Annual Progress Report

Project Code: 05-02 **Subcontract/Account No**. 557221
supported by 2003-38500-13505

Project Title: "Development of genetic markers to assess disease resistance in the eastern oyster"

Reporting Period: February 2005 - July 2006

Funding Level: $128,486

Participants:
Steven Roberts – research scientist in MBL's Scientific Aquaculture program with background in molecular biology
Roxanna Smolowitz – traditionally trained veterinary pathologist, has extensive experience studying bivalve disease
Richard Karney – Director of Martha's Vineyard Shellfish Group, Inc., holds culture experience with numerous bivalves
Inke Sunila – invertebrate pathologist, has extensive experience in the diagnosis of bivalve diseases and working with aquaculturists
Dale Leavitt – extensive experience in research and aquaculture of shellfish, hatchery management, and extension
Bill Walton – aquaculture specialist with research experience in shellfish biology and outreach/extension activities
Frederick Goetz – senior scientist with background in aquatic animal immunology and molecular biology

Project Objectives:
1. To demonstrate seed originating from local wild oysters, that have experienced heavy disease (Dermo) pressure, could significantly contribute to the development of disease resistance in cultured oysters.

2. To genetically characterize regional oysters (*C. virginica*) that are putatively resistant (more tolerant) to Dermo, in order to development genetic markers and to better understand mechanism involved in immunity.

3. To communicate with northeastern hatchery operations and help them to identify local, potentially Dermo resistant broodstocks.

Anticipated Benefits

1. Information related to the effectiveness of using of local broodstock (that have experienced heavy disease pressure) to successfully contribute to the regional oyster aquaculture effort.
2. Genetic characterization of *C. virginica* from the northeast region, that will contribute significantly to our understanding of mechanisms involved in disease resistance and aid in any breeding programs.
3. Simple laboratory tests to identify broodstock that have traits associated with disease resistance.
4. Website, fact sheets, and presentations containing information on how hatcheries can select premium broodstock locally, based on prior disease pressure and genetic markers.

Progress and Principal Accomplishments

One of the major causes of decreased production for the oyster industry is disease. The two primary diseases that affect the eastern oyster are MSX and Dermo. Both diseases invade the oyster's soft body resulting in death of the individual. The disease MSX is caused by the protozoan parasite *Haplosporidium nelsoni* and is present along the entire east coast. This parasite was originally given the name Multinucleated Sphere with unknown affinity (X). The disease Dermo is caused by the parasite *Perkinsus marinus.* In the last few years, the disease has markedly affected oyster culture in the more northern portion of the parasite's range (Connecticut, Rhode Island and Massachusetts) in addition to states already identified as problematic (New York to the Gulf of Mexico). Oyster disease is a particular concern of shellfish farmers in the northeast region not only due to periodic devastating oyster losses, but also because disease indirectly affects the industry by slowing financial investments. Realizing that oyster disease is a primary concern for the industry, the long-term goal of the proposed research is to assist in the development of disease resistant eastern oyster broodstocks.

A primary objective of our research is to demonstrate seed originating from local wild oysters, that have experienced heavy disease (Dermo) pressure, could significantly contribute to the development of disease resistance in cultured oysters. Previous research has demonstrated that genetic factors can be selected for that contributes to disease resistance in the eastern oyster. A majority of this work has involved hatchery-based selection practices with limited information on the performance of wild oyster populations that have survived heavy disease pressure. Therefore our focus is to characterize disease tolerance in local, naturally selected for oysters in relation to factors such as *P. marinus* infection, growth and genetics.

Oyster broodstock populations were identified on Martha's Vineyard, MA that had experienced heavy disease pressure, and that had not been exposed to Dermo in the recent past. Edgartown Great Pond (ETGP) was selected as the site to obtain oysters that had experienced heavy disease pressure. Edgartown Great Pond is a salt water pond that is separated from the ocean by a sand bar most of the year. As a result, little recruitment occurs from coastal waters. Dermo

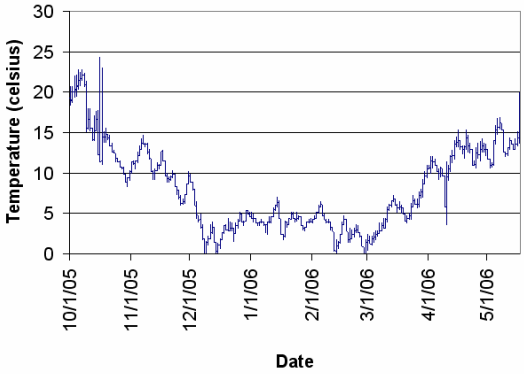

Figure 1. Temperature data recorded in Edgartown Great Pond at grow-out site.

prevalence and associated mortality have been very high in the pond for the past eight years, yet old animals are not hard to find. The control, or potentially susceptible oyster broodstock population was obtained from Tisbury, MA. There was limited Dermo occurrence at this site prior to selecting the broodstock. These two broodstock populations were spawned in July 2005, and seed from both broodstock populations was deployed. In July 2005, approximately 8000 oyster seed (4000 from ETGP stock, 4000 from Tisbury stock) were deployed in floating ADPI bags at two sites in Edgartown Great Pond. The average shell length of the oyster seed was 21 mm. In September 2005 the oysters were thinned out by adding additional ADPI bags at each site and evenly distributing the oysters. Since September 2005 there have been 12 bags at each site (6 with ETGP oysters and 6 with Tisbury oysters). In October 2005, oysters were counted and sixty oysters from each of the two groups were examined for *P. marinus* occurrence and abundance. *P. marinus* was observed in approximately twenty percent of all the oysters examined. However, the average abundance of the parasite in infected oysters was higher in the Tisbury group. **These results could suggest that *P. marinus* proliferates more in oysters from a population that had not experienced heavy disease pressure**. Floats were removed from the ADPI bags and they were submerged until May 2006 in order to avoid harsh conditions on the surface. In May, oysters were counted and samples brought back to the Marine Biological Laboratory for measuring and Dermo testing.

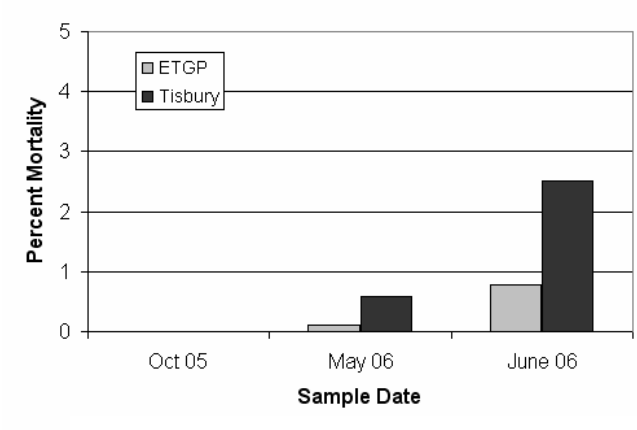

Figure 2. Mortality data for two oyster populations (Edgartown Great Pond and Tisbury) deployed in ADPI bags at our field site.

During the first year of grow-out, survival rates for all oyster groups were greater than 95% (Figure 2). However, increased survival was consistently observed for the Edgartown seed as opposed to the Tisbury group. **Once again, this demonstrates a difference in the EGTP and Tisbury oyster populations that could be associated with differential disease resistance and/or susceptibility.** Growth was not significantly different between the two oyster groups. As expected, slowest growth rates were observed during winter months with an average overall increase in shell height of 2.8 mm between October and May.

The second objective of this research project is genetically characterize regional oysters that are putatively resistant to Dermo, in order to development genetic markers and to better understand mechanism(s) involved in immune function. To begin to address this issue, we examined gene expression patterns in oysters provided to us by Marta Gomez-Chiarri and Dale Leavitt (participant on current project). Two wild populations were obtained from Rhode Island. These included oysters from Blue Bill Cove (Portsmouth) and Green Hill Pond (Charlestown). The Green Hill Pond strain had survived heavy Dermo pressure, where as the Blue Bill Cove strain had been exposed to very little Dermo pressure, if any at all. A third, proven disease resistant line of oysters were also characterized from the Haskin Shellfish Research Laboratory, Rutgers University. Oysters were kept for approximately one month in the same holding tank and were fed twice weekly. When transferred to a holding tank, several oysters were infected

with Dermo. This led to circulation of Dermo throughout the holding tank and equal exposure to the parasite for all oyster groups. After one month of growth, gill and mantle tissue samples were taken from each oyster for later RT-PCR analysis.

Select genes were identified based on their presumed function in the immune system to characterize in these oysters. Gill tissue was used for RNA extraction due to the prevalence of hemocytes which are the cells primarily responsible for immune function in oysters. One gene that was analyzed using real-time quantitative RT-PCR was a mitogen activated protein (MAP) kinase interacting protein (GenBank Accession number CD526707). The MAP kinase signaling pathway is important in innate immune function in both vertebrates and invertebrates. Interestingly, the level of gene expression was similar for the proven disease resistant strain (Rutgers) and the population from Green Hill Pond that had survived heavy disease pressure in the recent past (Figure 3). **One explanation for this is that there could be similarities in the immune response of the Green Hill Pond oysters and the Rutgers strain**.

The final major component of our work includes outreach. One way we have begun to meet this objective is the development of a website (http://www.mbl.edu/aquaculture/oyster). The purpose of this website is to keep the general public and shellfish farmers up to date on our activities and progress to date. This website includes some of our field data and photos of our routine field sampling. There is also a link to a local newspaper article that featured this project. We plan to keep this site up to date with future additions to include a copy of our progress report and presentation given at regional and national meetings.

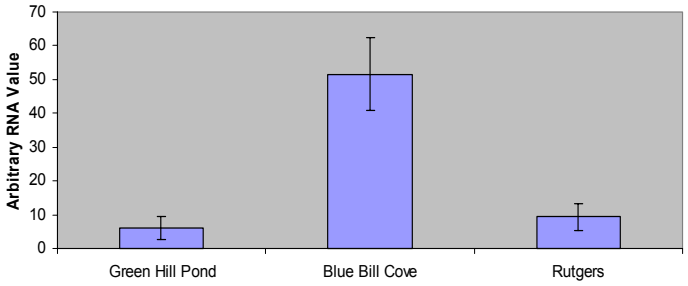

Figure 3. Gene expression levels of mitogen activated protein kinase interacting protein in two wild oyster populations (Green Hill Pond and Blue Bill Cove) and a proven disease resistant line (Rutgers).

Work Planned

Future work includes continual monitoring of our oysters deployed in Edgartown Great Pond. This will include documenting growth, survival, *P. marinus* prevalence and environmental conditions. Proven disease resistant strains from Rutgers University will also be deployed during the summer of 2006 to compare performance. Gene annotation is continuing with our Expressed Sequence Tag project on *Crassostrea* and over the next year, selected genes will be characterized in the oysters from our grow-out site in Edgartown Great Pond. Another major effort related to genetic characterization will include analyzing the differences in hemocyte gene expression patterns from a recently completed experiment conducted at the NMFS in Milford, CT by Gary Wikfors and Inke Sunila (participant on current project). Oysters from Connecticut with Dermo and oysters (*C. virginica*) from Washington State without Dermo, were subjected to mechanical stress. Hemocyte samples were harvested from each oyster and gene expression patterns will be characterized to better understand the relationship of immune function, disease and stress.

Impacts

While the results of our ongoing research have not been directly transferred to industry at this point, our data does suggest that shellfish farmers will be able to realize improved survival with local broodstock that has experienced persistent disease pressure. In addition, our gene expression data can be integrated into broodstock selection programs. These results would not only be beneficial to the oyster industry but could likely be used in developing superior broodstock in other shellfish.

Support

	NRAC-USDA Funding	Matching Support	Total Support
Year 1	$56,962	$13,979	$70,941
Year 2	$71,542	$13,979	$85,503
TOTAL	$128,486	$27,959	$156,445

Publications, manuscripts, or paper presented

Roberts, SB. 2006. Genomic approaches in characterizing shellfish disease: interrelationships between animal, human and ecosystem health. Cummings School of Veterinary Medicine at Tufts University, Annual Symposium: Marine and Aquatic Medicine & Conservation. North Grafton, MA. April 22, 2006.

Diner, E., Smolowitz, R., Gomez-Chiarri, M., Tammi, K., Leavitt, D., Roberts, S. 2006. Assessing disease tolerance in the eastern oyster using gene expression profiling. 26th Annual NOAA-NMFS Milford Aquaculture Symposium. Meridan, CT. February 28, 2006.