market no earlier than the year 2000. Selected individuals from the commercially spawned selected stock will be returned from production grounds to the hatchery for spawning either with broodstock selected from other family sets or with MBP selected broodstock or cryopreserved sperm from MBP broodstock. The flow of broodstock and genes from MBP to industry multiplying stations is depicted in Figure 3, which assumes a strict two-year generation cycle in MBP, but two or three year generation cycles for multiplied stocks grown on commercial grounds.

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X. APPENDICES

Appendix A. Attendees of WRCC-99 Meeting, Nov. 12-13, 1995, Newport, OR

S. Allen, Jr. (Secretary), Rutgers University, NJ
M. Blouin, Oregon State University, OR
G. Burreson, College of William and Mary, VA
K. Cooper, Taylor United Inc., WA
D. Dahman, Dahman Inc., WA
J. Davis, Baywater Inc., WA
B. Dewey, Taylor United, WA
P. Gaffney, Delaware University, DE
D. Hedgecock (Chair), University of California, Davis, CA
C. Langdon, Oregon State University, OR
J. Lannan, Oregon State University, OR
A. Robinson, Oregon State University, OR
L. Weber, Oregon State University, OR

Appendix B. Example of MOU between MBP and growers at test sites.

MOU BETWEEN MBP AND GROWERS AT TEST SITES

In order to facilitate co-operation between MBP and growers at each test site, the Executive Committee of MBP suggested that it would be prudent TO develop an MOU that could form the basis of the co-operative effort.

Responsibilities of MBP

Site selection:

MBP will work with the test site grower to select a suitable site.

Planting oysters

MBP will provide about 70,000 >8 mm spat for planting 500 oyster bags/cages (3/16th or 5 mm mesh) at 135 spat per bag.

- Spat will be provided in marked lots of 135 spat per lot
- MBP personnel will fill bags and will provide marked ties to label each bag
- MBP personnel will transfer spat from the 3/16" (5 mm) mesh bag/lantern net to a 3/8" (10 mm) or 5/8" (15 mm) mesh bag/lantern net for final grow-out to harvestible size.

Determination of family performance

MBP will determine the total wet weights of each of the 500 groups of oysters and count the number of oysters in each group after 18 months to two years of growth,

- MBP will randomly take 30 oysters from each bag (10 bags per family) from the 15 families with the greatest yield (4,500 oysters in total) and these will be placed in 150 labeled buckets.
- Oysters in the labeled buckets will be shucked by an industry-supplied shucker. MBP will measure the total meat volume of each of the 150 buckets of oysters, using a measuring cylinder and perforated weighted plunger. The color of the mantle edge and meat color will be noted.
- The sex of each oyster in each bucket will be determined. The relationship between sex (male, female or indefinite) and individual meat volume will be determined for a sub-sample of 5 oysters per bucket (750 in total). The correlation between meat volume and sex of the oyster will be determined and a sex-corrected total meat volume for each bucket will be determined.

•After removing 30 oysters for analysis, half the remaining oysters in each bag of the top 15 performing families will be retained by MBP for distribution to West coast hatcheries. The remaining oysters (approximately 50 per bag for the top 15 best performing families, and 137 per bag for the other families or a total of about 55,000 oysters) will be the property of the grower hosting the test site.
MBP will share performance data with growers and hatcheries to identify the best performing families at each test site for their use as broodstock.

NOTE: In the first year of MBP, total oyster wet weights per bag will be determined after 8 months as well as at 18 months to 2 years to determine if there a relationship between growth at 8 months and growth after about 2 years. If there is a relationship, then selection and breeding of 8-month old oysters could be undertaken to reduce the generation time and accelerate the selection process.

Responsibilities of the grower at each test site

Site selection

The grower will provide a site for 500 bags/cages or lantern net compartments for the culture of selected families of oysters for a period of not less than 2 years.
The grower will select the site in conjunction with MBP to reduce risks of losing oysters by accident or theft.

Growing and evaluating oysters

The grower will provide means of securing bags or lantern nets.
The grower will provide 500 1/8th in (3 mm) and 500 1/4 in (6 mm) or 5/8" (15 mm) mesh bags or lantern nets and necessary fasteners.

•The grower will service bags or lantern nets both to prevent sediment accumulation/fouling and re-distribute oysters within the bags.

•If possible, the grower will provide two shuckers to open selected oysters (about 4,500) for a period of about two days.