TAKING THE "X" OUT OF QPX DISEASE

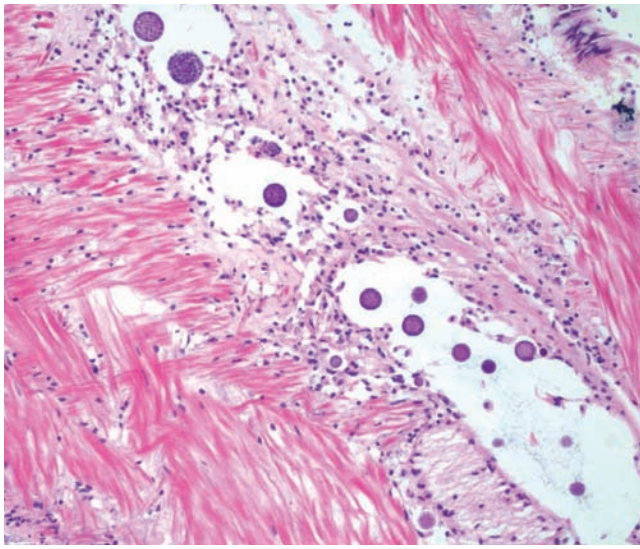
New York Sea Grant's mission is to provide scientific information that helps our coastal economies and environment. "When New York's shellfish industry started seeing large numbers of dead clams in Raritan Bay in 2002, we saw this an an important problem," says NYSG Assistant Director **Cornelia Schlenk**. "Timely research could play a critical role for managers making decisions about this valuable marine resource." That research recently bore fruit, shedding light on the puzzling disease affecting hard clams.

The story begins in 1987. The NYSDEC had introduced a shellfish transplant program, harvesting hard clams from uncertified waters of Raritan Bay off the coast of Staten Island and transporting them to certified Long Island waters. Once purged of bacterial contaminants through a natural cleansing process in designated areas in Peconic Bay, these sturdy bivalves could be re-harvested and eventually marketed as a safe food product.

The Raritan Bay Shellfish Transplant Program not only provided an opportunity for baymen to harvest shellfish from designated areas that were normally closed, but also protected public health by reducing shellfish populations in uncertified waters, making them less attractive to illegal harvest.

According to the NYSDEC's **Debra Barnes**, a marine biologist and Shellfish Management Unit leader in the Bureau of Marine Resources, it was one of the most successful shellfish management programs in the state. "The estimated wholesale value of the shellfish harvested in this program was more than $5 million annually. The program accounted for almost 45 percent of New York State's annual hard clam production and involved the participation of more than 160 shellfish harvesters and up to five shellfish cleansing operations located in Long Island Sound and Peconic Bay."

That transplant program proved effective until 2002, when a severe threat to the New York hard clam industry surfaced. Shellfish transplant harvesters began reporting large numbers of dead and dying clams in the waters off the coast of Staten Island. The diagnosis? A single-celled microscopic parasite that only infects northern hard clams (*Mercenaria mercenaria*), identified as Quahog Parasite Unknown and creating "QPX disease."



Lesions appear as white areas dotted with round clusters of QPX cells in this stained section of a hard clam siphon. Digital image by **Soren Dahl** as appears in *Journal of Shellfish Research,* August 2007. Reprinted with permission.

"The transplant program was immediately terminated on September 6, 2002, to prevent the potential introduction of the parasite to the cleansing sites and waters of Peconic Bays," says Barnes. She added that the shutdown of the program had devastating economic impacts on the program participants and permit holders who relied on this program for a significant portion of their income. "QPX disease affected almost 10,000 acres in Raritan Bay and was conservatively estimated to result in mortality of millions of hard clams."

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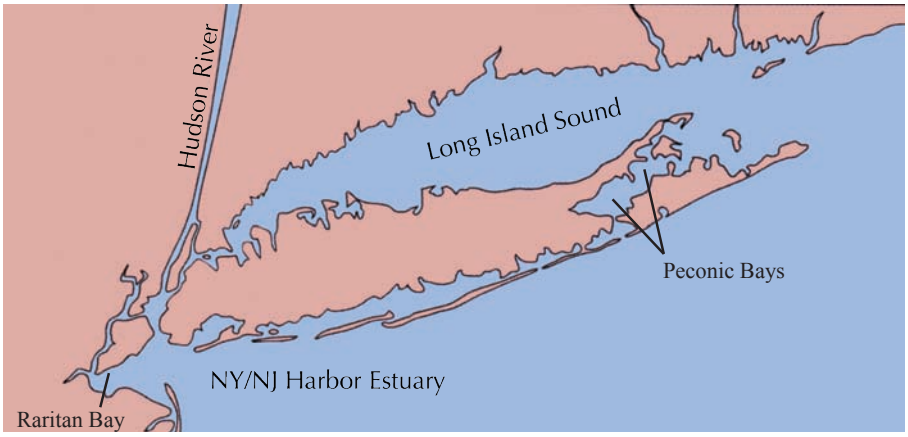
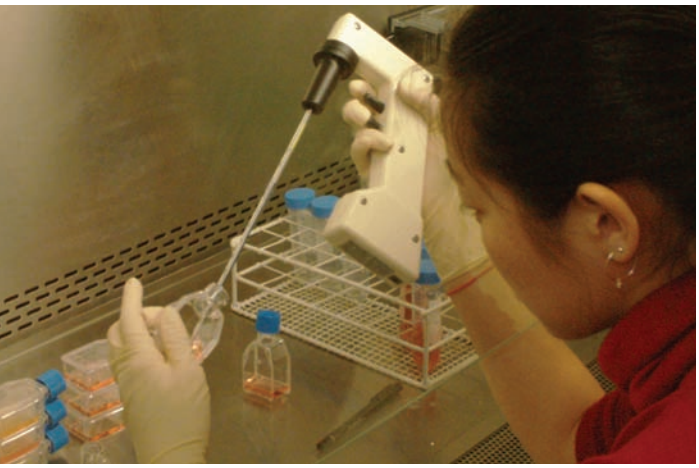
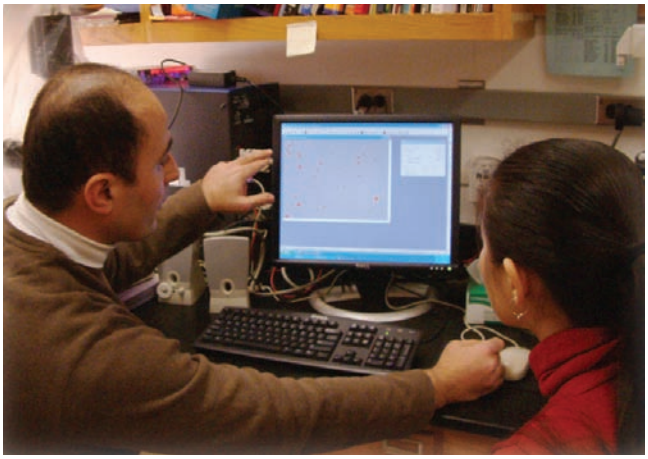


The NYSDEC Transplant Program taking clams from Raritan Bay to Peconic Bays was one of the most successful in the state until QPX caused clam mortalities in 2002. The program resumed in 2005.





Above, Sea Grant Scholar Qianqian Liu is transferring QPX culture. Isolating and maintaining the QPX parasite in living culture is crucial for most investigations of the disease. Using a digital camera, Bassem Allam and Qianqian Liu (at right) examine the quality of growth of the laboratory culture.

Since the closure, the NYSDEC and the Marine Animal Disease Laboratory (MADL) at Stony Brook University have developed and conducted an extensive QPX monitoring program in Raritan Bay, Peconic Bay and other areas of the marine district to determine the extent and distribution of QPX disease. Barnes notes that the transplant program remained closed until May 2005, when the NYSDEC re-opened 25 percent of Raritan Bay to transplant harvest based on the results of QPX monitoring, which found "null or low prevalence of QPX in hard clams collected from these areas."

QPX disease had caused mortalities in both cultured and wild populations of *Mercenaria mercenaria*, or hard clams, in the northeast in the 1990s. As its moniker suggests, little was known about this pathogen, a protistan parasite. A recent New York Sea Grant-funded study partially supported by NYSDEC, which has provided insight into the pathobiology of this parasite, may lay the groundwork for restoring the hard clam to health, as well as the industry itself.

This published work, titled "Laboratory Transmission Studies of QPX Disease in the Hard Clam: Development of an Infection Procedure," can be read in the *Journal of Shellfish Research*, August 2007 issue. The article, written by MADL team leader **Dr. Bassem Allam** and Sea Grant Scholar **Soren Dahl**, was based on Dahl's master's thesis. The article concludes, "This study has established an experimental infection method that can be used for future investigations concerning crucial aspects of the QPX/hard clam disease system." The investigators call this a major advance in the investigation of this important disease.

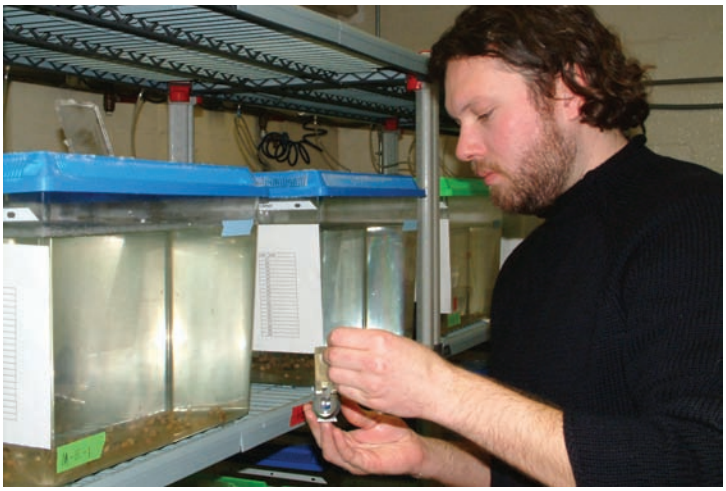
The main purpose of this NYSG-funded research was to investigate the ways the disease can be transmitted to clams and to replicate infection under laboratory conditions. Two different methods were used: cohabitation and inoculation.

For the cohabitation experiments, the research team used adult clams from sites where there have been QPX outbreaks–Raritan Bay (Staten Island, NY) and Cape Cod (MA). The infected adults were placed in with 2,400 seed clams from four different locations supplied by partners from Virginia Institute of Marine Science (VIMS) and Woods Hole. A control placed similar seed clams in seawater but without the infected clams. Over a year, mortality counts and histology samples were taken periodically. The results? The presence of infected clams did not cause disease in juveniles, which suggests that it takes more than just close habitation for transmission to occur.

However, the results from the inoculation experiments showed that QPX is a directly infective pathogen, as injection of cultured cells leads to infection and mortality. The experiments used year-old clams, some inoculated with a needle containing QPX and others merely exposed to QPX in the water column. When analyzed, the researchers found that a majority of inoculated clams had the disease, but not the clams exposed to the pathogen via the water. Although clams in the wild don't get the disease by inoculation, the lesions and other signs of the disease in the experiemental clams were the same as those observed in wild stocks.

The cohabitation experiments brought some unanticipated observations, too. "It was a very big surprise," says Allam. "We found that many heavily infected clams in the lab healed. There, we were controlling temperature, salinity and oxygen, however in the field there are fluctuations of these variables." For the naturally infected clams, the laboratory environment with its steady conditions may have been advantageous, promoting healing and resistance.

This observation is encouraging and may help to identify ways to manage or remediate clams infected



Sea Grant Scholar Soren Dahl examines water quality of the tanks holding experimental clams.
Photos by Paul C. Focazio

with QPX. Ultimately, environmental conditions could prove to be significant considerations in QPX disease mitigation.

"We work hand in hand with the state," says Allam. "Perhaps we can help find ways to transplant clams into bodies of water at an optimal, likely higher, temperature to promote clam health."

Barnes says that the diagnostic and research work conducted by MADL and funded by NYSG "plays a critical role in providing information needed for management and protection of the state's marine resources" and that the NYSDEC's important partnership with the MADL has enabled the agency "to obtain information on the distribution and prevalence of QPX in Raritan Bay and other locations in the marine district, which is vitally important for the management of these resources and support of our shellfish industry."

"I think the results of the research provide significant information on some of the unknowns about QPX transmission and also confirm the theory of QPX being a directly infective pathogen (opportunistic parasite)," says Barnes. "I was surprised by the laboratory results of the cohabitation experiments when they showed that QPX did *not* transmit from diseased adult clams to non-infected clams when held within the same tank."

Barnes notes that although these research findings will not likely change New York's shellfish transplant policies for the short term, "the results will be incorporated into the state's QPX monitoring program in order to maximize the harvest areas available for shellfish harvesters and at the same time minimize the risk of transmission to our natural clam resources." She adds that the results could also be used by town shellfish managers and private aquaculturists to develop Best Management Practices for hard clam restoration and culture.

What are the next steps? Other complementary research projects have already begun to examine variables like temperature, density and possible vectors that may cause disease transmission. The role of substrates (sediments or plants) that could possibly support the QPX pathogen outside of the clam was not incorporated into the inoculation experiments. Earlier in 2007, Stony Brook grad student **Deenie Bugge** (now at Dartmouth College) found that algae might be an important factor for the survival, growth and spread of QPX in the marine environment. A new line of research will now examine the possible role of sediments in transmission. Yet another project will examine the immune system of clams.

So can Allam and his team say that they've taken some of the X out of QPX by this series of experiments? "Yes," says Allam. "We haven't concentrated so much on the 'what' of this disease, but we have learned much about the 'how' of its transmission."

More on those unknowns is being filled in with the help of co-PI **Dr. Jackie Collier**. As reported in the September 2007 issue of *Diseases of Aquatic Organisms*, the research team, including Sea Grant Scholars **Hua** (Daisy) **Qian** and **Qianqian Liu**, examined a variable part of the QPX genome and found the same sequences in naturally infected clams as in experimental cultures of QPX. They have since used this information to develop a real-time polymerase chain reaction method that can be used to detect and enumerate the QPX organism in environmental samples and in hard clams. This new method is giving the team a new way to investigate the development of QPX disease in hard clams. These results will be described in research papers to be published soon. Stay tuned.

— Barbara A. Branca and Lynn Zawacki

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... for more photos of clam experiments and NYSG's 2003 brochure *QPX Disease in Hard Clams — Quahog Parasite Unknown*

HISTORY OF THE QUAHOG

Chowders, cherrystones, littlenecks–seafood lovers find these tasty, nutritional hard clams irresistible. For most of the twentieth century, the hard clam or northern quahog has been one of the most valuable seafood products harvested in New York.

Commercial harvesters dig for hard clams in shallow coastal bay waters in areas with a soft sandy bottom. But dig a little deeper into American history and you'll find that the hard clam was also valued by Native Americans and that it played a part in the economy of the colonies.

Native Americans collected oblong shells, which they polished and sawed into beads known as wampum (wampumpeag). The small, tubular beads were made of white or purple seashells; purple wampum beads were made from the growth rings of the Quahog shell. The beads were assembled into strings or woven into belts or embroidered ornaments. Wampum was valued because of the labor and skill it took to make. Indians used wampum for ceremonial purposes, oral traditions or as gifts, but not as currency.

In the early 1600s, however, European traders and settlers faced with a shortage of European currency turned to wampum beads as coin substitutes. It was used as legal tender in the original colonies, and the last recorded exchange of wampum as money was in New York in 1701.

— Lynn Zawacki