Final Report for Sea Grant Aquaculture Research Program 2010: Genomically enabled Crossbreeding to Improve Yields of Farmed Pacific Oysters

Dennis Hedgecock, PI and Project Leader, University of Southern California; Donal T. Manahan University of Southern California; Paul G. Olin, University of California, San Diego

Project Hypotheses

The hypothesis driving the applied science and outreach portions of the project was that double hybrid seed currently being produced by our primary industry collaborator, Taylor Shellfish Farms (TSF), can measurably improve production of Pacific oysters on California farms. The hypotheses behind the more forward-looking basic science part of the project were that (1) genes involved in protein metabolism are associated with growth heterosis (hybrid vigor) and (2) biochemical "biomarkers" of growth potential can be identified in the early (larval) stages of development.

Project Goals and Objectives

Our main goal was to increase U.S. production of shellfish through application of advanced biotechnology to the breeding of high-yielding Pacific oyster hybrids. We employed modern "omic" technologies for quantitative, global profiling of genomic variants, gene-transcripts, proteins, and metabolites, to discover—out of the millions of possibilities—the key genes and biochemical pathways that might serve as novel biomarkers for early detection of superior hybrids. Use of such biomarkers would increase the efficiency and speed with which commercial crossbreeding could improve yields of farmed Pacific oysters.

We distributed hybrid seed oysters produced in a commercial hatchery to three California farms located in different oceanic regimes. Farms reared hybrid seed alongside control seed, so that data on commercial yield, costs of production, and return on investment could be obtained.

Methodology

We used standard culture methods for the Pacific oyster established in our prior work (Pace et al 2006; Hedgecock & Davis 2007). Inbred parent lines and F_1 hybrids for the project were available from TSF. We used at both USC and TSF culture facilities to make F_1 hybrid

families, by controlled factorial crosses of inbred parent lines, and F_2 hybrid families, by sib-crosses within F_1 hybrid families. Inbred parent lines were selected on the basis of previous evidence for combining ability and differences between reciprocal hybrids.

At USC, F_1 hybrid families were reared in replicate tanks through the larval stage. Growth and physiological parameters, such as energy allocation, protein metabolism, and other biochemical processes were measured to test for the feasibility of identifying biomarkers for growth. Samples were taken for sequencing of gene-transcripts, to a depth of ~12 million reads for each inbred or hybrid population, and for proteomic and metabolomic analyses. Gene expression data were analyzed by methods similar to those used by Hedgecock et al. (2007).

We made large sets of F_2 families, measured their growth monthly, and collected DNA from large numbers of individuals (n>200) from each family, in order to genotype **s**ingle **n**ucleotide **p**olymorphism**s** (SNPs) and to map **q**uantitative-**t**rait **l**oci (QTL) for growth. This experimental design has greater power than single-family QTL experiments.

Commercial quantities of hybrid seed, ~100,000, enough to stock thousands of growout bags, were distributed to farms for assessments of yield. Sea Grant Extension staff and project researchers recorded numbers and biomass of seed stocked and harvested per bag. Production costs and relative economic gain of hybrid vs. control seed were assessed.

Progress and accomplishments towards meeting goals and objectives

Sets of F_1 reciprocal hybrid crosses were made at USC's Wrigley Marine Science Center on Catalina Island, and larvae from these crosses were used for biochemical and physiological assays. Cost of protein synthesis, which is a key parameter required to understand the physiological basis of growth efficiencies in larvae of different genotypes and which we measured for the first time in developing oyster larvae, was found to be independent of genotype or temperature (Manahan et al. 2012; Lee et al 2012). This work was extended to triploid larvae and wild-type diploid larvae supplied from the commercial TSF hatchery. In addition, extensive

metabolomic profiles were completed by comprehensive analyses of all small molecular weight metabolites in larvae of contrasting growth phenotypes (Applebaum et al. 2012). In parallel, a suite of samples are also currently being analyzed for a full transcriptomic analysis of gene expression (millions of mRNA molecules) in larvae of different ages and sizes. The gene sequencing has been completed for this part of the project and the bioinformatic analysis is proceeding. Combined, these "omic" analyses will be used to identify possible biomarkers for genotype-dependent growth efficiencies. Prediction of whole organism phenotype from individual genes is certainly complex, as our analysis is indicating (Manahan et al. 2014). Still, we have made significant progress in identifying key physiological processes that may predict differential growth potential (Pan et al. 2013, 2014).

Individuals (n=1456) from six F_2 families, including two pairs of F_2 families from reciprocal F_1 hybrids and two F_2 families sharing one or two grandparents with these reciprocal pairs, were individually tagged in June, 2012. Individual growth was followed monthly to produce a set of samples that affords a powerful first comparison of growth QTL, across related F_2 families. This QTL analysis was initiated and partially completed in a requested extension year. To accomplish the QTL analyses, we developed new methods for genotyping SNPs with high-throughput, next generation Illumina HiSeq sequencing. We made 14 genomic libraries, each comprising about 3.5% of the Pacific oyster genome; seven libraries contained DNA from 48 barcoded individuals and seven had DNA from 96 barcoded individuals. The libraries include duplicate DNA samples of the 12 parents of the six mapping families, which will enable estimation of genotyping error. Altogether, we obtained 2.4 billion, 100 bp single-end reads from the 14 libraries (an average of 172 million sequences per library); this is the equivalent of about 80 human genomes. Bioinformatics processing of this massive amount of data is in progress, but we estimate preliminarily that we will have between 8,000 and 15,000 SNPs per family. Libraries for the remaining individuals (n≈400) are in progress and will be sequenced, using alternative sources of funding.

The extension component of the project borrowed a strategy from the history of hybrid corn production in the U.S. We distributed 100,000 "double hybrid" oyster seed, which were produced by a TSF commercial cross of robust F_1 hybrid broodstock, to each of three California oyster farms. We also distributed to each farm 100,000 seed from a "control" stock selectively bred for yield and survival of summer mortality in South Puget Sound, WA. We demonstrated, first, the ability to track experimental groups through each of the three commercial production systems, permitting the first collection of such performance data. The double hybrid stock yielded more biomass per growing unit than the control stock, at all three sites, taking density into account (10.9 kg *vs.* 10.7 kg, P=0.04; 11.0 kg *vs.* 10.6 kg, P=0.075; and 3.63 kg *vs.* 3.50 kg, P=0.029). In addition to increased yield, hybrids had reduced variance in average individual weight and survival, compared to controls, on at least two farms; crop uniformity could be an economically significant advantage of hybrid oyster seed for commercial production. The control group had slightly better survival than the hybrid group at all three sites (0.97 *vs.* 0.95, P=0.006 at the northern farm; 0.91 *vs.* 0.87, P=0.033, at the central farm; and 0.97 *vs.* 0.96, P=0.025, at the southern farm), suggesting that prior selection on the control stock for survival of summer mortality had been effective. We calculated the economic value of yield improvement at all three farms (see below). A presentation of the results was made at the joint annual meeting of the Pacific Coast Shellfish Growers Association and the West Coast Chapter of the National Shellfisheries Association, in Tulalip, WA, September 25-27, 2012.

Project outcomes and impacts

Perhaps, the greatest outcome of this project has been an increase in industry appreciation of the value of genetic improvement. The three California farms harvested substantially more oysters than they might have, using unimproved, wild seed stock. One farm, in particular, reported a much higher survival rate (96-97%) in both the control and hybrid groups than they have normally experienced in recent times (~75%). This observation is, in part, the motivation

behind a newly funded Sea Grant project (R/SSFS-01, "Determining the genetic and molecular bases of oyster resistance to an oyster-killing virus, Ostreid herpesvirus 1").

Average individual weight gain of double hybrid oysters was 2.2, 6.5, and 8.4 percent greater than that of controls on farms in Morro Bay, Agua Hedionda Lagoon, and Tomales Bay, respectively. Because the control stock was also selected for growth and survival and is not yet in commercial production, a preliminary estimate of economic gain based on increased double hybrid yield is conservative, yet highly encouraging. If hybrid seed were widely adopted by California shellfish growers and if annual revenues (~$14 million) were to increase proportionally, from $0.3 to $1.2 million could potentially be added to the income of California growers. For the west coast industry, with an estimated value of around $94 million, widespread use of double hybrid oysters could generate an additional $2.0 to $8.0 million in industry revenue.

Data on superior performance and uniformity of hybrid seed helped to persuade Taylor Shellfish Farms to re-double their investment and effort in an in-house commercial crossbreeding program. Moreover, our scientific advice (Manahan et al. 2012) to rear larvae at lower temperatures, in order to permit energetic scope for resistance to stress—for example, under scenarios of ocean acidification—was heeded by the TSF hatchery, resulting in better production than in previous years. This is just one example of the practical and outreach significance of our project. On a larger scale, we have worked closely with our industry partners to provide scientifically based advice about global environmental change in the ocean (Manahan and Hedgecock 2013; Applebaum et al. 2014; Hedgecock and Manahan 2014). Avoiding possible negative impacts on production will be essential to support the growing demand for seafood in the future.

References

Applebaum, S.L., J.W. Lee, and D.T. Manahan, 2013. Global metabolite profiles as predictors of physiological traits in bivalve larvae with genetically-determined differential growth rates. Integrative and Comparative Biology 53: E6

Applebaum, S.L., T.-C. Francis Pan, D. Hedgecock and D.T. Manahan, 2014. Biological responses to global change: Separating the nature and nurture of energy allocation. Integrative and Comparative Biology (manuscript in review).

Hedgecock, D. and D.T. Manahan, 2014. Controlling Nature & Nurture: Why experiments with wild type are insufficient for understanding adaptation to environmental change. Society of Integrative and Comparative Biology. Annual Meeting, published abstract.

Lee, J.W., S.L. Applebaum, and D.T. Manahan, 2013. Differential energy allocation for protein synthesis is genetically determined during marine larval development. Integrative and Comparative Biology 53: E123

Manahan, D. T. and D. Hedgecock, 2013. Developmental physiology: Predicting "Winners and Losers" to environmental change. Integrative and Comparative Biology 53: E135

Manahan, D. T., J.W. Lee, and S.L. Applebaum, 2012. Well-fed but metabolically starving: implications of genotype for energy allocation in larvae of Crassostrea gigas. Journal of Shellfish Research 31: 317

Manahan, D.T., S.L. Applebaum, and F. Pan, 2014. Genes, genomes and proteins – the challenge of making physiological predictions. Society of Integrative and Comparative Biology. Annual Meeting, published abstract.

Pan, F., S.L. Applebaum, and D.T. Manahan, 2013. Amino acid transport as an index of growth potential in larvae of the Pacific oyster, Crassostrea gigas. Integrative and Comparative Biology 53: E161

Pan, T.-C. F., S.L. Applebaum and D.T. Manahan, 2014. Genetically determined variation in developmental physiology of bivalve larvae (Crassostrea gigas). Physiological and Biochemical Zoology (manuscript in review).