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## Research Summaries

### **Title**

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# Gene Expression Predictors of Summer Mortality Syndrome in Pacific Oysters

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## SUMMARY

Native to Japan, the Pacific oyster (*Crassostrea gigas*) is a major component of the U.S. shellfish industry, hailed as Washington's most valuable bivalve. NOAA estimates that from 1984 to 2005, oyster farming generated approximately \$96.2 million annually (not limited to the Pacific oyster).

However, a common oyster disease, summer mortality syndrome (SMS), has been known to destroy more than half (52% to 63%) of a farm's expected annual harvest. Though this disease first appeared in Japan in the 1950s, it later spread to North America and Europe, creating serious problems for growers. Combating the disease could save oyster growers, here and abroad, millions of dollars annually.

In this project, the biologist sought to investigate the causes of SMS in Pacific oysters. His goal was to identify possible links between SMS and gene expression patterns by collecting fluid samples from oysters in the summer. He also observed how the oysters were affected by higher water temperatures and full vs. partial submersion.

## METHOD

The scientist began his research in the summer of 2007 in Shelton, Washington, a location historically known for having high rates of oyster mortality. He travelled to the location at 2-3 week intervals from June to August, using what he called an "innovative repetitive sampling approach" to test the oysters for SMS, without impacting their survival.



Sample oysters at Totten Inlet, Wash.

By drilling a small hole in the oyster's shell, the scientist was able to extract hemolymph (a fluid in the circulatory system) with a needle and place it directly in a microcentrifuge tube. The samples were then frozen on dry ice until the research team returned to the University of Southern California. In 2007, they collected a total of 768 samples from 205 oysters.

Once back at the lab, the hemocyte RNA was hybridized into a cDNA microarray, at which point an algorithm was used to identify gene expression patterns.

In addition to the gene expression analysis, the scientist also exposed animals to environmental stress associated with summertime conditions, including thermal stress, hypoxia (lack of oxygen), different salinity levels, hypercapnia (high levels of carbon dioxide) and oxidative stress, to test any effects they may have on SMS.

In 2008, the scientist returned to Shelton to collect more samples following the 2007 protocol and found that mortality rates were higher, but still much lower than expected.

## RESULTS

In 2007, only 31 of the 342 oysters that were tracked died. This 9% mortality rate was far lower than the scientist expected. In 2008, the mortality rate rose to 25%, which the scientist believes may have been linked to the sporadic nature of the disease or the slightly warmer summer temperatures.

Despite the high rates of survivorship, he was able to identify about 60 genes that exhibited extreme differences in oysters that died versus those that lived. These differences were reliable indicators of the likelihood of mortality weeks before the oysters actually perished.

In 2008, the scientist experimented with the oysters' need for water by submerging some oysters and partially submerging others. He found that while 25% of the partially submerged oysters died, only 12% of the submerged ones perished. He also found that when water temperatures rose above 36 degrees Celsius, mortality rates jumped dramatically.

## CONCLUSION

The scientist believes the low mortality rates observed during this project can be attributed to the sporadic nature of the year-to-year severity



The inside of a Pacific oyster.



Hemolymph testing technique being conducted on an oyster.

of SMS. Through communication with other researchers and farmers, he found that mortality was relatively low overall in 2007 and 2008. Though these data would have been more compelling had there been more oyster deaths, the scientist believes that his analysis will help identify the root causes of the syndrome.

In addition, he can now accurately predict the mortality rate for a particular set of oysters using gene expression. The next step is to identify a common gene expression in all sets that will indicate an animal's chances of survival.

Although the data have not yet been fully analyzed, the faster-than-normal growth of farmed oysters is one of the scientist's hypotheses for their high mortality rates. Cultured oysters are fed and kept fully submerged in order to help them reach sexual maturation quickly. It is possible that this fast growth causes metabolic problems. The scientist hopes to identify a process to create disease-resistant oysters that could grow to large size.

## APPLICATION

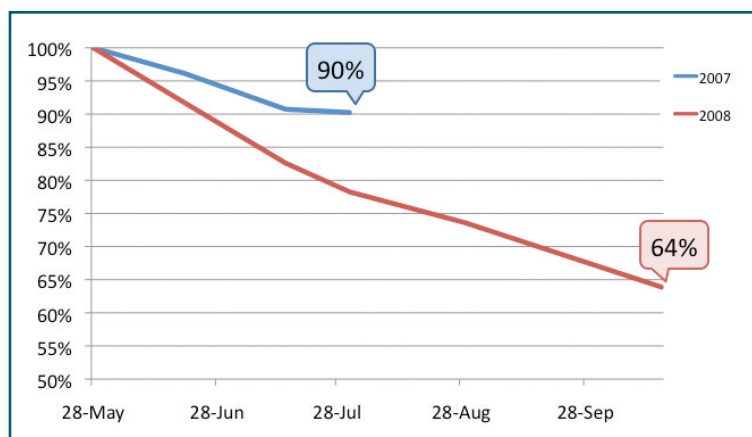
The economic repercussions of discovering a method of breeding disease-resistant oysters would be staggering. Currently, oyster farmers often harvest smaller oysters (which are less likely to contract disease) to prevent losses due to SMS. This strategy, however, reduces production and profits. If scientists were to find a method of controlling the disease at the genetic level, farmers could raise larger, healthier oysters.

## PRESENTATIONS

Chaney, M. and A.Y. Gracey. Correspondence between tissue function and gene expression in *Crassostrea gigas*. National Shellfisheries Association 100th Annual Meeting: A Century of Shellfish, 2008.

Chaney, M. and A.Y. Gracey. Exploring summer mortality disease characteristics in *Crassostrea gigas* through gene expression. National Shellfisheries Association 101st Annual Meeting, 2009.

Chaney, M. and A.Y. Gracey. Interpreting disease physiology with molecular patterns in the Pacific Oyster. National Shellfisheries Association 102nd Annual Meeting: Aquaculture, 2010.



Survivorship rates from two summers of testing.

## STUDENT

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