Patrick Gaffney
Professor
University of Delaware
College of Marine Studies

Testimony
Before the Committee on Resources
Unites States House of Representatives

Hearing on the Status of the Eastern Oyster (*Crassostrea virginica*)
and the Petition to List the Eastern Oyster as Endangered
or Threatened under the Endangered Species Act

July 19, 2005

The eastern oyster *Crassostrea virginica* inhabits estuarine and coastal waters from the Gulf of St. Lawrence to the Yucatan Peninsula, tolerating a wide range of temperature and salinity. More than fifty years ago oyster biologists postulated the existence of distinct "physiological races", adapted to their local environmental conditions. It was not clear, however, whether differences in features such as growth rate or spawning season reflected underlying genetic differences among populations, or merely acclimation to local conditions occurring during an oyster's development.

During the 1980s, researchers examined geographical patterns of variation in tissue proteins and came to the conclusion that oysters from Cape Cod to Corpus Christi were genetically very similar, while peripheral populations in Nova Scotia and southern Texas (Laguna Madre) were distinct. These findings were interpreted to mean that the primary oyster population (Cape Cod to Texas) was genetically homogeneous as a result of gene flow facilitated by the dispersal of planktonic larvae, which spend several weeks in the water column before settling on hard surfaces and metamorphosing into juvenile oysters. The northern and southern peripheral populations were hypothesized to be genetically distinct as a result of long-standing isolation by hydrographic features (temperature, salinity and currents).

This view of a single large unit stock ranging from Cape Cod to Corpus Christi was challenged in the early 1990s as the result of several lines of evidence. Techniques for assessing genetic variation in populations had advanced considerably in the previous decade, and now it was possible to examine genetic variability at the DNA level, which afforded higher resolution and less bias than the analysis of protein variation. A seminal study from the lab of John Avise at the University of Georgia using mitochondrial DNA showed a deep genetic division between oysters from the Atlantic coast and those from the Gulf of Mexico. This division dates back several million years, to the late Pliocene and Pleistocene. A similar genetic break between Atlantic and Gulf is seen for a variety of organisms, and marks the two populations as "evolutionarily significant units". This picture has been developed further using other genetic markers (nuclear DNA) in studies by students of Avise and in my laboratory.

During the last decade, my laboratory has continued to examine geographic variation in both mitochondrial and nuclear genes throughout the global range of the eastern oyster. In addition to confirming the genetic distinctness of the Gulf and Atlantic populations, we have asked whether population subdivision exists *within* the Gulf and Atlantic regions. Experimental evidence that genetically distinct stocks or subpopulations might exist within the Atlantic was provided in 1991 by researchers at the Rutgers Haskin Shellfish Laboratory, who showed that despite six generations of culture in Delaware Bay, oysters of Long Island Sound origin maintained their original (Long Island) pattern of gonadal development and spawning. My laboratory subsequently found evidence of genetic differences between North and South Atlantic oysters, using both mitochondrial and nuclear DNA markers. However, the difference is much less pronounced than that separating Atlantic and Gulf oysters, which may indicate that this pattern of population subdivision has developed much more recently, and may be confounded by the human movement of oysters up and down the Atlantic seaboard. Figure 1 shows the distribution of mitochondrial DNA sequence variants (haplotypes) detected by restriction enzymes, indicating geographic variation in haplotype frequencies with both Gulf and Atlantic

regions. Along the Atlantic coast, some haplotypes are distributed widely but are most common in either the north Atlantic (blue) or south Atlantic (red).

A similar picture emerges from direct sequence analysis of a single mitochondrial gene (Figure 2). We see clear separation between Gulf Coast and Atlantic haplotypes, while Atlantic coast haplotypes are more closely related to each other, and are more widely distributed. For example, the common north Atlantic haplotype (marked DB) is found from Canada through Virginia. Sequence analysis of other regions of the mitochondrial genome show the same pattern.

Although the currently available data are limited in geographic coverage and sample size, nuclear genes tell a similar story. A two-dimensional plot illustrating genetic relatedness (Figure 3) shows a cluster of north Atlantic populations ranging from North Carolina to New Brunswick (blue) separate from south Atlantic populations (orange) and Gulf Coast populations (green). Outliers include a western Florida site, Cedar Key (which also possesses a mix of Atlantic and Gulf Coast haplotypes) and Texas.

My interpretation of the genetic data described here is that the species *Crassostrea virginica* is subdivided into two major "evolutionarily significant units", or subspecies. There is additional population structure within each of these, but the degree of genetic differentiation is smaller, and the boundaries are currently not well defined. The south Atlantic population occupies the coast from Cape Canaveral northwards to somewhere in North Carolina (perhaps Cape Hatteras, a well-known biogeographical boundary). The north Atlantic population ranges from North Carolina to Canada. (Preliminary data also suggest that some populations in Canada appear to be genetically distinct, probably owing to small size and isolation.)

We have not found any indication thus far that the Chesapeake Bay oyster population is genetically different from oysters found in Delaware Bay or other north Atlantic sites.

On a separate issue, it may be appropriate to comment on the possibility of hybridization between the eastern oyster and the Asian oyster *C. ariakensis*, which may be introduced into Chesapeake Bay. We have shown that the Asian oyster cannot form viable hybrids when crossed with the eastern oyster. Cross-fertilization can be achieved in a hatchery, but the embryos die after 7-10 days. Thus the possibility of hybridization between the two species seems negligible.

However, the potential for cross-fertilization does raise concerns about interactions among the two species, if the Asian oyster were to be introduced. That is, if the two species lived side by side and spawned at the same time, it is possible that cross-fertilization would result in the loss of large numbers of gametes from both species. For this to happen, two things are necessary. First, the two species must spawn at the same time, so that the eggs and sperm from both species are in the water together. Second, cross-fertilization must be able to occur when eggs and sperm from both species are in the water together. In this situation, it is likely that eastern oyster eggs will be more successful at fertilizing eastern oyster eggs than Asian oyster sperm are; if this is the case, gamete mixing will not lead to gamete wastage. Experiments are now underway to evaluate reproductive interactions among the two species.
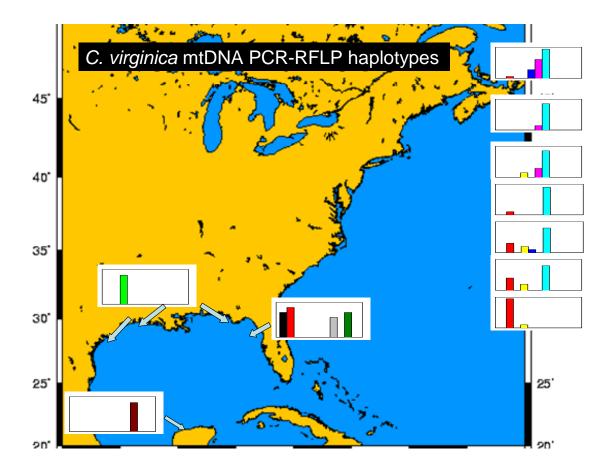
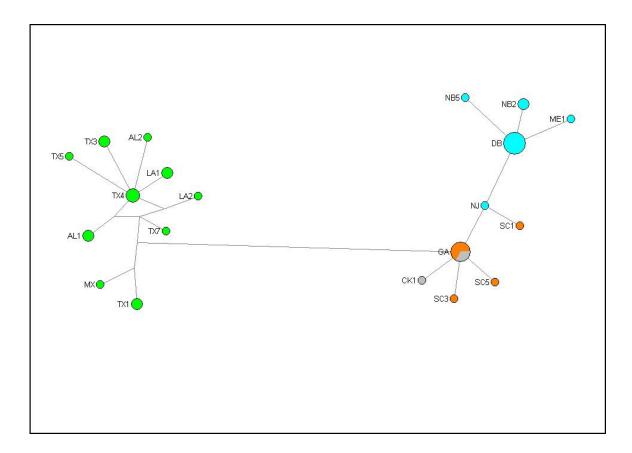Figure 1. Frequencies of mitochondrial haplotypes detected by restriction enzymes.

Figure 2. Mitochondrial DNA haplotypes fall into three clusters: Gulf Coast (green circles, including Alabama, Louisiana, Texas and Mexico), South Atlantic (orange circles, including Georgia and South Carolina) and North Atlantic (blue circles including Chesapeake Bay, Delaware Bay, New Jersey, Maine and New Brunswick). The common South Atlantic haplotype is also found in one Gulf Coast location, Cedar Key (western Florida, grey).
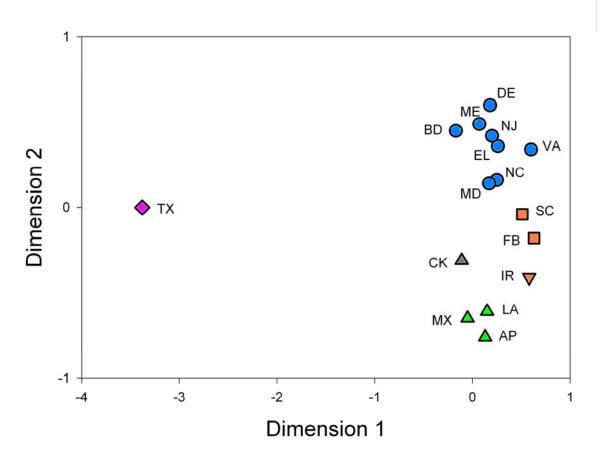
Figure 3. Nonmetric multidimensional scaling of genetic distances among eastern oyster populations, based on four nuclear genes.