Improving Pacific Oyster Broodstocks

Reporting Period	4/01/97–3/31/98		
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Funding Level	$ 77,000		
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PROJECT OBJECTIVES

The project had the following objectives in year 1: (1) to test the performance of hybrids produced by controlled crosses of existing WRAC inbred lines, at a commercial scale, in different environments, and in comparison both to existing commercial stocks and to select families in the Molluscan Broodstock Program (MBP, Hatfield Marine Science Center, Newport, Oregon); (2) to create triploids from combinations of two and three inbred lines and test their early hatchery and nursery performance; (3) to measure the metabolic performance of inbred and hybrid larvae at whole organism, cellular, and subcellular levels to determine the metabolic basis of hybrid vigor and to enable subsequent correlation of larval metabolism with growth to market size. Owing to a delay in the MBP spawning schedule, a fourth year 1 objective, to make new inbred lines from the pedigreed families being produced by the MBP, was postponed until year 2. Oregon State University (OSU) was an unfunded participant in year 1.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Objective 1: Crosses Among Inbred Lines and Testing of Hybrids

A 3x3 factorial cross of inbred lines was made at Bodega Marine Lab, University of California-Davis (BML, UCD) on June 17, 1997. The six hybrid families from this cross were destined for an experimental growth trial with random-bred families produced by the OSU's Molluscan Broodstock Program (MBP). Hybrid seed were planted on the lease of Hog Island Oyster Co., Tomales Bay, California, in early August and held in nursery cages until December 1, when they were returned to BML for counting and weighing. Five of the hybrid families yielded enough seed for the MBP grow-out experiment, and sub-samples were separately bagged and tagged for transfer to Wescott Bay Oyster Co., Washington, the site of the MBP growth trial. Spat were returned to Tomales Bay, however, while we awaited an importation permit from the Washington Department of Fisheries and Wildlife (WDFW), which was finally issued on March 10, 1998. In the meantime, the severe El Niño storms of February 1998 overturned the seed rack, causing suffocation of the seed and loss of this experiment.

A second large-scale test of hybrid performance was initiated at the Taylor United, Inc. (TUI) hatchery on North Dabob Bay, Washington, after some logistical improvisation. In early summer of 1997, WDFW decided not to allow importation of BML WRAC inbred broodstock for experimental hybridizations at TUI. This policy was reversed in late 1997, but permitting problems continue to plague our research, as will be described in the Year 2 report. So broodstock from two inbred lines were imported, instead, from OSU to TUI, in July. The two lines were hybridized and cultured alongside several typical

mass-spawned TUI stocks. About 100,000 1 mm spat from this cross were reared in an upwelling nursery system at TUI and 50–70,000 1–2 cm seed oysters were planted in mid-November 1997 in Thorndyke Bay, Hood Canal, Washington.

Objective 2: Creation and Testing of Triploids from Inbred Lines

The WDFW ban on importation of Tomales Bay oysters to TUI also affected the plan for the VIMS team to make triploid oysters from inbred broodstock at the TUI hatchery. This work was instead conducted at the BML. Triploids were produced, by standard methods, in three experiments conducted in July and August. In the first two experiments, crosses among the same three inbred lines (lines 1 and 9 from cross 95x2; line 5 from cross 96x1) produced triploids having various 1-way, 2-way, and 3-way combinations of inbred genomes, as shown below (underlining emphasizes the maternal contributions):

Female parents	1 = Line 93-21	Male inbred parents 9 = Line 93-0	5 = Line 89-5
11 = 1x1 inbred	**1**11	**9**11	**5**11
19 = 1x9f_1 hybrid	**1**19	**9**19	**5**19
91 = 9x1F_1 hybrid	**1**91	**9**91	**5**91

Spat were obtained from all three spawns and were planted in Tomales Bay in September. The first experiment yielded at least small numbers of seed from all nine triploid groups, while the second experiment yielded the subset shown in bold. The third spawn, which used males and females of inbred lines 89-5, males of lines 93-2 and 93-21, and females of the hybrid families 2x5 and 5x2 (from cross 96x1), produced all nine possible triploid combinations in a matrix similar to that shown above. The average percentage of triploids over all three spawns, as determined by flow cytometry, was 86%, with a range of 65–95%. The experimental design enables comparisons of completely inbred triploids (AAA) vs. 2-way (AAB) or 3-way (ABC) hybrid triploids and of triploids from reciprocal hybrid females (AAB vs. ABA or ABC vs. ACB). Comparisons of triploids with corresponding diploids are possible in all groups, since cytochalasin B is not 100% effective. Surprisingly, across all three experiments, the completely inbred triploids did not consistently have the lowest relative larval survival or growth, but differences between larvae from reciprocal hybrid females were remarkably consistent, especially in survival. For example, survival after one week was always higher, on average 33% higher, in triploid larval cultures from the 1x9-hybrid female than from the 9x1-hybrid females.

Seed from these crosses are being reared for use, in summer of 1999 (year 3), as broodstock for the triploid x diploid crosses needed to produce tetraploids. Two-year-old oysters are required for these experiments, as the fecundity of triploid oysters is extremely low and variable.

Objective 3: Comparisons of growth and physiology of inbred and hybrid larvae

Three separate hybridization experiments were made by the USC team at BML in June and July. The first experiment was the same 3x3 diallel cross of lines 3, 5, and 7 as described under objective 1. Six days after fertilization, a 2x2 subset (the 5x7 factorial cross: 5x5, 5x7, 7x5, 7x7) of the full 3x3 diallel was selected and restocked at ca. 2 larvae/ml with tank replication. Only oxygen consumption measurements were made thereafter on crosses involving line 3. The second and third experiments were each replications of the full 2x2 diallel cross between lines 5 and 7. The repeated use of lines 5 and 7 for all 3 spawns was intended to

confirm the stability of heterosis using different individuals from the same genetic lines.

Growth of larvae was measured by taking shell lengths of 50 randomly sampled larvae every other day from each 100-1 cone. The following traits were measured in addition to larval growth: rate of oxygen consumption; *in vivo* food clearance rate; *in vivo* food retention efficiency; pattern of protein expression (2-d gels); relative abundance of various protein groups (gels); metabolic cost of shell deposition; *in vitro* activity of enzymes (carbonic anhydrase, trypsin, glucoronidase, galactosidase, Na^+, K^+-ATPase); protein content; lipid classes and lipid content; DNA content; taurine content.

As expected from previous work, heterosis was consistently observed, with both hybrid crosses (7x5 and 5x7) growing faster than the inbred crosses (5x5 and 7x7) in all three experiments. However, unlike previous years, hybrid larvae did not show increased feeding rates relative to inbred larvae. This observation reinforces the conclusion that differential feeding rate is neither a necessary nor a sufficient explanation of growth heterosis. Other physiological parameters also failed to show any difference between inbred and hybrid larvae (though the data themselves are primary contributions to fundamental larval physiology). Assay of carbonic anhydrase activity and the direct incorporation of $^{45}Ca^+$ into larval shells, for example, showed that the cost of shell deposition is only 5–10% of total metabolism, with no difference evident between inbreds and hybrids. Protein content, lipid classes, and lipid content did not vary between inbreds and hybrids. Activity of Na^+K^+-ATPase was not different between inbreds and hybrids. Activities of digestive enzymes (trypsin, glucoronidase, galactosidase), DNA content, and taurine content remain to be analyzed.

Also, as expected from previous work (Vavra et al., 1998), more often than not respiration and protein turnover show differences between inbred and hybrid larvae. For example, although there were large inbred-hybrid differences in larval growth rates in our 1997 cultures, there was no difference in the rates with which inbred and hybrid respiration increased with age (12.47 vs. 12.84 pmol 0_2 $h^{-1}d^{-1}$, respectively). This observation suggests that hybrids were metabolically more efficient through the larval stage (more growth for the same increase in metabolic rate).

In this work year, we examined embryonic development (0–48 h postfertilization) and found evidence for every early expression of heterosis. In the first experiment, a clear pattern of slower early development in inbreds was evident. Mean shell length for 15 hybrid cultures 24 hrs after fertilization was 78.6 ± 0.6 µm; the comparable average length of larvae from nine inbred cultures was 72.4 ± 0.4 µm. Moreover, while none of the hybrid cultures contained trochophore larvae at 24 hrs, all inbred cultures had trochophores larvae, averaging 14.6% of larvae. In the second experiment, this pattern was not evident, perhaps because fewer larvae were sampled, and they were sampled after all cultures had progressed to the D-hinge stage. Oxygen consumption rates measured at the trochophore/D-hinge developmental transition clearly show significant heterosis for metabolic rates. At 15 h post fertilization, inbred embryos (5x5 and 7x7) had a respiration rate that was almost three times higher than the hybrids (94.1 vs. 1.5 pmol 0_2h^{-1}). Although respiration rates remained higher in inbred D-hinge larvae, by 30 h post fertilization, inbred respiration rates were only 1.5 times higher (9.4 vs. 6.7 pmol 0_2h^{-1}). These results suggest that (1) metabolic efficiency plays a larger and more consistent role in growth heterosis than any other physiological parameter thus far investigated, and (2) physiological correlates of heterosis are expressed during embryogenesis. Clearly, there is need for more research on early development, for the prospect of evaluating oyster growth potential at 24 hrs postfertilization is exciting indeed.

During year 1, 18 protein labeling experiments were performed to characterize by 2-D gel electrophoresis differences between inbred and hybrid larvae with respect to the synthesis and turnover of specific proteins. In general, there appears to be high turnover of 5–7 small acidic proteins associated with slow-growing larvae. The use of a protease inhibitor has provided an experimental approach for quantifying absolute differences in protein turnover, but these gels have not yet been quantitatively analyzed. Difference in protein turnover also appears to be a consistent component of greater hybrid metabolic efficiency and growth heterosis.

WORK PLANNED FOR YEAR 2

No major changes are anticipated in the scope of work specified for year 2 in the original proposal for this project. With the maturation of the late-planted 1996 year classes of MBP families in 1998, it will be possible to begin inbreeding these families so that a direct test of the relative gains obtained through crossbreeding and selection can be made in the last year of this project. Aside from the postponement caused by MBP's late start, and delays in obtaining WDFW import permits, which necessitated minor adjustments in the TUI and VIMS tasks, this project met all of its objectives on schedule.

SUPPORT

FISCAL YEAR	WRAC-USDA FUNDS	OTHER SUPPORT				TOTAL SUPPORT
		UNIVERSITY	INDUSTRY	OTHER FEDERAL	TOTAL	
97	77,000	(PI FTE)	5,000[a]	70,715[b]	75,715	152,715

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

[b] Hedgecock and Manahan portions of total award (direct plus indirect costs) from NRICGP-USDA, grant number 95-03914, for October 1996 through September 1997

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

Publications in print

McGoldrick, DJ and D Hedgecock. 1997. Fixation, segregation, and linkage of allozymes in inbred families of the Pacific oyster *Crassostrea gigas*: Implications for the causes of inbreeding depression. *Genetics* 146:321–334.

McGoldrick, DJ. 1997. An Experimental Investigation of the Genetic Basis of Heterosis in the Pacific Oyster *Crassostrea gigas* (Thunberg). PhD Dissertation, Genetics, University of California, Davis.

Guo, X, D Hedgecock, WK Hershberger, K Cooper, and SK Allen, Jr. 1998. Genetic component for protandric sex in a *Crassostrea* oyster. *Evolution* 52:394–402.

Vavra, J, N Appelmans, D Hedgecock, and DT Manahan. 1998. Physiological components of hybrid vigor in larvae: A study using inbred and hybrid oysters (*Crassostrea gigas*). *Physiological Zoology*, In press.

Bayne, BL, D Hedgecock, D McGoldrick, and R Rees. 1998. Feeding behavior and metabolic efficiency contribute to growth heterosis in Pacific oysters (*Crassostrea gigas). J Exp Mar Biol Ecol*, In press.

Manuscripts

Pace, D, P Leong, A Marsh, and DT Manahan. 1998. Growth, feeding rates, energy metabolism and enzyme activities in Pacific oyster larvae with altered degrees of heterozygosity. In preparation for submission to *Biological Bulletin*.

Marsh, AG and DT Manahan. 1998. Genetic determinants of protein metabolism and turnover in inbred vs. hybrid larvae of *Crassostrea gigas*. In preparation for submission to *Biological Bulletin*.

Marsh, AG, MA Sewell, and DT Manahan. 1998. Energetics of embryogenesis on oysters (*Crassostrea gigas*) evidences higher metabolic costs in inbred vs. hybrid lines. In preparation for submission to *Biological Bulletin*.

Papers presented

Pace, DA, PK Leong, AG Marsh, and DT Manahan. 1997. Genetic manipulation of growth, feeding, and metabolic processes in bivalve larvae (*Crassostrea gigas*). *American Zoologist* 37:148A. (Presentation at the National Meeting for the Society of Comparative and Integrative Biology, Boston, MA, Jan., 1998).

Hedgecock, D, D Brun, and F Bonhomme. Genetic mapping in oysters: Problems with non-Mendelian segregation and complete interference. Plant and Animal Genome VI, San Diego, Jan. 18–20, 1998. (Abstract

available on the World Wide Web: http://probe.nalusda.gov:8300/pag/6/review/hedgecock/html.
Hedgecock, D. Genetic improvement of farmed Pacific oyster on the US West Coast by selection and cross-breeding. Aquaculture '98, Las Vegas, Feb. 15–19, 1998, invited symposium speaker.
Hedgecock, D. Genetic linkage analysis of the Pacific oyster. Japan Aquagenome Society Meeting, Tokyo, April 1, 1998, invited symposium speaker.