Restoring Oysters to U.S. Coastal Waters

A NATIONAL COMMITMENT

OYSTER DISEASE RESEARCH PROGRAM
NATIONAL SEA GRANT COLLEGE PROGRAM

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A REPORT FROM THE NATIONAL SEA GRANT COLLEGE PROGRAM

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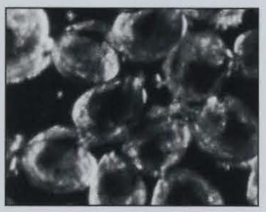


The Oyster Disease Research Program (ODRP)

A Congressionally-mandated program to support research, outreach and management efforts to better serve restoration of healthy populations of oysters in the nation's coastal waters, ODRP began in 1990 with oversight by the National Oceanic and Atmospheric Administration's National Marine Fisheries Service and its Chesapeake Bay Office; it is now administered by the National Sea Grant College Program.

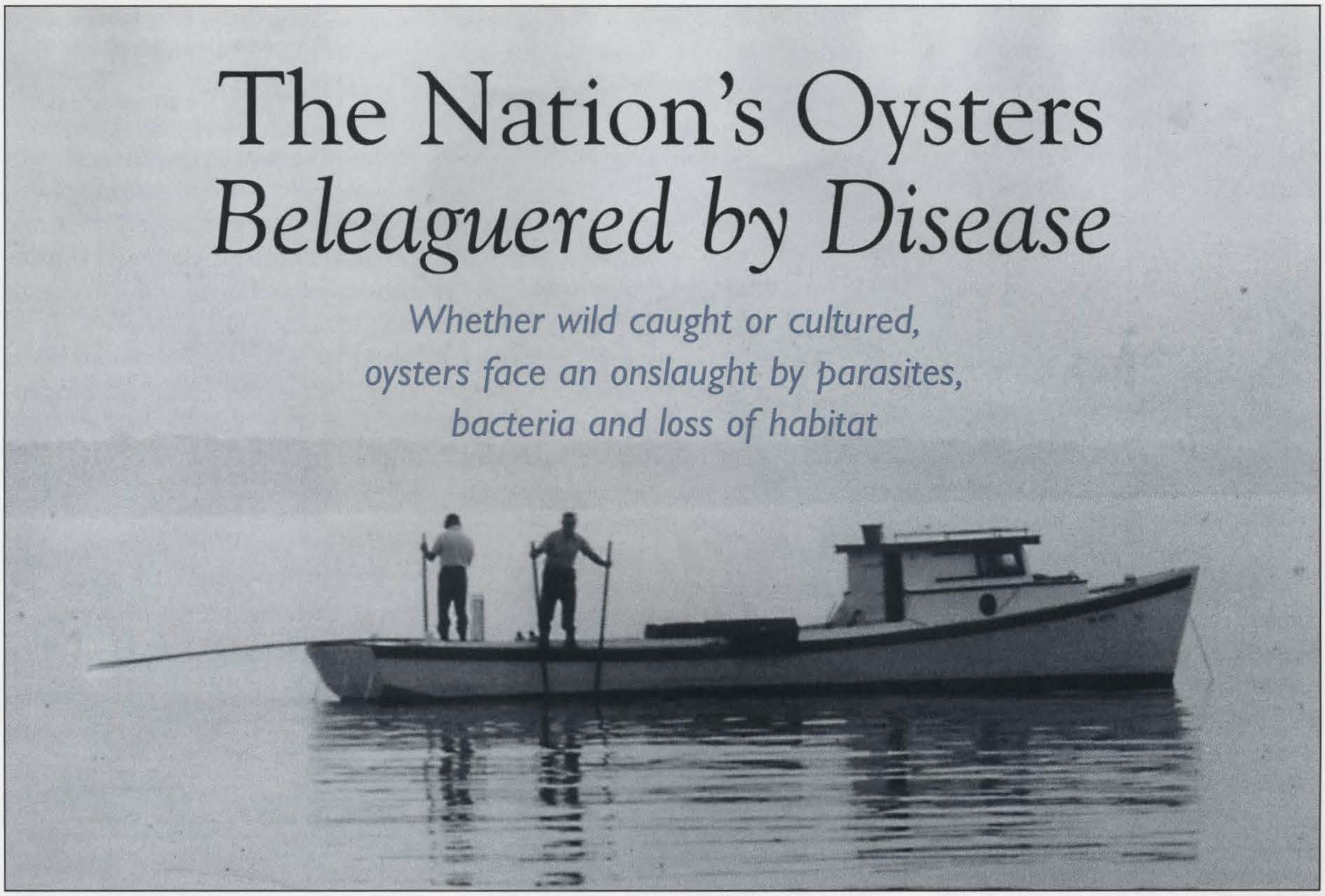
Through competitive proposals, ODRP supports research to develop the following: (1) optimal strategies for managing around disease; (2) better understanding of the processes of parasitic infection; (3) improved understanding of the oyster's immune system; (4) hatchery techniques for producing disease-resistant strains; (5) molecular tools to better monitor the onset and presence of disease.

For more information about research findings and current projects, contact the National Sea Grant College Program, 1335 East-West Highway, Silver Spring, Maryland 20910 or visit the ODRP website:

http://www.mdsg.umd.edu/NSGO/research/oysterdisease/

The Nation's Oysters *Beleaguered by Disease*

Whether wild caught or cultured, oysters face an onslaught by parasites, bacteria and loss of habitat



For more than a century, commercial oyster harvests have had a major economic influence on many of the nation's coastal communities with annual dockside values totaling in the tens of millions of dollars. These numbers rise significantly when support industries are included — processing plants, boat building, equipment manufacturing and other services that range from retailing to food service. All told, the oyster business has employed thousands of workers. Along the Atlantic coast, in particular, this is no longer the case.

The wild oyster fishery, which has helped define a way of life in the mid-Atlantic and Gulf Coast regions, saw the first signs of decline in 1956 when a mysterious parasite — it was initially referred to as MSX because of its multispherical appearance — killed more than 90 percent of the oysters in Delaware Bay. By the next year, MSX began making its way up the lower Chesapeake Bay, and over the next several years, it spread farther up the Bay into Maryland waters.

On the west coast, where the industry is based primarily on aquaculture, growers do not depend on natural sets of oysters; rather, oysters are spawned in the hatchery and then planted on private grounds. While growers in Washington state, Oregon and California have been successful in developing an industry based on a non-native species (the Pacific oyster, *Crassostrea gigas*), intensive production has also resulted in increased prevalence of many infectious diseases known as "summer mortality" — these diseases now threaten the future of the industry.

Early in the 1940s, oysters in Gulf Coast waters began falling to a protozoan parasite, *Perkinsus marinus* (more familiarly known as Dermo because it was originally classified as a fungal species, *Dermocystidium*). In the mid-80s, Dermo began killing oysters in the Chesapeake Bay — over the next decade, the parasite spread throughout the Chesapeake, sometimes inadvertently transported by replenishment programs and commercial operations that moved oysters from reproductively rich bottom grounds to areas more favorable for growout. By the early 90s, Dermo had infested virtually every major oyster bottom in the Bay. A resilient parasite, Dermo has continued to move up the Atlantic coast; it has struck Delaware Bay and been seen as far north as Maine oyster grounds.

Harvest records tell part of the story. In Maryland, for example, the annual catch fluctuated between two and three million bushels a year from the 1960s through the 1980s. Over the last eight years, the annual catch has averaged a mere 150,000 bushels, a 90 percent decline. With declining oyster harvests has come

Left: Oysters form the base of important habitats and help to sustain biological diversity. Opposite page: The processing industry and related businesses have also suffered with the decline of oysters in coastal waters.

a diminished industry infrastructure: shucking houses, businesses that served harvesters and processors in the fall and winter months, economic changes and something more elusive — a way of life in which social and work patterns that were clearly tied to the region's historical past and ecological present.

A Keystone Species

The heavy loss of oysters to disease and the dismantling of habitats through more than a century of harvesting and landborne pollution have also impacted water quality that depends, at least in part, on robust oyster populations and the bottom-dwelling communities that form around them. Only in recent years have we begun to appreciate what the loss of oysters and their reef structures have meant to coastal sounds and estuaries from Maine to the Gulf of Mexico.

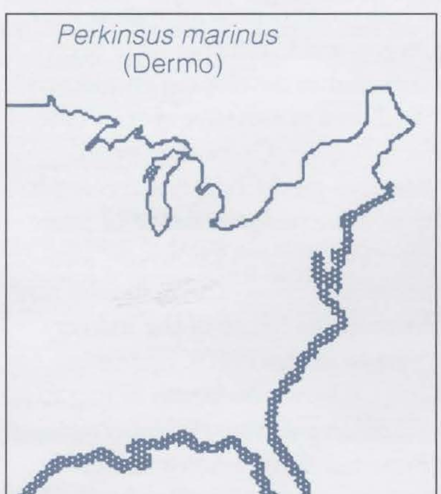
Oysters are keystone species in a number of aquatic ecosystems, meaning that they play a significant role in converting organic matter, namely single-celled algae, to energy. An adult oyster, for example, can filter 40 to 50 gallons of water daily in warm months — in so doing, they ingest algae, thus removing that living matter from the water. Though algae (also called phytoplankton) form the base of the food chain and are critical for organisms higher up in the chain, too many algae can present a problem for ecosystems that cannot assimilate them.

A major problem in many estuaries like the Chesapeake is two-fold: nutrients flooding in by land and air and a lack of assimilative organisms to handle them. The result is excessive growth of phytoplankton. Without enough filter feeders like oysters, the algae that go uneaten are left to decay. The dead algae, when metabolized by microbes, contribute to oxygen depletion, a major cause of poor water quality, especially along the bottom, where many species live.

Clearly, more oysters and improved habitat in the Chesapeake could help remove algae and contribute to improved water quality; meanwhile, improved water quality could also lead to an invigorated oyster fishery, based on sustainable harvesting.

Until recently, the prospects of any restoration were hardly conceivable in the mid-Atlantic region. Not only had Dermo and MSX dec-

A resilient parasite, Dermo was first observed in Gulf Coast waters and has been moving up the Atlantic coast.



In these maps dark shading indicates regions where Dermo and MSX diseases are epidemic among a large number of organisms (epizootic) or have occurred in particular localities (enzootic); light shading indicates regions where parasites have been reported, but are not causing recognizable mortalities.

imated virtually every major oyster ground in Chesapeake and Delaware bays, but except for ad hoc actions to try and manage around disease, a full-scale plan to combat these diseases was virtually nonexistent. Such a comprehensive plan is essential. Scientists have lacked a fundamental understanding of the biology of the parasitic organisms, let alone knowledge about the factors that make oysters so vulnerable. Though researchers at the Rutgers University Haskin Shellfish Laboratory had bred oysters resistant to MSX, these oysters were not resistant to Dermo. When Dermo began spreading through mid-Atlantic waters, the Rutgers oysters proved to be vulnerable, thus precluding their use in commercial hatchery operations.

A Coordinated Counterattack

While research on oyster disease through the 70s and 80s increased the scientific understanding of such issues as the oyster's immune system, that research was piecemeal, consisting of individual studies largely funded by the National Science Foundation and individual Sea Grant programs. There was no coordinated, nor consistent long-term support that would enable scientists to develop innovative research efforts — nor did those piecemeal efforts focus on the critical relationship between scientific findings and their practical application by growers and management agencies.

In 1989, however, Congress enacted legislation which recognized that if there was to be any real chance of returning oysters to coastal ecosystems, then it would take consistent support for research and, equally important, education efforts to demonstrate the application of that research. The Oyster Disease Research Program (ODRP) is the result — its aim, the development and application of a body of knowledge to help restore oysters to coastal systems in the United States.

Over the past five years, this research has made enormous progress that has already put surviving oysters back in the water — ODRP has significantly improved approaches to managing around disease and has furthered the scientific understanding of the dynamics of disease. In addition, new molecular probes are now coming on line that will soon give east coast oystermen a rapid means to test for threats of Dermo and MSX and west coast growers diagnostic tools to detect Pacific oyster nocardosis, the widespread disease that periodically causes significant mortalities, especially in cultured species during periods of warm temperature.

Over the past five years, research on oyster disease has made enormous progress.

Scientists have been breeding oysters that are more tolerant of both MSX and Dermo — several seasons of field trials are pointing to new directions that commercial growers, private and public, are already beginning to employ.

Will we see successes in oyster restoration in the next several years? The answer is yes. In addition to improved prospects for disease-resistant oysters, educational efforts by public agencies, citizens groups and Sea Grant programs focusing on the key role of oyster habitats for aquatic health have excited public participation in oyster gardening in the mid-Atlantic. Citizens in Maryland and Virginia are growing oysters along docks and on leased grounds that can then be planted and used as broodstock for new generations of oysters.

Will these and other efforts restore oysters to levels of the mid-80s in Chesapeake Bay, let alone to what they were fifty years ago? The answer is no. It has taken more than a century of steady harvesting and rising pollution to eliminate oysters — it may take decades more to even approach large-scale restoration.

While restoration capabilities will depend on laboratory and field research, such as those supported by the Sea Grant Oyster Disease Research Program, widespread restoration will also depend on other actions, from rebuilding oyster habitats to ongoing efforts to slash contaminant and sediment runoff from the land. Even more, bringing back the eastern oyster will take continued public recognition that oysters are keystone organisms and an understanding that if there is to be any chance of sustaining the health of coastal systems, oyster restoration will be critical.

Breeding Disease Resistance in the Hatchery

Fast-track research has led to eastern oysters bred for resistance to MSX and Dermo

If disease-devastated oyster populations were left to themselves for a long enough time, chances are that eventually enough would survive from one year to the next to serve as foundation stocks for natural, ongoing replenishment. This is because individual oysters, like all organisms, vary with regard to growth, reproduction, and susceptibility to disease. Even under the deadliest attack of *Haplosporidium nelsoni* (MSX) and *Perkinsus marinus* (Dermo), some eastern oysters survive.

Is this tolerance to the impacts of disease due to heredity or environmental influences? Or both? Scientists don't yet know.

How long would oysters need to be left? Ten years, twenty, a lifetime, two lifetimes? There is no basis for predictions.

Such a waiting game of unknown duration is impractical if not untenable, whether for aquaculturists, public harvesters, or resource managers working to bring oysters back for ecosystem restoration. That is why the Oyster Disease Research Program has supported fast-track research on developing strains of the eastern oyster that are hardier and more tolerant of disease. That work has taken advantage of hatchery technology to help oysters do what they might do otherwise, if left alone. Researchers have been speeding up that process — they use traditional breeding practices to spawn adult survivors of disease. These survivors are then reared to maturity and spawned to produce the next generation.

"The results of our work are coming in," says Standish Allen, "and over the next several years they could begin to have an impact on the success of oyster restoration and commercial farming." Allen, formerly at Rutgers University and now director of the Aquaculture Genetics and Breeding Technology Center at the Virginia Institute of Marine Science, has been a catalyst in a region-wide effort that has brought researchers throughout the mid-Atlantic together to work with resource managers and growers to begin testing hatchery-developed oysters under varying conditions of disease pressure.

"We have gone from what was originally my own project," says Allen, "to a truly regional mandate. With support from the Oyster Disease Research Program," he adds, "we have evolved from a grant to a collective regional mission."

Selecting for Disease Resistance

Beginning in the years after MSX invaded Delaware Bay in 1956, nearly eliminating the oyster industry there, Rutgers scientist Hal Haskin began spawning survivors of MSX in the hatchery. The young oysters that survived to maturity were then spawned; those that reached maturity were then spawned again. For the past several decades, scientists at the Rutgers Shellfish Lab continued this breeding program, rearing pedigreed lines of oysters that would tolerate MSX, while working to avoid the potential problems of inbreeding. Those lines were used for both research and for commercial aquaculture.

Then in 1992, *Perkinsus marinus* invaded Delaware Bay; the specially-bred stocks for MSX had little resistance against this new parasite and were hard hit. Though many died, still there were some survivors — and it is those survivors and their progeny that have been serving as the foundation stocks for developing oysters resistant to both MSX and Dermo.

Allen and his collaborators are using these dual disease-resistant oysters in a regional project to evaluate the growth and survival of different oyster lines in the mid-Atlantic. Called CROSBreed for Cooperative Regional Oyster Selective Breeding, the program involves Allen, Susan Ford of Rutgers University, Kennedy Paynter of the University of Maryland Department of Zoology and Don Meritt of the University of Maryland Center for Environmental Science, Mark Luckenbach and Eugene Burreson of the Virginia Institute of Marine Science, and Pat Gaffney of the University of Delaware College of Marine Studies.

CROSBreed researchers have been testing the specially-bred Rutgers oysters at three sites — one in Delaware Bay and two in the Chesapeake, in Maryland's Choptank River and Virginia's Mobjack Bay.

Opposite page: a spawning oyster releases thousands of eggs which will be fertilized by sperm from male oysters. Above: researchers Standish Allen of the Virginia Institute of Marine Science and Ximing Guo of the Rutgers' Haskin Shellfish Research Laboratory examine oysters specially bred to resist MSX and Dermo.

Researchers have bred eastern oysters for resistance to MSX and Dermo — these CROSBreed oysters are now growing in Chesapeake and Delaware bays.

CROSBreed: Offering Hope

In August 1995, nearly 75,000 young oysters (called spat) were divided among three test sites, the Cape Shore Laboratory in Delaware Bay, the Choptank River in Maryland, and at Wachapreague in Virginia. These sites had 4,000 oysters from each of the five strains (20,000 total) of the Rutgers-bred oysters, as well as 4,000 wild oysters from Delaware Bay; in addition, each site deployed about 4,000 local spat. In total, some 100,000 oysters were planted for comparing the response of disease-resistant lines with native oysters to the challenges of disease.

How are these CROSBreed oysters doing?

"At regular intervals from fall 1995 through fall 1997, samples from all groups at all sites were examined for MSX and Dermo, as well as growth and survival," says Pat Gaffney. "In general, the CROSBreed lines have had better overall performance than the unselected controls from New Jersey wild stocks."

Analyzing the combined effects of both diseases over these last two years has been more difficult, largely because MSX was hardly a factor at any of the oyster sites — probably because of unusually low salinities in these areas. MSX was not observed in the Maryland and Virginia sites, while infections in New Jersey's Delaware Bay were at a low frequency in the fall of 1995 (about two percent) and below five percent until summer 1997, when they reached 14 percent.

Among the findings, says Gaffney, are these:

New Jersey, Delaware Bay. By the fall of 1996, Dermo was prevalent in 40-50 percent of the sampled CROSBreed oysters and there were significant differences among the five lines. For example, 71 percent of sampled oysters were infected in one line, while the other showed lower rates of infection, 15-35 percent. Though this pattern persisted through the summer of 1997, by August 98 percent of the sampled oysters were infected, though the intensity of parasitic cells varied.

Virginia, Chesapeake Bay. By fall of 1996, Dermo was present in 9 percent of sampled oysters, but by summer 1997, disease had spread to 79 percent. All of the CROSBreed lines fared better than the local Virginia controls, especially line 1, which was the worst performer at

Selectively-bred oysters are demonstrating better performance than wild unselected oysters.

the New Jersey site. By fall 1997, 95 percent of sampled oysters were infected and, again, significant differences existed among lines.

Maryland, Chesapeake Bay. Scientists found little Dermo infection on this Choptank River bar — less than 2 percent. The few light infections appeared to be randomly scattered among the five different lines.

Because of the low levels of MSX infection, says Gaffney, it is difficult to evaluate the MSX resistance of the selected lines. Dermo infection, on the other hand, was high. Does this heavy Dermo infection mean that the CROSBreed oysters are ineffective for resisting disease? We have to remember, Gaffney says, that the CROSBreed lines were derived from MSX-resistant lines that have been under selection for resistance to Dermo for only one to two generations at most. "That these the lines contracted Dermo," he says, "is not surprising. It may be that resistance to Dermo involves resisting death at a high infection level rather than resisting infection per se."

Gaffney speculates that if MSX had been present at the three sites, the overall performance of the CROSBreed lines would have been more remarkable. To get a better indication of how oysters do under high MSX pressure, the researchers are now moving the Maryland site from the lower salinity Choptank River site to the higher salinity waters off Deal Island. Because the Virginia and New Jersey sites were similar in salinities, the Virginia site is being moved to even higher salinity waters near the sea. "We have shown that the CROSBreed lines were better or equal to local controls," says Allen. "The stock seems genetically robust. Our testing over these next couple of years will tell."

With signs pointing to the success of CROSBreed oysters, Allen is now working with Sea Grant Extension agents on the east coast to get disease-resistant oysters into the hands of hatchery operators.

In an ODRP funded project, researchers will conduct workshops for commercial hatcheries on the principles of managing CROSBreed oysters. "We are offering these oysters to hatchery operators," says Allen, "with the provision that they attend the workshop. They'll learn about principles of genetics, especially as they relate to maintaining these and other strains of selectively bred oysters."

These workshops could catalyze a change in the relationship between commercial hatcheries and scientists like the consortium of ODRP researchers. Allen is enthusiastic. "The benefits for oyster aquaculture on the east coast," he says, "could be considerable."

Sperm, stripped from a male oyster in the hatchery, will be used to fertilize eggs released by female oysters.

Prospects for Disease-Free Seed

Spawning uninfected oysters in hatcheries may be one way to manage around disease

Is it possible to plant disease-free oysters in low salinity waters — where disease may be present but is generally less virulent than in higher salinity waters — and bring them to harvest before Dermo or MSX kills them?

In principle, says Don Meritt of the University of Maryland Center for Environmental Science (UMCES), the answer is yes. "The problem," he says, "is in knowing for sure that we are beginning with disease-free seed."

Because most productive grounds in the Bay are infected with Dermo and are subject to getting MSX early in their growth, the best way to produce oysters that are without disease, says Meritt, is in hatcheries from stocks that are themselves free of disease.

Meritt is doing just that at the UMCES Horn Point Laboratory hatchery, working together with researcher Ken Paynter of the University of Maryland at College Park, the state Department of Natural Resources, and the Oyster Recovery Partnership.

The Partnership, which grew out of a unique consensus agreement in Maryland among watermen, aquaculturists, resource managers, legislators, scientists and environmentalists, has developed a long-range plan for restoring oyster populations and thus improving Chesapeake Bay habitat. The agreement calls for the use of disease-free hatchery seed in the reconstruction of oyster bars in the Bay system, and also sets limitations on transporting, planting and harvesting oysters.

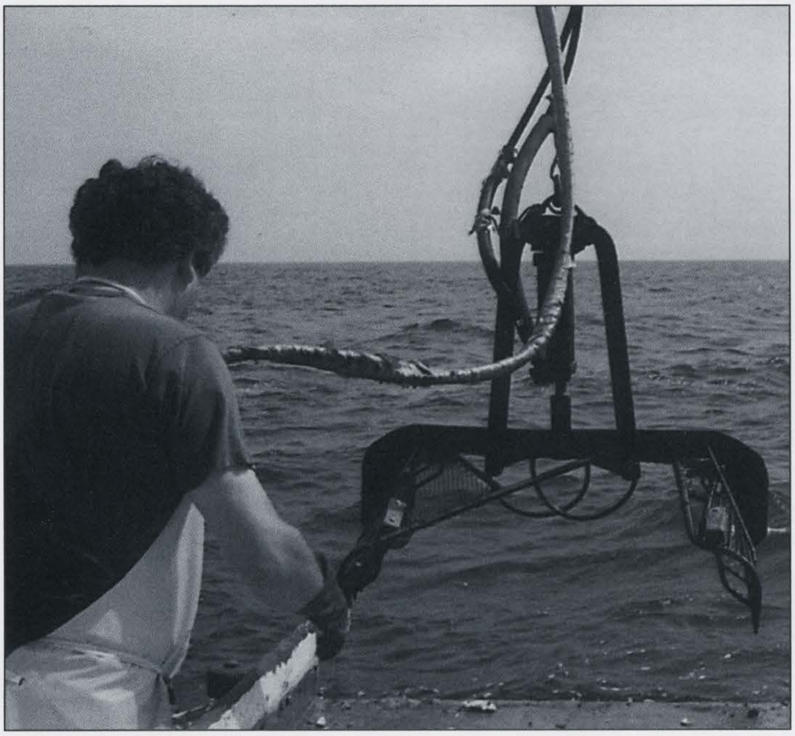
In 1998, Meritt and colleagues produced more than 20 million oyster spat at the Horn Point hatchery — while this is a major achievement for a research hatchery, it is still only enough to plant some 20-30 acres of bottom ground. Considering that Maryland alone has some 270,000 acres of designated public oyster grounds (though most no longer produce harvestable oysters), long-term repletion efforts face an enormous task.

Nevertheless, important inroads are being made: hatchery-reared seed planted in the Choptank and Severn rivers have been assisted by low salinities these past two years, the result of high precipitation which has helped moderate the intensity of disease. Meritt is working with Paynter to measure the success of this project, which also employs hundreds of volunteer students each summer season whose efforts include unloading thousands of bags of oyster seed from setting tanks and transporting them to boats where they are off-loaded into the river.

After a month or so, when oysters have grown hardier, students transport the bagged oysters upriver, where they release the spat-laden shells for planting on oyster bars.

"We've been tracking the oysters planted in the Choptank for three years now," says Paynter. "They haven't grown very fast, which is due to the low salinity; the up side, though, is that they've been essentially disease free for that time." So far, so good, he says. "The oysters are about two inches long now on average and mortality has been quite low, even though the salinity dipped to one part per thousand in 1996."

While commercial culture of oysters has been a risky proposition in the mid-Atlantic region, Meritt is finding commercial growers in the Bay interested in the potential for managing around Dermo with disease-free seed. Economics is the key — if growers have leased grounds to grow their oysters and can get a high enough price for their labors, new ventures will be getting underway.



Modeling Around Disease

Simulation models can give managers the ability to assess the effects of different environments and different restoration strategies on the virulence of oyster disease

The natural set of new oysters in Chesapeake Bay reached record numbers in 1997, surprising scientists and managers alike. That was the good news — the bad news is that the outlook for these young oysters is not promising. Why? Because the oyster diseases Dermo (*Perkinsus marinus*) and MSX (*Haplosporidium nelsoni*) can be so virulent that young oysters may not survive the three years it generally takes to reach market size.

To help head off this bleak prognosis, both resource managers and commercial growers are looking for better forecasts of year-to-year variations in disease. This means new computer models — specifically, a dual-disease simulation model that will mimic the dynamic ebb and flow of MSX and Dermo in the estuary and their impact on oyster populations. In the long run, the goal is to help rescue susceptible oysters that would otherwise fall victim to Dermo or MSX.

Disease Virulence and the Environment

Just how virulent disease is likely to be each year depends on a suite of factors, ranging from environmental and climatic conditions to direct human impacts — commercial harvesting, for example, or large-scale movement of oysters and shell by resource management agencies.

Even oysters specially bred to tolerate both MSX and Dermo (see "Breeding Disease Resistance in the Hatchery") may be more vulnerable given certain environmental conditions. Whether the goal is commercial production or large-scale restoration for ecological purposes, the need to manage around disease virulence has become paramount. Simulation models that can give managers the ability to assess the effects of different environments and different restoration strategies on the virulence of oyster disease are likely to play a key role in restoring oysters to estuarine waters.

Already models developed by Eileen Hofmann and John Klinck at Old Dominion University, in collaboration with researchers Eric Powell and Susan Ford at Rutgers University and Steve Jordan at the Maryland Department of Natural Resources (DNR), are making it possible to assess the relative outcomes of disease virulence and pressure under varying conditions — outcomes that are not necessarily intuitive, says Jordan, director of Maryland DNR's Cooperative Oxford Laboratory.

What lies behind these shifts in patterns of disease? What affects an oyster's growth, its reproductive success? Its chances for survival?

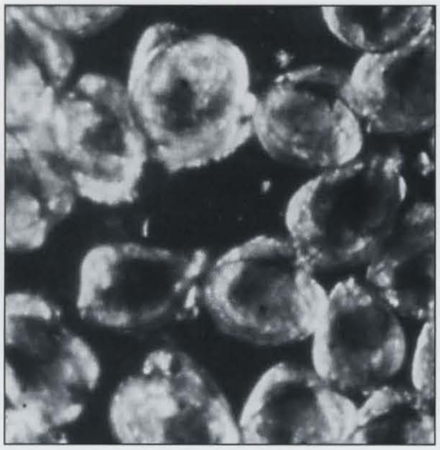
Planting Shells and Moving Oysters

In most estuaries there are regions that from year to year will receive heavier sets of new oysters than other areas. This occurs for a number of reasons, among them, salinity levels and circulation patterns that may entrain both newly-spawned oyster larvae and the algae they feed on. Resource managers and growers try to take advantage of these natural sets by placing large volumes of clean shell (called cultch) in these areas to provide more surface area for setting oysters.

In Maryland, for example, the Department of Natural Resources dredges thousands of tons of fossil oyster shell annually for planting. Once spawning season is over and the free-swimming oysters have attached themselves, metamorphosed and hardened (as spat), the seed oysters are hauled up and moved to public grounds throughout the Chesapeake for growout. Waterman can harvest oysters some three years later — except that many disease-ridden oysters do not live that long. Since the early 1990s, Dermo has decimated entire populations and before that MSX sporadically entered the Bay, especially in areas of high salinity, beginning in the late 1950s in Virginia.

In Delaware Bay, the state resource agency works with private operators to move seed oysters from public grounds that receive good natural sets of new oysters to leased grounds in high salinity waters. As Eric Powell points out, "the oysters grow more rapidly to market size there because food supply averages are greater, and high salinities are biologically more favorable to growth."

Before the spread of MSX in the mid-1950s, Delaware growers left oysters on the high salinity leased grounds for more than a year. But MSX began killing oysters before they reached market size — it forced the industry, says Powell, to move oysters from higher salinity leased grounds after only a few months. "Unfortunately," says Powell, "while this procedure limits mortality, it also limits growth."



What affects an oyster's growth, its reproductive success, its chances for survival?

Nevertheless, the approach was generally successful, and growers were careful to move oysters from low salinity grounds in May or June, and then to harvest them from September to December.

Beginning in the 1990s, however, Dermo invaded Delaware Bay and competed with MSX as a killer of adult oysters. Practices that had worked to combat MSX were no longer working for both diseases, and oysters were succumbing between the May-June transplanting and the fall harvest.

Were growers at a dead end? Or were there management alternatives? According to Powell, simulation modeling in Delaware Bay had shown that the spring bloom of algae was extremely important for the growth of oysters to reach market size. Powell notes that the degree to which a spring bloom can be used by oysters may be "crucial" in determining the success of transplanting "when survival is limited by disease." It is such factors that Hofmann and her colleagues wanted to capture in developing mathematical models.

Modeling the Development of Disease

Hofmann originally developed a model for Galveston Bay, Texas to help resource managers predict potential impacts from dredging operations and resulting changes in freshwater flow. The model projected the impact of salinity changes on oyster growth and on the prevalence and intensity of Dermo infection. It did not take into account the impact of MSX, which, though a scourge in the Mid-Atlantic, is of less concern in the Gulf of Mexico.

To adapt the Texas model to the Chesapeake and Delaware bays, accounting for the effects of both Dermo and MSX, Hofmann needed detailed field information such as the prevalence of MSX spores during the winter on different sized oysters under varying salinities. Support from the Oyster Disease Research Program helped researchers gather that data, and led to Hofmann's collaborations with scientists in Delaware and Maryland to take on the heady work of adapting the original model.

"We have one of the best data sets on the recruitment of new oysters and disease," says Steve Jordan. In place since 1990, the Maryland monitoring program includes measurements on MSX infection during different seasons and over a whole range of oyster sizes.

"We couldn't have done our model without that long time-series of data," says Hofmann. In fact, because of the information that the model requires, Maryland DNR has modified its monitoring program. Employing electronic positioning systems that make use of satellites to give highly accurate location information, Maryland has aligned its oyster monitoring with the Chesapeake Bay restoration program's water quality monitoring. "They were never coordinated because they were done for different

Handtonging for oysters has been a way of life in coastal waters on the Atlantic and Gulf coasts for more than a century.

reasons," says Jordan. "Our collaborative project spurred us to do this."

Models and Management

While oystermen in Delaware Bay were already working with Powell and Susan Ford to identify the best period for moving spat from seed grounds to leaseholds, the model shed new light on the all-important element of timing. "With the model," says Powell, "we looked at whether it is better to transplant oysters in spring or fall, given different circumstances, including the presence of disease."

What would be the differences in growth and survival, the researchers asked, if oysters were transplanted in November, rather than the following spring? What if they were planted in December or January or February?

Powell and his colleagues ran a series of simulations to analyze the role of disease and predators in determining the success of transplanting, and compared them with simulation results in which oysters were harvested directly from the seed beds.

Those simulations showed that transplanting in May resulted in the lowest harvest yield, while transplanting in November led to a high harvest the following August. "The reason," says Powell, "is that oysters apparently get the benefit of the large spring bloom of algae in the higher salinity waters." If, however, oystermen delay harvesting until late autumn, when prices are higher, the model shows that they would also be faced with considerably higher mortalities.

Waiting for an autumn harvest is especially risky, Powell says, if the principal source of mortality is Dermo disease rather than predation. Ultimately, a decision about when to harvest cannot be made by the model alone. Powell points out that oyster growers must balance "the increased price in the autumn with the increased loss through predation and, particularly, disease." Simulation models will simply help them understand the odds.

Looking Forward

An unexpected outcome of Hofmann's simulation model is that it is also helping researchers track down how MSX is transmitted. Unlike *Perkinsus* which releases spores that can then be filtered by nearby oysters, the means by which MSX infects other oysters remains a mystery. Though they have not yet discovered it, scientists have long thought there is an MSX "carrier," an intermediary host that causes infection.

> *"You cannot set management strategies based simply on what you see this week or what you've done in the past."*

In fact, for the simulation model to work, says Hofmann, "we had to put one [an MSX carrier] in so that we could reproduce what we were seeing in the field," though she cannot say what that carrier is.

The model results suggest it is an organism that has a relatively short life span, one that responds to variations in salinity.

Using Hofmann's information, researchers at the Virginia Institute of Marine Science (VIMS) have been searching for secondary carriers, employing molecular techniques developed through the Oyster Disease Research Program that enable them to sift through many microscopic organisms in Chesapeake Bay (see "Diagnosing Dermo and MSX"). "We have already found positive samples" says Burreson, meaning that genetic material from MSX is present in the sediment and water column. "Whether these are free spores or developmental stages [of MSX] we don't yet know," he says. Burreson's team is proceeding with its analysis.

Beyond the capability for projecting outcomes of different transplanting and shell planting strategies, Hofmann's simulation models have much broader implications for resource management of shellfish. "They demonstrate how important climate is in regulating diseases such as *Perkinsus* and MSX," says Hofmann. With the apparent warming trends globally, *Perkinsus marinus* has been extending its range — where its northern limit was once Chesapeake Bay, Dermo disease is now being detected as far north as New England.

"We have to manage the disease populations with a long-term climate perspective," Hofmann says, "which means that you have to be aware of such occurrences as an El Niño or other climatic effects — you cannot set management strategies based simply on what you see this week or what you've done in the past." Factors such as changes in freshwater inflow to estuaries — which control salinity and vary year to year — have a huge impact on oyster reproduction, on survival and on disease. Oyster bars that historically have shown large natural sets of new oysters may well be affected by shifting climatic conditions, which can affect water circulation patterns.

"The future is challenging us to develop flexible management approaches in all of our fisheries," says Hofmann, approaches that can take into account year-to-year changes in environmental conditions and the effects they have in ecological food webs — with such approaches as the dual-disease simulation model, she says, we are developing the kinds of tools we need to meet those challenges.

Oyster Foes East & West

Whether they are microbes like bacteria and protozoans or larger organisms such as oyster drills, parasitic worms and crabs, oyster predators are natural to any ecosystem. The daily warfare between predator and prey is one of attack and defense — over a long period, gains and losses will often average out. That has not been the case for the eastern oyster, *Crassostrea virginica*. In the mid-Atlantic, this species has been on the losing end for more than a decade to two protozoan parasites, MSX and Dermo. Its west coast cousin, the Pacific oyster, *Crassostrea gigas* — a species first brought from Japan in the early years of the century and now cultivated largely through hatchery production — can tolerate the impact of these two diseases; however, it often experiences high summer mortality. The following is a summary of some of the diseases that have been the focus of the Oyster Disease Research Program.

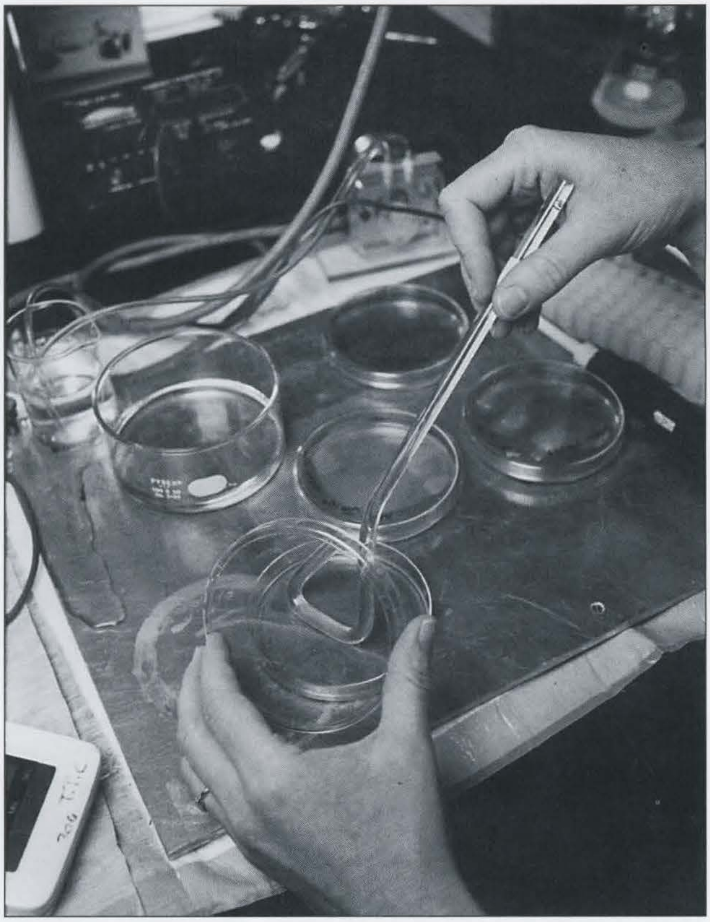
MSX. Its first appearance in mid-Atlantic waters was in Delaware Bay in 1956 where it ravaged oyster beds; the next year it arrived in the Chesapeake. Though the single-celled organism has been identified as a protozoan, *Haplosporidium nelsoni*, its life cycle and means of infecting oysters still remain as mysterious now as they did forty years ago. Unlike Dermo, MSX cannot be transmitted from oyster to oyster but new molecular tools are now making it possible to hunt for carriers of the parasite among estuarine organisms.

Dermo (*Perkinsus marinus*). First observed in Gulf Coast oysters in the 1940s and in the lower Chesapeake in the early 1950s, Dermo, like MSX, is now present on virtually every harvesting area in the Chesapeake Bay: this is largely the result of the widespread transfer of oysters from one location to another in an effort to renew unproductive bottom grounds. While studies have shown a higher virulence of Dermo in the mid-Atlantic than in the Gulf waters, genetic research has revealed differences between *Perkinsus* in mid-Atlantic locations and within Chesapeake Bay itself. Other bivalves host *Perkinsus*, but whether those are forms transmittable to oysters is currently under investigation.

JOD (Juvenile Oyster Disease). A disease that seems to affect only oysters cultured in hatcheries, it strikes only young oysters, typically less than 30 millimeters in size. In the Long Island area, JOD usually hits in late June to early July, while further north in Maine, oysters are not struck until mid-August or early September. The first indication of JOD is an abrupt stop in growth — within three to five weeks most oysters are dead. Though researchers suspect that JOD is a single celled organism, the cause and origin of JOD are not yet known. JOD is transmissible from one oyster to another and so far there is no simple cure. Commercial growers in the northeast have been working with researchers to develop successful techniques for managing around JOD's devastating effects.

Summer Mortality. Mass mortality of Pacific oysters occurs sporadically and inexplicably on west coast oyster grounds, especially when water temperature rises. Many shellfish growers are concerned that these mortalities pose a major threat to the industry. Researchers believe that the deaths may be the result of several factors, although there is little understanding of the cumulative effects of various environmental stresses on the oyster's disease tolerance. ODRP-supported research has been identifying bacteria populations such as *Nocardia*, which are thought to be a major cause of summer mortality. Molecular diagnostic methods for identifying and detecting *Nocardia* are under development and will enable growers to diagnose and screen spat that are infected by these bacteria.

Combatting Disease in the Cell



Are oysters more vulnerable now because of ecological changes that have resulted, either directly or indirectly, from human activities?

Why are Dermo and MSX so successful in killing the eastern oyster or, conversely, why has the oyster been so unsuccessful in defending itself? Parasitic organisms like *Perkinsus marinus* (which causes Dermo disease) and *Haplosporidium nelsoni* (which causes MSX) are, after all, natural constituents of aquatic ecosystems, and some scientists speculate that *Perkinsus* itself may have been in the Chesapeake Bay long before it began devastating oyster populations in this last decade.

Are eastern oysters more vulnerable now because of ecological changes that have resulted, either directly or indirectly, from human activities — for instance, overharvesting, elevated pollution levels and habitat destruction? Have there been natural changes to mid-Atlantic waters that have contributed to the oyster's vulnerability? Or have the parasites themselves improved the weaponry with which they invade, attack and ultimately defeat their host?

Answers to such questions depend on an understanding of how the oyster's immune system acts to defend itself against the biochemical weapons that MSX and Dermo marshal against it, as well as the means by which the parasites are able to evade the oyster's counterattack. For years, scientists have been frustrated by the difficulty of observing a battle which takes place within the very cells of the host, in which the weapons are protein molecules. But recently the tools of biotechnology have given us the means to follow the struggle, and to gain vital insights into the attack strategies of both oyster and parasite. Consistent support from the Oyster Disease Research Program has allowed scientists to initiate the painstaking studies of this cellular war of oyster and parasite, and already this work has led to some remarkable discoveries.

As understanding grows, these new insights hold the key to practical techniques — from therapeutic agents to genetic manipulation — that could give the eastern oyster a better-than-fair chance to combat diseases against which it is now helpless.

Cellular Machinations

Unlike vertebrates, molluscs such as the oyster do not possess a sophisticated immune system which produces targeted antibodies against invading disease organisms. The oyster's primary defense against such invaders is phagocytosis, a process in which specialized blood cells called hemocytes recognize a foreign invader, ingest that invader and then internally produce an oxidizing substance such as hydrogen peroxide to destroy it. Scientists have long known that hemocytes, which are analogous to white blood cells in the human body, though far less sophisticated, play a major role in fending off protozoan parasites like *Perkinsus*. Robert Anderson of the University of Maryland Center for Environmental Science (UMCES), found that when hemocytes are exposed to Dermo cells, the hemocytes engulf them but don't release the toxic compounds — as a result the parasites survive and proliferate. Why?

Dermo may survive for a number of reasons, Anderson says. It may prevent the the hemocytes from triggering the oxidizing compounds, it may for some reason be able to withstand them, or it may itself produce substances that render the hemocyte's defenses harmless. New molecular tools have been making it possible for Anderson and other scientists such as Gerardo Vasta at the University of Maryland's Center of Marine Biotechnology (COMB) and Muhammad Faisal at the Virginia Institute of Marine Science (VIMS) to better study the chemical armaments that both oysters and *Perkinsus marinus* deploy in their mutual battle.

Many of these studies depend on large amounts of *P. marinus* cells, which can now be produced "by the bucketful," says Anderson, thanks to breakthroughs in culturing techniques supported by the Oyster Disease Research Program. Growing *P. marinus* in the laboratory under controlled conditions makes it possible to study its life cycle and how different environmental conditions (e.g., salinity, temperature, heavy metals, chemicals) affect its growth and behavior. Most importantly, they can begin to tease apart the means by which *P. marinus* actually causes Dermo disease — how it invades its host, how it suppresses the response of the oyster's immune system, how it multiplies and, eventually, kills the oyster.

Proteases and Blockers

Researchers soon discovered that, unlike the eastern oyster, the Pacific oyster (*Crassostrea gigas*) is better able to withstand and fend off the blistering attacks of Dermo and MSX. That's the reason why, says Muhammad Faisal, research in his lab has focused on explaining these different responses. If we can identify the mechanisms that account for the critical difference between these two species, he explains, "it will be the first step towards developing measures to help the eastern oyster better resist these diseases as well."

In laboratory studies, Faisal found that Pacific oysters infected with Dermo showed an increase in the production of hemocytes and blood plasma proteins. But just as in the eastern oyster, Dermo cells were capable of surviving phagocytosis by *C. gigas* hemocytes. Researchers in his lab found that *P. marinus* readily grows and divides in hemocytes of infected Pacific oysters, apparently suppressing its hemocyte functions as effectively as it does those of the eastern oyster. This finding, Faisal says, strongly suggests that "factors other than hemocytes may be important in the [Pacific] oyster's resistance." These factors are probably proteins in the oyster's blood (more properly called hemolymph) which in some manner inhibit the ability of Dermo to invade host cells or to multiply.

"The new methodologies give you a chance to count three million cells, not 300."

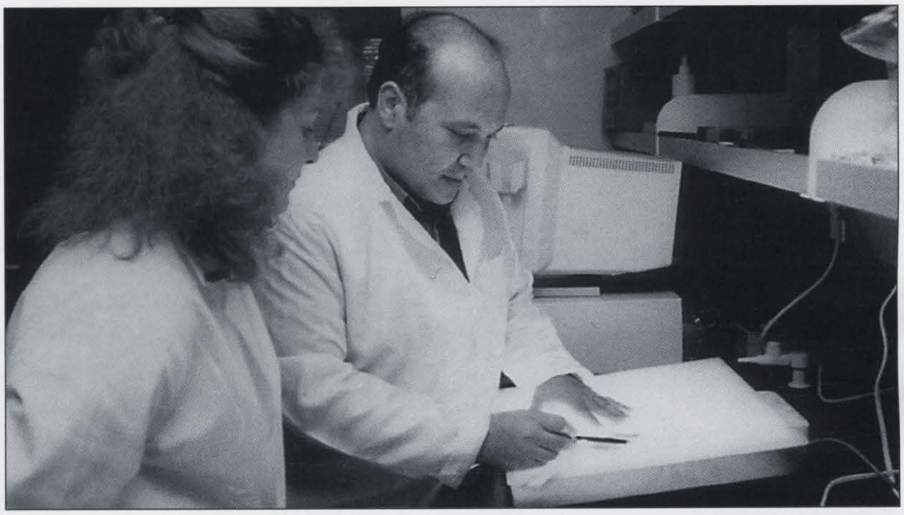
Gerardo Vasta (left) and H. Ahmed at the University of Maryland Biotechnology Institute's Center of Marine Biology are purifying virulence factors from the oyster parasite Perkinsus marinus.

As part of their attack, notes Faisal, parasites such as Dermo cells release enzymes called proteases that are capable of digesting proteins, thus weakening the host's tissues as well as undoing its molecular defense mechanisms. For example, Faisal and his co-workers have found that *P. marinus* proteases suppress activity of oyster hemocytes. In a counterattack, the oyster's hemolymph produces protease inhibitors, enzymes which in turn break down the proteases fired off by *P. marinus*.

The evidence is mounting, says Faisal, "that protease inhibitors play a vital role in the Pacific oyster's defensive arsenal." This finding is especially striking when contrasted with the eastern oyster: the sharp increases in plasma protein concentrations following exposure to Dermo cells, are seen only in Pacific oysters. For instance, in one set of experiments, he found the inhibitory activity of Pacific oysters against *P. marinus* proteases to be three times greater than the eastern oysters.

If further research confirms these initial findings, Faisal is hoping that it may be possible to develop "protease blockers" that would act something like antibiotics in fighting the parasite, at least under controlled conditions. These materials could be delivered to the oyster in its food, or in tiny microcapsules called liposomes. Various practical approaches are now being investigated in Faisal's laboratory.

Also being tested is whether eastern oysters which show the most protease inhibitory activity in the lab (albeit lower than that of *C. gigas*) will prove less vulnerable to Dermo disease in the field. This could provide a means of predicting disease resistance, and contribute to the eventual development of resistant genetic strains.



Muhammad Faisal has been pursuing the role that proteases, enzymes released by Perkinsus marinus, *play in suppressing the oyster's defense mechanisms; his aim is to develop a therapeutant, a protease "blocker" that could help the oyster better defend itself.*

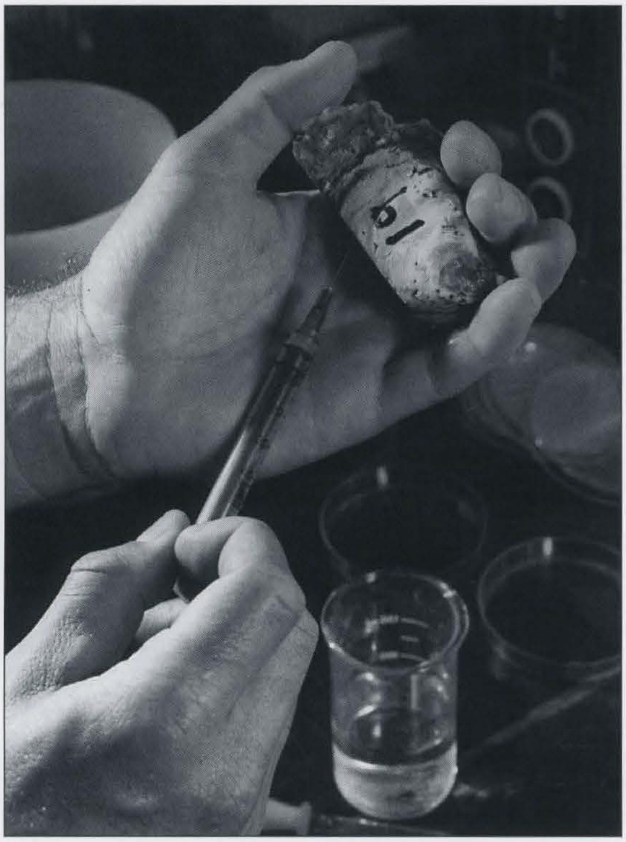
An Arsenal of Weapons

The arsenal of a complex parasite like *Perkinsus marinus* is not limited to a single weapon. Scientists are finding that it has many ways of evading or defusing the defense mechanisms of its oyster host. They have also discovered that there are several strains (or types) of *P. marinus*, which differ in virulence and possibly in their modus operandi. The general strategy, explains Dr. Gerardo Vasta, of the University of Maryland's Center of Marine Biotechnology, appears to be that "the best defense is a good offense." The parasite produces a wide variety of proteins with different actions, all of which contribute to its successful survival within the oyster by disrupting the normal functioning of the oyster's phagocytes. The diversity of these compounds, says Vasta, suggest that they are directed towards challenging the hemocytes "up front" and putting them at an immediate disadvantage, instead of simply responding to the hemocyte's initial attack.

Vasta is on the trail of these "virulence factors." He and his coworkers are investigating what determines the ability of a parasite to combat the oyster's defenses, and how quickly it can respond to challenges from the oyster's immune system. Even more basically, exactly how does the parasite invade? Why are some *P. marinus* strains (such as those from the Gulf coast) apparently less damaging than those from the Chesapeake Bay — is it the parasite which differs, are Gulf oysters more resistant, or does the very different Gulf Coast environment reduce the parasite's virulence?

Why are some Perkinsus *strains (such as those from the Gulf coast) apparently less damaging than those from the Chesapeake Bay?*

Surprisingly, it appears as if the oyster hemocytes themselves may be the key to the initial infection. Current thinking is that infectious *P. marinus* cells in the surrounding water are taken up by the oyster as it feeds. In the oyster's gut, the parasite crosses the epithelium of the stomach or the intestine into the body of the oyster. It is not known whether this invasion is entirely passive (through phagocytosis) or whether the parasite can also actively penetrate the oyster's cells. The first scenario is highly likely. Unlike the case in many other parasitic diseases (where the parasites "hide" within the host), the invading *P. marinus* is apparently readily recognized by the oyster's hemocytes. In fact, it almost appears as if the parasite "wants" to be recognized and engulfed by the hemocytes, says Gerardo Vasta.



In studies on the cellular functioning of the immune system, a researcher extracts circulatory fluid from the oyster's adductor mussel.

The parasite possesses cell surface features (such as sugar molecules) which attract the hemocyte, and enhance phagocytosis. Once inside the hemocyte, *P. marinus* evades or (more probably) inhibits the cellular defense mechanisms, multiplies, and spreads throughout the oyster's tissues. The invader excretes various enzymes which break down the oyster's proteins, providing food for the multiplying parasite. As the disease progresses, hemocytes, oyster tissue and parasite cells are sloughed into the oyster's gut, and released into the water column, thereby spreading the infection.

In research supported by the Oyster Disease Research Program, both Robert Anderson and Fu-Lin Chu, of the Virginia Institute of Marine Science, observed that while oyster hemocytes usually produced bursts of superoxides when engulfing foreign organisms — typically killing the invaders — they did not do so when ingesting *P. marinus* cells. Vasta's research has shown that the parasite has the ability to produce several enzymes which inhibit the hemocyte's "oxidative burst." His laboratory is now investigating the genetic basis for the production and regulation of these and other factors which contribute to the virulence of Dermo. Which of the many "weapons" employed by *P. marinus* are the most important in controlling the disease process? Does this vary among the different strains of the parasite? What about the effects of environmental variables? In the future, this information may allow development of biotechnological applications for control of *P. marinus*, as well as clarify some still-puzzling aspects of its life cycle.

Exposure to various contaminants (all of which are found in waters or sediments of the Chesapeake) inhibit the oyster's immune responses.

One interesting finding is that many other common Chesapeake Bay bivalves, such as the hard clam *Mercenaria* and the Baltic clam *Macoma*, are also hosts to genetically distinct forms of *Perkinsus*. Whether these animals can also serve as a "reservoir" for oyster Dermo disease is, however, unknown. With support from the Oyster Disease Research Program, this lead is now being investigated in the laboratory and field by Vasta and Greg Ruiz of the Smithsonian's Environmental Research Center.

Environment and Disease

One provocative hypothesis which has developed from ODRP and related research is that environmental degradation of the Chesapeake Bay has exacerbated the spread of Dermo disease. For example, Fu-Lin Chu and her co-workers exposed oysters to water containing contaminants extracted from sediments of the Elizabeth River in Virginia. She observed that these oysters contracted Dermo disease earlier, and had more extensive infections, than oysters exposed to clean water.

Similarly, Anderson observed that exposure to various contaminants (all of which are found in waters or sediments of the Chesapeake) inhibit the oyster's immune responses, in particular the hemocyte's ability to generate a parasite-killing "oxidative burst."

Nutrient overenrichment may also be affecting the oyster's immune system, in a subtle manner. Some of the parasite's most effective weapons are a suite of enzymes which essentially "shut down" the production of the killing superoxide burst by the oyster hemocytes. Gerardo Vasta notes that one essential element in the production of these enzymes is iron — each enzyme molecule contains a single atom of iron.

In fact, since iron is also essential to the production of many important oyster proteins, the parasite must compete with its host for this important element — a lack of iron would slow proliferation of *P. marinus*, as well as affect its ability to evade the oyster's immune response.

In the saline waters of Chesapeake Bay, under well-oxygenated conditions, free iron concentrations are low, and most iron is bound into very stable iron-phosphate complexes in the Bay sediments. However, in warm months much of the deeper waters of the Chesapeake are extremely low in oxygen (due to nutrient enrichment and decay of resulting algal blooms). Under these conditions, the iron-phosphate compounds break down, releasing free iron into the water column. This ready availability of free iron may help stimulate the proliferation of Dermo disease in Chesapeake Bay. Certainly there is a seasonal component to Dermo virulence which might reflect the greater iron availability (and uptake of the element by oysters) found in summer months.

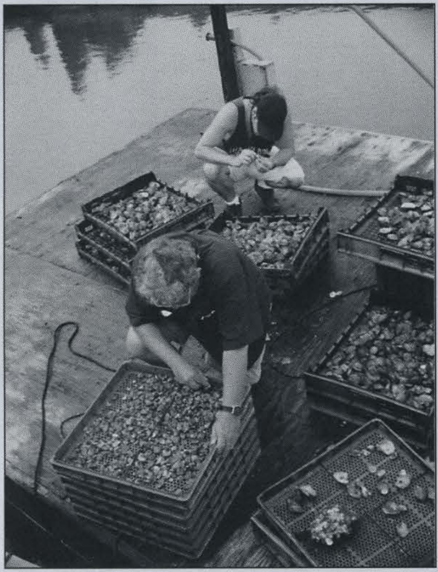
Results from earlier field studies which show a higher rate of Dermo infection in oysters in deeper bars are also suggestive of environmental influence on Dermo disease. A number of researchers have found that oysters infected with *Perkinsus* have significantly more acid blood, a condition called acidosis. There is evidence that acidosis enhances the action of some of the parasite's potent weapons (e.g., protease), and speeds the rate of infection. Ken Paynter, of the University of Maryland College Park, points out that environmental factors can also cause acidosis, which would exacerbate disease. Among these factors are high salinity, high temperature and low levels of dissolved oxygen — all characteristic of the Chesapeake Bay in summer.

These hypotheses are now being tested in a controlled manner, with support from the Oyster Disease Research Program, by Vasta, Anderson and Chu in the laboratory, and by Paynter and others in the field.

For example, in the laboratory Vasta and his co-workers are studying the mechanism by which *Perkinsus* acquires iron from its host (or the environment), and how iron controls the production of enzymes needed for parasite survival and growth. One goal is to develop a means for blocking infection or parasite proliferation (possibly through use of iron chelators), which could be employed in aquaculture. Initial tests are promising: in culture, iron chelators were shown to inhibit the multiplication of parasite cells, whereas the addition of soluble iron enhanced their growth, supporting the initial premise.

The most intriguing aspect of these hypotheses is that current efforts to reduce nutrient and toxicant pollution of the Chesapeake Bay and other estuaries, and to restore a well-oxygenated water column throughout the year, may have the unexpected side benefit of reducing the incidence of Dermo disease as well.

Getting around Juvenile Oyster Disease



"JOD was like a wild fire spreading through my racks — I ended up with 93 percent mortality out of my first batch of oysters." This is Rob Parrino of Paradise Point Oyster Farms describing the impact that Juvenile Oyster Disease had on his oysters in 1988. Parrino was not alone: until these last several years oyster farmers from New York to Maine were losing nearly entire crops of oysters to disease.

We have turned that situation around now, say Jay Lewis and Austin Farley, researchers at the National Marine Fisheries Service Cooperative Oxford Laboratory in Maryland, and growers are again experiencing good survival of hatchery-reared oysters. Though the JOD organism has yet to

While Juvenile Oyster Disease continues to resist identification, oyster growers have developed techniques to manage around its impact

be identified, scientists have worked with growers to apply techniques from observational and experimental research for circumventing disease. Juvenile Oyster Disease has been confined to cultured oysters in the northeast with very little evidence of infestations in wild oysters.

The first known outbreak occurred in 1984, in oysters that were spawned in Maine. By 1990, the disease was seen in first-year oysters throughout the Northeast, from New York to Maine. Growers found that affected oysters generally ranged in size from five to 30 millimeters and had a noticeable ring of conchiolin on the inner shell surface(s). Survivors often had "shell checks," marks that indicated where JOD first occurred.

The disease usually strikes after water temperatures rise and remain above 20°C and in salinities above 18 parts per thousand. Oysters that grew larger than 30 millimeters in length without succumbing to JOD generally grew to maturity.

These observations were the first clue in one potential way to circumvent the impact of JOD. Researchers reasoned that by spawning oysters in heated hatchery water early in January through March, they could jump start growth and get juvenile oysters to a length larger than 30 millimeters before the onset of high summer temperatures. The approach worked. As Robert Rheault of Moonstone Oysters says, "we always try to get our seed oysters as early in the spring [as possible]. As soon as the water temperature hits 10°C we like to have our seed in the pond."

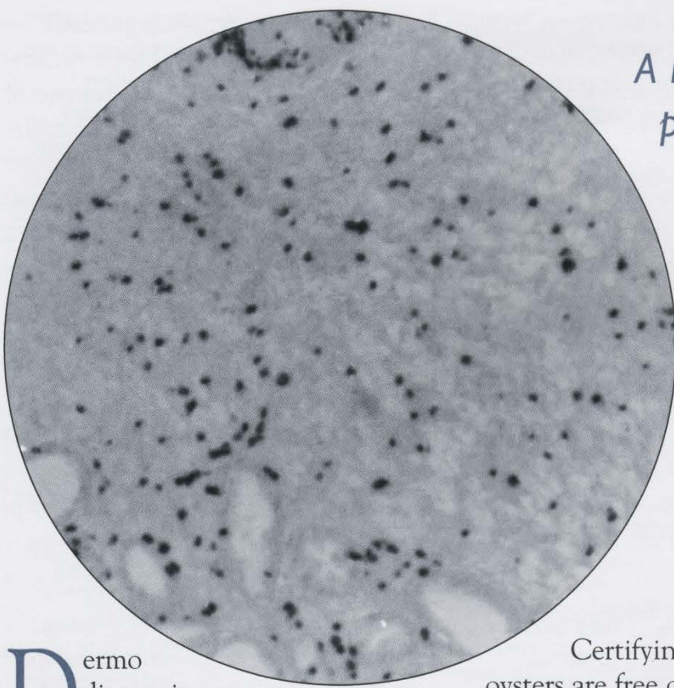
Opposite page and below: Frank M. Flowers & Sons on Long Island Sound has been one of the beneficiaries of research on Juvenile Oyster Disease (JOD). Above left: Shell checks indicate when surviving oysters first contracted JOD.

Meanwhile researchers came up with another successful strategy for beating JOD, one that Dave Relyea of Frank M. Flowers and Sons, Inc. says is probably the most effective, namely to produce resistant oysters from broodstock that has survived the disease and that has shown strong evidence of shell checks.

Because of the disease-resistant broodstock, the Flowers hatchery has produced record numbers of juvenile oysters without significant mortality; the company has also helped other local growers by providing them with resistant seed. Relyea says that "some of the work the scientists have done seems to prove that our native oysters that have been exposed to JOD have much more resistance than animals that have not been subjected to it." This is one reason oysters produced in local waters may survive better than those developed and brought in from other locations. As Aaron Rosenfield of the Cooperative Oxford Laboratory says, "the most fundamental way of avoiding JOD is to not bring in oyster seed from JOD-infected regions or locations. This helps prevent contamination."

Also, research by Gregg Rivara, Cornell Cooperative Extension Laboratory in Southhold, New York, has shown that increased water flows in upweller culture systems reduce JOD mortality.

While managing around JOD through early spawning and employing disease-resistant stocks has been extremely successful, scientists are still trying to identify the organism that causes the disease, and to cultivate it in the lab. If successful, their work will lead to the development of reliable molecular tools to screen for diseased animals; this capability could give growers a means for selecting broodstock to maintain resistance in subsequent generations.



A new generation of molecular techniques promises rapid detection for the first time

New Tools for Diagnosing Disease

Dermo disease is inescapable on most harvestable grounds in mid-Atlantic estuaries: though oysters may be infected in the first several months, disease can progress slowly and many oysters will survive for at least a couple of years before succumbing, usually in the third year. But oysters may reach harvestable size sooner, depending on the availability of food and other environmental conditions.

The ability to rapidly detect the progress and intensity of *Perkinsus marinus* (Dermo disease) or *Haplosporidium nelsoni* (MSX) would give resource managers and commercial growers a chance to take proactive measures. For instance, in an area where disease is especially intense and oysters are not likely to reach harvestable size, it might make economic sense to move them to less salty waters and mitigate the progress of the disease (see "Modeling Around Disease").

Of equal importance is the ability to detect infections in adult oysters or hatchery-reared seed before they are transferred to regions free of disease — such transfers in the past may have contributed to the spread of Dermo and MSX.

Certifying that hatchery oysters are free of disease must be a cornerstone of some restoration programs. How certain can we be that young oysters first spawned in hatcheries, placed overboard in Bay waters to harden, and then planted for growing to maturity (growout) are free of Dermo disease?

Multiple-stage testing depends on the availability of detection tools that we haven't had in the past — tools that can serve as a ready alert to managers and growers that infection is present, or that it is spreading.

Molecular Probes

Since the 50s, scientists have depended on what is still the standard method for determining whether oysters are carrying *Perkinsus marinus* cells. Called Ray's fluid thioglycolate medium (RFTM), this procedure is labor intensive, time-consuming and relatively insensitive; moreover scientists have discovered in the last couple of years that it is not necessarily specific to *P. marinus*.

"There are several other *Perkinsus* species," says Gerardo Vasta, "and the RFTM analysis cannot distinguish among them. Moreover," he says, "there have to be numbers of cells in the oyster tissue to be able to detect the presence of *Perkinsus*." Reliance on such traditional analysis has shifted, largely because of support by the Oyster Disease Research Program for developing sophisticated molecular diagnostic technologies.

Vasta and other researchers — among them, Muhammad Faisal and Eugene Burreson of the Virginia Institute of Marine Science and Christopher Dungan at the Cooperative Oxford Laboratory — have been designing a suite of highly sensitive probes that can detect just one or two cells of a parasite in a newly set oyster, no bigger than a pencil point. On the west coast, Arthur Gee and Ralph Elston at Pacific Lutheran University in Tacoma, Washington, have developed a probe for detecting Pacific oyster nocardiosis (PON) — this widespread disease consists of different *Nocardia* bacterial species that afflict adult Pacific oysters. PON can be especially lethal at high temperatures.

A molecular probe is a very sensitive way to identify an organism such as a *Perkinsus* cell. One scientist has characterized the probe as a flag that you plant on a particular cell or organism to distinguish it from a background of similar cells or organisms.

Probes tag the DNA or RNA of an organism and are specific to a species. Vasta and his co-workers, for example, have identified and isolated a segment of DNA that is unique to *P. marinus*, cloned the segment, and developed probes (short chains of nucleic acids) which will bind with this specific target. These probes are labeled with a more readily detectable molecule such as a radioactive compound.

They have also designed primers based on this DNA sequence for detection of *P. marinus* through a technique called PCR (polymerase chain reaction) amplification. To analyze for the presence of *P. marinus* by PCR, DNA is first extracted from the oyster's mantle, rectal tissues or oyster hemocytes, the disease-fighting blood cells in the circulating fluid (hemolymph) — this initial extract contains a mix of DNA from both oyster and parasite. The PCR amplification codes the *P. marinus* DNA segment millions of times, thus raising the concentration of the target DNA segment in the sample to detectable levels on agarose gels stained with ethidium bromide. This method is specific for *P. marinus* and can detect a single parasite cell in 30 milligrams of oyster tissue.

Vasta has compared this PCR probe to the standard RFTM tests and discovered that about 15 percent of oysters deemed "disease-free" by the older method actually harbored the parasite, a serious concern for managers and growers.

The current test is only semi-quantitative, providing a general estimate of the intensity of the infection. The next step is truly quantitative PCR techniques, which Vasta, as well as Eugene Burreson, and others are pursuing with support from the Oyster Disease Research Program.

Antibody Probes

Another promising diagnostic tool is a rapid assay, or quantitative analysis, based on antibodies produced by a mouse or rabbit that has been inoculated with *P. marinus* cells. Antibodies are proteins — they are generated in the blood of a host organism, when invaded by a foreign organism, in order to destroy invaders. Chris Dungan has been developing this diagnostic approach in which antibodies are labeled with a fluorescent dye or linked to a specific enzyme.

In the presence of any *Perkinsus* cell in an oyster tissue sample, the labeled antibodies bind to it. This reaction can produce a color change in the sample or a fluorescent complex detectable by microscopic examination. Dungan has successfully employed immunoassays which disclose the presence of *Perkinsus* cells in both oyster tissue and in Chesapeake Bay waters.

However, because his initial polyclonal detection antibodies bind to a range of *Perkinsus* species, he found the test not specific enough for *P. marinus*. New culture techniques for the oyster parasite will allow the production of pure *P. marinus* cell extracts, and the promise of rapid, precise and easily-conducted immunoassays for Dermo disease.

Even the mysterious parasite *Haplosporidium nelsoni*, known as MSX, has begun to yield its secrets to scientists, who have been frustrated for decades by their inability to grow the organism in laboratory cultures, or even to determine how it is transmitted from oyster to oyster. Many researchers feel that this protozoan may have an intermediate host, or "carrier," though this too is unknown (see "Modeling Around Oyster Disease"). Researchers in Burreson's lab have developed a DNA probe and a PCR-based assay specific for MSX

Even the mysterious parasite known as MSX has begun to yield its secrets to scientists.

*On the west coast, the oyster industry is largely based on hatchery-reared Pacific oysters (*Crassostrea gigas*). Though high summer mortalities are thought to be the result of multiple stresses, scientists are developing molecular diagnostic probes for identifying* Nocardia *bacterial populations which are suspected as a major cause of mortalities.*

that are far more sensitive to the presence of the parasite in oyster tissue or hemolymph than the standard microscopic examination techniques.

In order to identify the hypothetical carrier of MSX, they are using the PCR assay to detect the organism in environmental samples taken from areas where MSX is prevalent. A positive PCR result indicates that *H. nelsoni* DNA, and thus the parasite, is present somewhere in the sample. Positive samples are then placed on microscope slides and mixed with the MSX DNA probe in a procedure called in situ hybridization. Only the *H. nelsoni* cells will stain purple-black, showing the location of the organism within the sample.

The ultimate result of this research will be a toolkit of rapid diagnostic tests that scientists, managers and growers can use in the field. These tests should lead to widespread improvements in managing around disease, reliable certification of disease-free spat and improved screening of disease-resistant adult oysters for brood stock.

Glossary

***Crassostrea gigas*.** Pacific oyster, originally imported by West Coast oyster growers from Japan; it is now cultured in hatcheries.

***Crassostrea virginica*.** Eastern oyster (also referred to as American oyster); inhabits waters from Maine to the Gulf of Mexico.

Cultch. Oyster shell or other material on which free-swimming larvae set and grow.

Dermo, *Perkinsus marinus*. Protozoan parasite that thrives in high salinities and causes significant oyster dieoffs. Unlike MSX, Dermo is transmitted from one oyster to another.

Eyed larva. Last stage of a freely-swimming oyster that is ready to set, or attach to cultch and undergo metamorphosis to juvenile oyster, or spat.

Growout. The period between setting of oysters and maturity to market size, generally one to three years.

Hemocytes. Disease-fighting cells in the hemolymph of marine invertebrates; analogous to white blood cells in vertebrates.

Hemolymph. Circulatory fluid in invertebrates, comparable to the blood system in mammals.

Immunity. An organism's resistance, natural or acquired, to onset of disease resulting from infection.

Invertebrate. Animals lacking an internal skeleton or spinal column, such as the oyster and crab.

JOD (Juvenile Oyster Disease). An unidentified disease that has primarily affected young oysters cultivated in hatcheries in the northeast.

Larva, larvae. Free-living, pre-adult stage of an organism; when first spawned, an oyster larva will swim and feed before it is developmentally ready to seek a permanent home.

MSX, *Haplosporidium nelsoni*. Parasite that flourishes in high salinity waters and is responsible for heavy oyster kills in mid-Atlantic waters.

Oyster seed. Young oysters that have successfully survived the larval stage and are planted by growers either on private or public grounds.

Parasite. An organism such as MSX or Dermo living on or in another host organism to its advantage and the disadvantage of the host.

Pathogen. Any disease-causing organism.

Protozoan. A single-cell organism, usually free living but sometimes parasitic like MSX.

Selective breeding. Process by which human beings rear animals and plants with particular traits that an organism will pass on to offspring. For example, in oysters, breeding for resistance to disease or for faster growth.

Spat. Young oyster larva that has metamorphosed to become a juvenile oyster.

Spat set. The attachment of oyster larvae to cultch and the successful completion of metamorphosis.

Summer mortality. Mass mortality of Pacific oysters occurring sporadically and inexplicably on west coast oyster grounds — especially when water temperature rises; thought to be caused by bacteria populations such as *Nocardia*.

Sea Grant, Coastal Ecosystems and Oyster Restoration

The National Sea Grant College Program represents a unique partnership among the nation's academic research communities, coastal industries, state government and the National Oceanic and Atmospheric Administration. Thirty Sea Grant programs — one in every coastal and Great Lakes state — form the core of a nationwide network of over 200 participating institutions that draw on the expertise of more than 3,000 scientists, researchers, educators, students and outreach specialists.

While individual Sea Grant programs support research and education efforts on a host of issues, the National Sea Grant College Program's Oyster Disease Research Program and the new Gulf Oyster Industry Research Program are specially targeted at reversing the decline of oysters due to disease and other environmental pathogens.

Sea Grant's objective is to marshal the nation's highest quality researchers for developing a suite of practical approaches to mitigate the impacts of disease. In working with industry and government, the aim is to restore the commercial and ecological viability of oysters in U.S. waters, specifically *Crassostrea virginica* on the east coast and Gulf coast and *Crassostrea gigas* on the west coast.

Restoring Oysters to U.S. Coastal Waters provides snapshots of recent scientific achievements of the Oyster Disease Research Program.

For information about Sea Grant and details on the Oyster Disease Research Program and the Gulf Oyster Industry Research Program, contact the National Sea Grant College Program, NOAA, 1335 East-West Highway, Silver Spring, Maryland 20910 or visit the ODRP website at http://www.mdsg.umd.edu/NSGO/research/oysterdisease/

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