

IMPROVING PACIFIC OYSTER BROODSTOCKS

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PROJECT OBJECTIVES

The project had the following objectives in year 3: (1) to test the performance of Pacific oyster (*Crassostrea gigas*) hybrids, produced by controlled crosses of existing WRAC inbred lines, at a commercial scale, in different environments, and in comparison to both existing commercial stocks and select families in the Molluscan Broodstock Program (MBP, Hatfield Marine Science Center, Newport, Oregon); (2) to make new inbred lines from the pedigreed families being produced by the MBP, using cryopreservation and brother-sister matings; (3) to compare MBP-select broodstock with elite hybrids; (4) to create triploids from combinations of two and three inbred lines and test their early hatchery and nursery performance; (5) to create tetraploid lines of varying genetic composition from these triploids and test their triploid and tetraploid progeny after mating to diploid and tetraploid stocks, respectively; (6) to measure the metabolic performance of inbred and hybrid larvae at whole organism, cellular, and sub-cellular levels to determine the metabolic basis of hybrid vigor and to enable subsequent correlation of larval metabolism with growth to market size.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Objective 1: Testing Commercial Performance of Hybrids

The first test of hybrids in commercial production comprised three hybrid seed lots and their controls (Dabob Bay wild seed), which were produced at the TRI hatchery and shipped to a Taylor site in Samish Bay, WA, for remote setting and replicated long-line rearing. Unfortunately, growers combined seed from the control and one hybrid group and set them on the same cultch lot. Interim yield data for the three groups, obtained in January 2000, showed no significant differences in yield among the three hybrid groups or between tidal heights, considering the number of oysters per individual mother shell. Without a control group, we cannot assess the relative merits of the hybrid seed, but the Samish Bay growers were pleased with the crop.

1999 Commercial Production of Hybrid Oysters. We made two sets of hybrid families in summer 1999 at the TRI hatchery, using WRAC inbred lines. Brood stocks were from inbred lines made at BML and OSU. We crossed lines in a 4×5 factorial design in July, 1999. Larvae reared in the hatchery, metamorphosed under epinephrine without attachment to cultch, reared in upwelling/downwelling nursery silos, and were planted in Thorndyke Bay in fall 1999. We made a second factorial cross of lines five lines in September 1, 1999, but larvae from California brood stock did not survive.

Microsatellite marker analyses of all parents used in crosses at TRI in 1998 and 1999 revealed con-

tamination in two OSU inbred lines and one BML inbred line. There were no parents or too few siblings available to verify parentage of five other lines. We disposed of all families made from contaminated stocks in 1998 and 1999. Because of this experience, we now type microsatellite markers before or immediately after controlled crosses in order to insure the pedigree of all lines reared.

Evaluation of the performance of inbred versus outbred families. Although our objective is to test hybrids, we expect inbred lines to have depressed yields, *i.e.* the converse of hybrid vigor, in comparison to normal oysters. We made six inbred families, by sib-mating top performing MBP families, and planted these in replicated field trials alongside non-inbred controls. The non-inbred controls consisted of (1) select MBP families, (2) non-pedigreed, random seed oysters from industry, and (3) families from random pair crosses of wild Dabob Bay stock. The cohort reared in Totten Inlet, WA (summer 1998) and Yaquina Bay, OR (spring 1999). As expected, the inbred families had significantly lower standardized mean yield over the two growout sites than the MBP select families or the commercial seed, which were not different from each other. Offspring of "wild" oysters had an average yield between the selected/industry and inbred families. In Year 4 of this project, we are using these same inbred families, at the TRI hatchery, for experimental crossbreeding and inbreeding.

We initiated two other comparisons of inbred and outbred oysters. In fall 1999, we planted 12 inbred families from MBP stock alongside selected, out-crossed MBP families in Tomales Bay, Yaquina Bay, and Prince William Sound, AK. In spring 2000, another six inbred families were planted with out-crossed MBP families in Prince William Sound and Wescott Bay, WA. These replicated yield trials will provide robust data on the comparison of inbred and outbred oysters on commercial farms.

That the yield of inbred oysters is significantly lower than that from outbred families suggests the converse, *i.e.* that crossbreeding should produce hybrids with significantly higher yields than standard industry seed. Explicit comparisons of hybrids and controls are in progress or planned. At this point, however, industry should take care to avoid the accidental inbreeding known to occur under commercial hatchery conditions when oysters of unknown pedigree are spawned en masse.

Objective 2: To make new inbred lines from MBP families

Interstate stock transfer issues. Movement of research stocks among states presents logistical problems from the standpoint of animal health. Fortunately, replication of MBP stocks in various sites will enable us to bypass the need for interstate transfer. At the same time, risk of spreading SSO, which contaminates MBP stocks in Yaquina Bay, can be reduced to acceptable levels by shipping D-hinge larvae from OSU to TRI for rearing.

Cryopreservation of sperm for self-mating to produce inbred lines. We have cryopreserved sperm sampled from 31 males from MBP stocks. Next year, we will re-examine these individuals to see if any have become females. Cryopreserved sperm will be used to self-fertilize sex-reversed females to produce inbred lines.

Inbred lines from brother-sister matings. We have produced inbred lines from brother-sister matings of about 30 top performing MBP families. In most cases, at least four individuals were used to make each brother-sister mating and most crosses resulted in production of spat.

At the TRI hatchery, we attempted to propagate new inbred lines from MBP broodstock imported from Tomales Bay, CA, but, of the five factorial crosses attempted, only two produced inbred families and none survived to planting. Poor survival in these crosses is attributable, again, to deterioration in the gamete quality of oysters transported from California.

Objective 3: To compare MPB-select stock with elite hybrids

In 1999, we carried out crosses among three "elite" WRAC inbred lines, creating hybrids that were planted together with top performing MBP families, in Wescott Bay and Alaska. We will likely harvest this cohorts in fall 2001 or spring 2002; the data will provide explicit and well-replicated comparative data on the yield of inbred and hybrid oysters relative to first-generation selected families.

Objective 4: To create and test triploids crossbred from inbred lines

We produced more triploids, from the same inbred lines used in the previous two years, to determine the repeatability of differences between triploid progeny made from inbred vs. hybrid females or between reciprocal hybrid females. We conducted two replicated experiments, using standard methods for cytochalasin B inhibition of the second meiotic division of fertilized eggs. Crosses among males and females from two inbred lines and females from two or three reciprocal hybrids produced triploids with 1-way (AAA), 2-way (ABA, or AAB), or 3-way (ABC, ACB) combinations of inbred genomes. Although differences in survivorship and larval growth among treatment groups were not significant, two trends noted over the past two years were supported. Inbred, 1-way triploids (AAA) tend to have lower survival than 2-way or 3-way triploids, and triploids from reciprocal hybrid females tend to be different (ABA π AAB).

Objective 5: To create and test crossbred tetraploid lines

In order to produce an F₁ tetraploid, a triploid female must be crossed with a diploid male, followed by the retention of the first polar body. Unfortunately, the triploid females created in 1997 at BML did not yield enough eggs for this task. Low and variable triploid fecundity presents a hurdle to producing polyploid hybrids that must be surmounted by producing much large numbers of crossbred triploids than was possible in this project.

Objective 6: Comparisons of growth and physiology of inbred and hybrid larvae

During the 1999 summer season, we made seven independent experimental crosses for physiological analyses. The major objective of these crosses was to further investigate physiological and biochemical parameters that accompany differential growth rates between hybrid and inbred oyster larvae. Two broad areas of interest were: 1) elucidating early signals of fast growth between hybrid and inbred larvae (*i.e.* from 10–48 hours post-fertilization); and 2) examining differences in energy usage and acquisition in hybrid and inbred larvae.

Biochemical measurements to detect early signals of fast growth included biochemical composition of embryos and trochophore larvae (*e.g.* protein content vs. lipid content), total DNA and RNA content, mitochondrial density (as measured by citrate synthase activity), and digestive enzyme activity. Physiological rates measured at early developmental time points were respiration rates and protein turnover. Owing to the number of samples taken over the seven crosses, results from these analyses are still forthcoming.

We placed major emphasis on examining differences in energy usage and acquisition between hybrid and inbred larvae in the 1999 season. Both measurements correlated in previous experiments with the difference in growth rates between hybrid and inbred larvae of *C. gigas*. We designed studies to look at both processes simultaneously, in order to understand better whether faster hybrid growth is through more efficient use of metabolic energy, increased ability to capture and absorb food, or a combination of both. Hybrid larvae had significantly higher algal clearance rates than inbred larvae for the same size shell length throughout larval development. Indeed, hybrid larvae had nearly double the clearance rate of inbred larvae (26.3 $\mu\text{l hr}^{-1} \text{larva}^{-1}$ in hybrids as compared with 13.2 $\mu\text{l hr}^{-1} \text{larva}^{-1}$ in inbreds) at a shell length of 225 μm (the average shell length of study). Absorption efficiencies of hybrid and inbred larvae, which were measured concomitantly with their feeding rates, were not significantly different. These results signify that higher feeding rates of hybrid larvae are translated into higher amounts of actual energy absorbed, thereby increasing larval “scope for growth” (*i.e.* the amount of energy available for growth after maintenance costs have been met). Large differences in feeding rates were also seen between reciprocal hybrid crosses, which supports a major premise of our new WRAC proposal, that elite lines for crossbreeding can be predicted from larval growth and performance.

In summary, the physiological data obtained from 1999 show that inbred and hybrid larvae have large and consistent differences in the physiological processes underlying growth heterosis. Specifically, feeding ability accounts for 61% of the variance in growth rate among inbreds and hybrid larvae. Hybrid larvae are able consistently to maintain a higher scope for growth than inbred larvae by possessing higher feeding rates while maintaining similar absorption efficiencies.

SUPPORT

FISCAL YEAR	WRAC-USDA FUNDS	OTHER SUPPORT				TOTAL SUPPORT
		UNIVERSITY	INDUSTRY	OTHER FEDERAL	TOTAL	
99	106,100	(PI FTE)	5,000 ^a	35,756 ^b	35,756	146,856

^a Estimated in-kind contributions by Taylor United, Inc.; labor and materials for culturing hybrid larvae

^b Portion of total award (direct plus indirect costs) from NRICGP-USDA, grant 99-35205-8260, for October 1999 through March 2000.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED*Publications in print*

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Manuscripts

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- Launey, S. and D. Hedgecock. High genetic load explains segregation distortion and heterosis in a bivalve mollusc. submitted to *Nature*.

Papers presented or to be presented

- Hedgecock, D. Tying together the loose ends of bivalve population genetics. Invited plenary paper, Bivalve Genetics Session, World Aquaculture Society '99, April 1999, Sydney, Australia.
- Hedgecock, D. and J.P. Davis. 2000. Improving Pacific oyster broodstock through crossbreeding. National Shellfisheries Association, March 2000, Seattle, Washington.
- Pace, D. and Manahan, D.T. 2000. Genetic variance and feeding rates in bivalve larvae. National Shellfisheries Association, March 2000, Seattle, Washington.
- Marsh, A.G., Moore, M., Manahan, D.T., 2000. Genetic variation, protein metabolism, and physiological performance in bivalve larvae. 4th International Larval Biology Symposium, June 2000, Santa Cruz, California.