



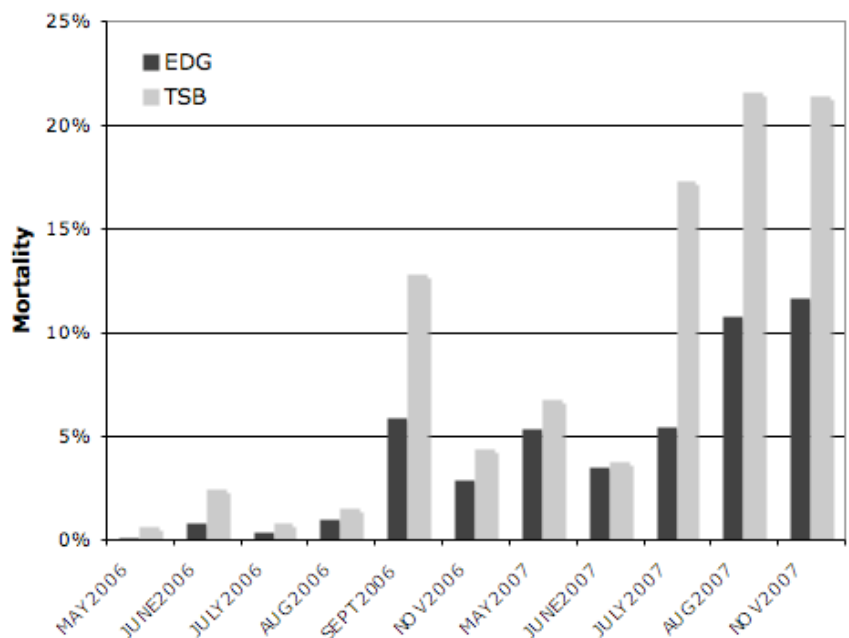
## Surviving Dermo: Progress towards Growing a More Resistant Oyster

Dermo (*Perkinsus marinus*) is a common and occasionally devastating disease of American oysters (*Crassostrea virginica*) found along the US East coast from Maine to Florida and along the Gulf coast. First documented in the 1940's in Chesapeake Bay, the disease has spread northward affecting oyster farmers throughout its range. Typically observed in oysters at least one year old, moderate to heavy infections reduce growth, lower the oyster's condition, reduce reproductive potential and, of course, cause death. Attempts to control the disease have largely focused on not transplanting infected seed and taking advantage of low salinity (< 9 ppt) areas where the disease does not do as well. Unfortunately, many oyster farmers don't have access to low salinity growing areas and are affected by disease.

This has led to a demand for Dermo resistant oysters. In a recent study, Dermo resistance was assessed in two populations of oysters from Martha's Vineyard (MA, USA) and the results demonstrate that shellfish farmers will be able to realize improved survival with local broodstock that has experienced persistent disease pressure. Though heavily affected populations have long been thought to be good sources of disease-resistant brood stock, this study conclusively demonstrates this. In addition, the research identified specific genetic markers in oysters that indicate disease resistance, allowing shellfish hatchery managers to be confident that their broodstock is tolerant to Dermo. Finally, as a side benefit of this research, a new lower cost, high volume method of testing for Dermo was developed, using quantitative PCR.

### Heavy Disease Pressure Produces Resistant Broodstock

In this study, performance of two oyster populations was compared over 18 months in Edgartown Great Pond, MA. One population (EDG) was from broodstock that had survived consistent disease pressure. A second population (TSB) was from broodstock that were kept in a hatchery and exposed to limited disease. As shown in the figure, the percent mortality was much greater over time in the naïve TSB oysters, compared to the EDG oysters. These results indicate that shellfish hatcheries can obtain superiorly performing broodstock by selecting from local, wild population that have survived heavy disease pressure, thus being assured of providing disease-resistant stock to oyster farmers.



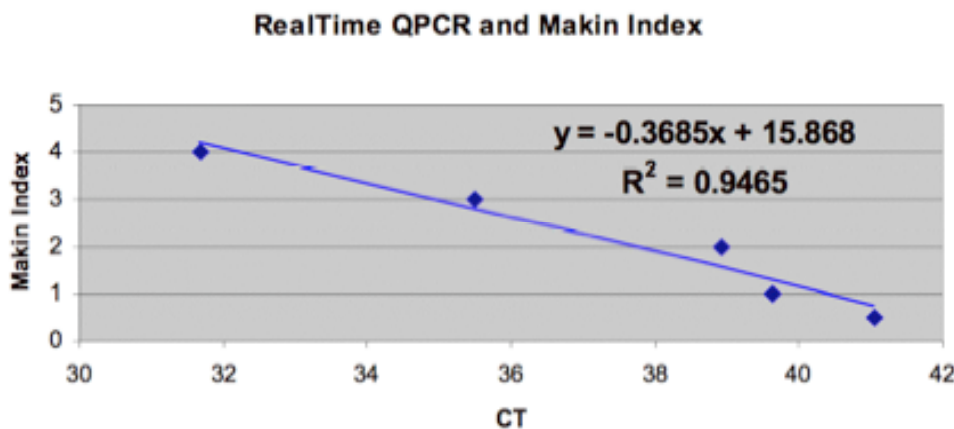
### Identifying Dermo Resistant Oysters with Genetic Markers

Exact disease history for a body of water is not always known, of course, so relying on broodstock collected from a water body is not a sure thing. In order to assist in the selection of oyster broodstock, genetic markers have been developed. This means that samples of oysters from an area could be collected and submitted to a lab to see if the population has markers associated with resistance to Dermo. Specifically, conventional quantitative PCR can be done to determine if certain gene expression patterns correlate with improved performance<sup>1</sup>. If so, then the tested population is a good candidate for Dermo-resistant broodstock.

### New Low Cost Method to Test for Dermo

As a benefit of this study, a high-throughput, low-cost alternative for Dermo diagnosis was developed that quickly and accurately determines the presence and intensity of Dermo in an oyster population.

The test is a molecular assay that uses quantitative PCR with dual-labeled probe technology<sup>2</sup>. Importantly, this new test was compared directly with the conventional Makin Index with significant correlation, indicating that this new test provides the same information but both more quickly and cheaply than standard methods.



### Promising Directions

In addition to the progress made, it was observed that moderate physical stress activated the oysters' immune defense pathways and increased number of cells responsible for attacking pathogens, such as Dermo. This suggests that there *could* be positive, short term effects of limited physical stress (e.g., tumbling) on immune function. This has not yet been demonstrated to increase survival and, of course, physical stress could potentially harm oysters; therefore, this particular area requires more research before it can be recommended to oyster farmers.

<sup>1</sup> The gene expression assays are carried out using conventional quantitative PCR analysis. Some of the genes with expression levels that correlated with improved performance include MAP8K IP (Accession # CD526707), BCL-X (Accession # CD649038), CIAPIN (Accession # DQ403192) and HMG (Accession # EU437744).

<sup>2</sup>Standard assay conditions for this technology are used with the following primer and probe sequences;

fw: CGC CTG TGA GTA TCT CTC GA

probe: FAM-CGC AAA CTC GAC TGT GTT GTG GTG-BHQ1

rv: GTT GAA GAG AAG AAT CGC GTG AT

For more information go to:

<http://fish.washington.edu/research/genefish/robertslab/dermo.html> or <http://tinyurl.com/22nhbs>