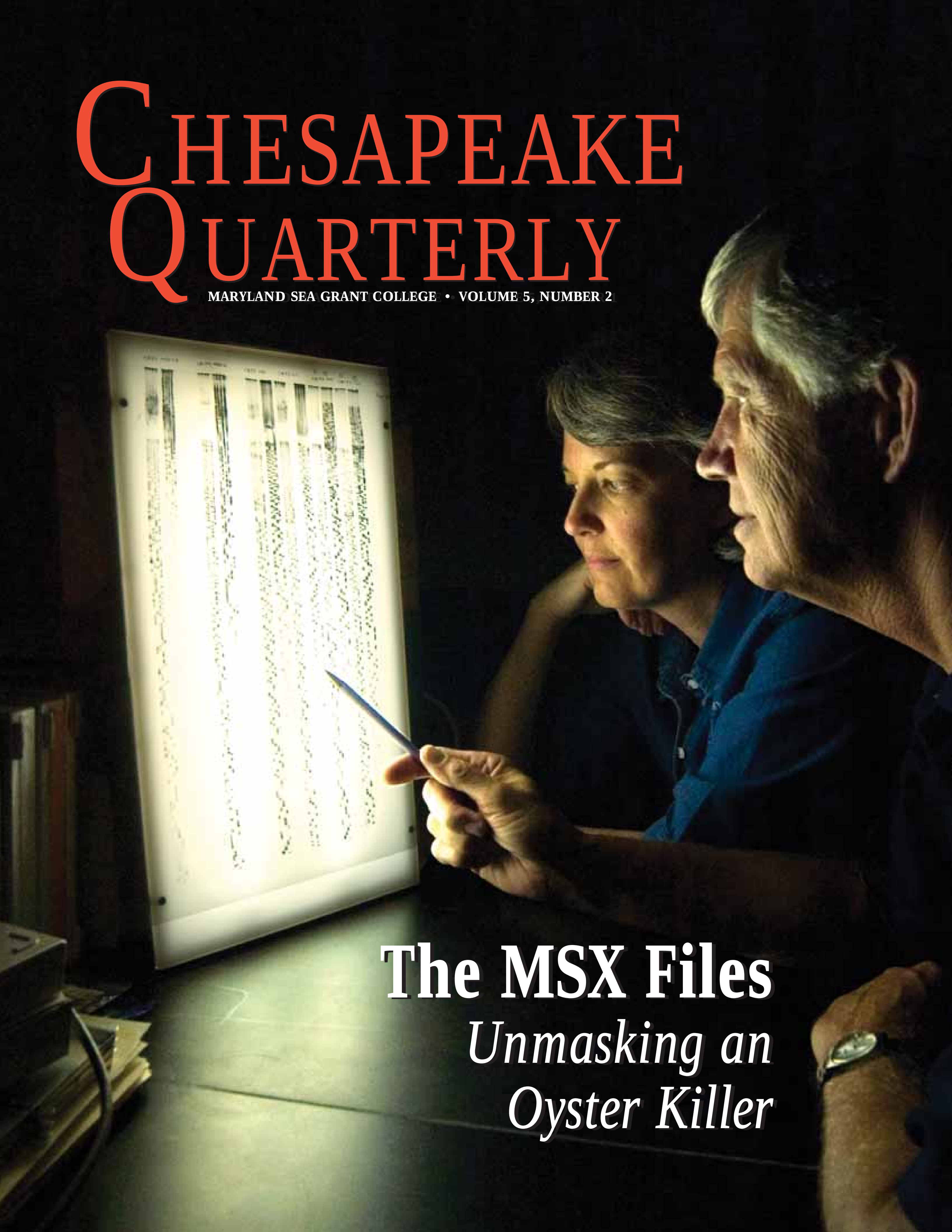
CHESAPEAKE QUARTERLY

MARYLAND SEA GRANT COLLEGE • VOLUME 5, NUMBER 2



The MSX Files

Unmasking an Oyster Killer

contents

Volume 5, Number 2

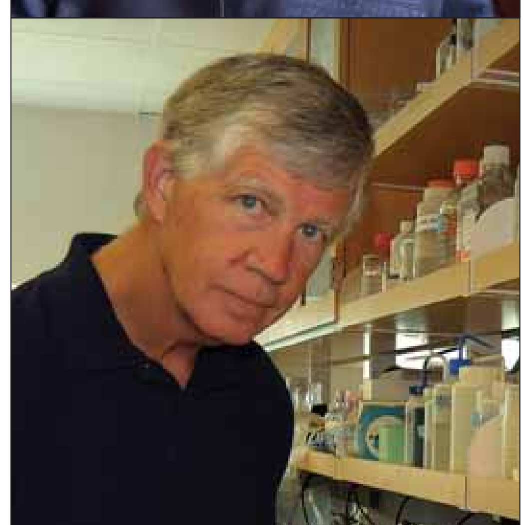
CHESAPEAKE QUARTERLY September 2006

Chesapeake Quarterly is published four times a year by the Maryland Sea Grant College for and about the marine research, education, and outreach community around the state.

This magazine is produced and funded by the Maryland Sea Grant College Program, which receives support from the National Oceanic and Atmospheric Administration and the state of Maryland. Managing Editor and Art Director, Sandy Rodgers; Contributing Editors, Jack Greer and Michael Fincham; Science Writer, Erica Goldman. Send items for the magazine to:

Chesapeake Quarterly
Maryland Sea Grant College
4321 Hartwick Road, Suite 300
University System of Maryland
College Park, Maryland 20740
301.405.7500, fax 301.314.5780
e-mail: mdsg@mdsg.umd.edu

Cover photo: *Gene Burreson and Nancy Stokes read an X-ray film showing the sequence of a key section of the DNA of MSX, the parasite that devastated oyster populations in both Delaware and Chesapeake bays. Though X-ray films have now given way to computer screens, the earlier technique provided a key to finally figuring out the probable origins of the MSX parasite.* PHOTOGRAPH BY MICHAEL W. FINCHAM.



Lessons of History: Hubris and *Humility*

THE STORY OF MSX

The bottom of Chesapeake Bay is a battleground littered with the empty shells of dead oysters. Gone, for the most part, are the living oyster reefs that once created habitat for small fish and crabs and helped filter Bay waters now cloudy with excess plankton.

Tongers and dredgers and police once fought over those reefs in the infamous Oyster Wars of the 19th and early 20th centuries. But today the combatants include watermen and oyster growers, scientists and state agencies and environmental organizations. And the core battles rage not over how to harvest the reefs, but how best to rebuild them. Should we replant the Bay with native oysters — or with non-natives imported from China?

The answer may depend in part on another question: what killed off the oyster reefs of the Chesapeake in the first place? The suspects include disease, overfishing, sedimentation, habitat destruction, and water quality changes. Of all the well-known oyster killers, the most dramatic in recent decades was MSX, an epidemic that began in the late 1950s. An unknown parasite, MSX was a terrible swift sword, slashing harvests by 90 percent on the oyster grounds of Delaware Bay and by 75 percent in the Virginia Chesapeake in its first three years. The result was disaster: an oyster-killing disease that altered the ecology of two estuaries and nearly wiped out oyster industries in three states.

Where did this killer parasite come from? When Gene Burreson and Nancy Stokes of the Virginia Institute of Marine Science found a way to solve this long-standing mystery, their findings represented a landmark in the annals of Chesapeake Bay science, but a landmark that was a long time coming. Although scientists first began investigating the origins of MSX in 1958, the 20th century would come to an end before Burreson and Stokes could eventually publish their findings. In the year 2000, they finally identified this deadly parasite as a foreign invader.

Why did it take more than 40 years to answer such a key question? Faced with a parasite of unknown origins, marine biologists in the 1950s and 1960s found themselves working at the limits of their current tool set, waiting for a breakthrough. In the history of science, when new research tools come online, new discoveries soon follow — after new telescopes start up, new galaxies appear in the depths of space; after deep submersibles plunge to the sea floor, hydrothermal vents appear in the depths of the world's oceans.

For Burreson and Stokes and biologists everywhere, the invention of a new tool called PCR — short for polymerase chain reaction — created a revolution in their science in the early 1990s. Using the new DNA-based tools of molecular biology, Burreson and Stokes were at last able to trace the origins of the MSX epidemic to a parasite that invaded from elsewhere, probably as a hitchhiker on a non-native oyster.

If there are lessons to be learned from that long history, one of them might be this: breakthroughs can breed hubris, what physicist Freeman Dyson called "a technical arrogance that overcomes people when they see what they can do with their minds." Innovations like PCR and novel technologies for oyster aquaculture open up discoveries and expand the options for commercial and ecological restoration, reviving a dream in the oyster industry of planting the Chesapeake with faster-growing non-natives.

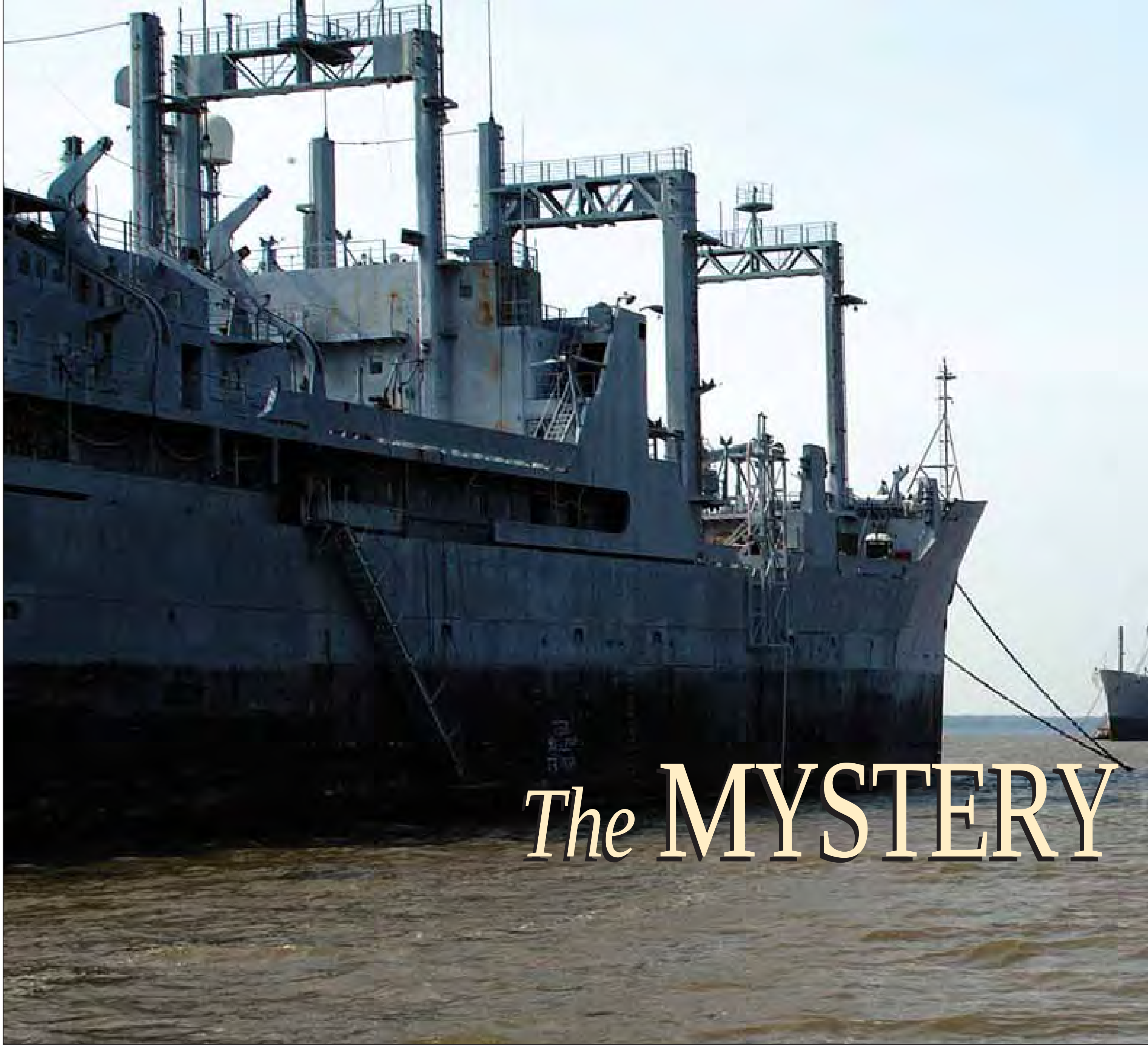
That dream focuses now on a Chinese oyster called *Crassostrea ariakensis*, but the vision has been around for 60 years, spurred by the commercial success of Japanese oyster aquaculture in the Pacific Northwest. If it could be done elsewhere, the thinking goes, it could be tried here also.

Humility, however, might be another lesson from history, humility in the face of the unknown and the untried. The devastation of MSX dates back 50 years now, and 40 of these years passed before scientists, thanks to a breakthrough, finally solved the puzzle of its unknown origins.

Ironically enough, the scientists responsible for breakthroughs often see — more clearly than most — the limits of their knowledge. Some researchers warn that we'll never have enough knowledge ahead of time to predict accurately the outcome of widespread introductions. If we want to know the answer, we have to run the experiment.

The restoration of Chesapeake oysters will be exactly that: a huge, system-wide experiment in the country's largest estuary. And the outcome is unknowable.

— *Michael W. Fincham*



The MYSTERY

COMING FAST ACROSS THE WATER HE CAN see the old fleet floating at permanent anchor. Gene Burreson is riding shotgun in a small runabout that is slicing straight across the James River, headed for the world's largest mothball fleet, an armada of decaying Navy ships that are clearly headed nowhere.

Tall, big-boned, and hatless, Burreson squints against the bright sun bouncing off the water. At 6'4" he towers over his boat driver and three assistants crouching in the forward cockpit for the bouncy ride across the river. From here the ships are a distant scraggle of grey hulks humped up against a brown-green river and distant, dark green trees.

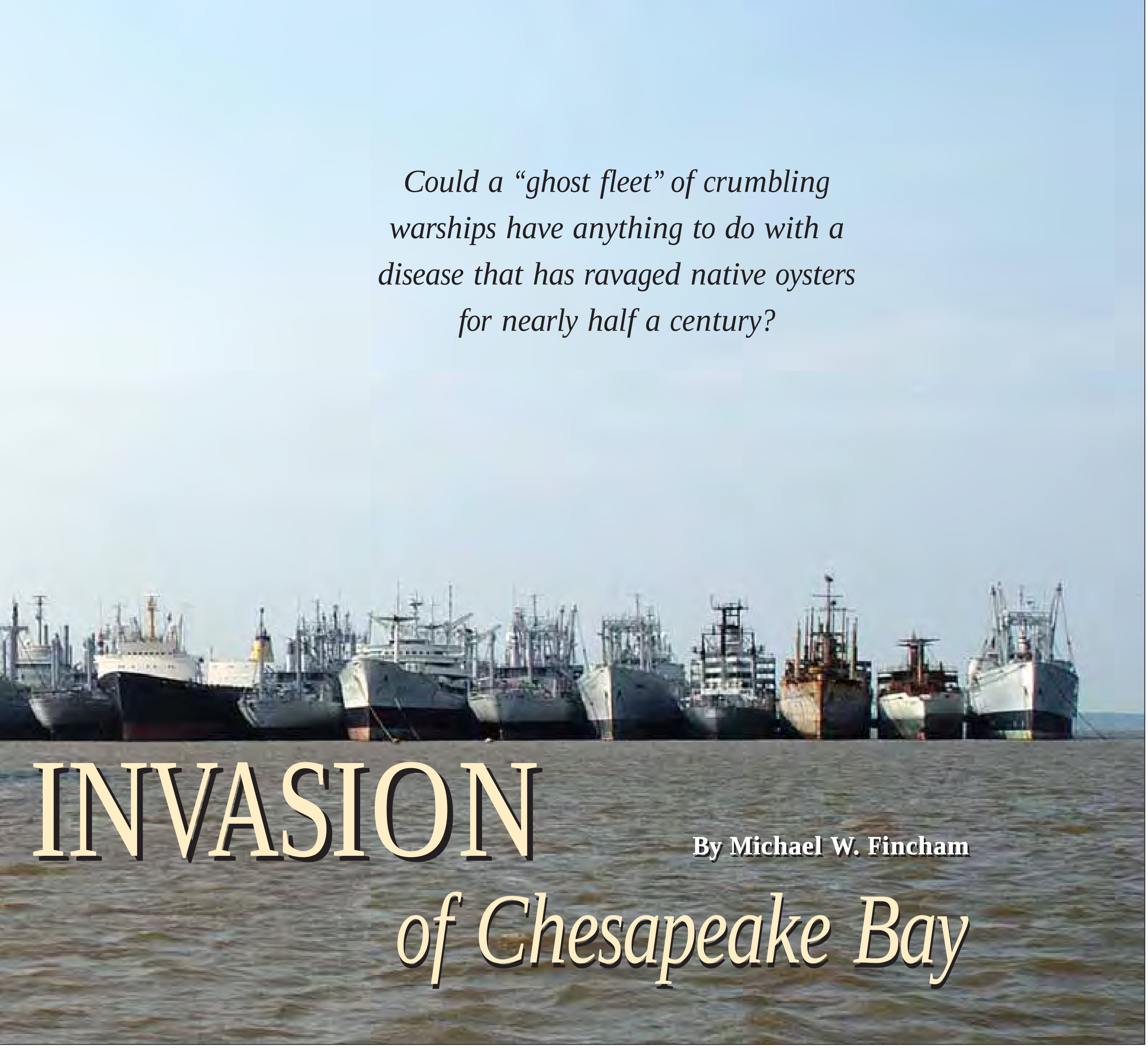
Burreson is an oyster biologist with the Virginia Institute of Marine Science (VIMS) and his target today is not the fleet but what lies beneath. Along the bottom of the river, sprawled under and around and south of the Navy ships are huge oyster reefs with names like Deep Water, Horsehead and Wreck Shoals. Reefs like these once made the James River the mother lode for new seed oysters for Chesapeake Bay. "It's amazing," Burreson tells his crew, "they've got this reserve fleet anchored over what were once the most productive oyster grounds in the world."

Closing with the fleet, Burreson and his crew find mothballed ships lashed together side by side, with sharp, pointed bows tied to fat, round sterns. The U.S. Maritime Administration calls this collection the James River Reserve Fleet. Locals call it "the Ghost Fleet." These rusting hulls carried men and women and weapons to World War II, Korea, and Vietnam. Several ships made it to the first Gulf War. Now they float here, motionless, empty of people and cargo, their waterlines riding high above the river. Up top they carry a jungle gym of smoke stacks and radar dishes and radio towers with no signals to send. From the window of a control bridge sunlight flares out like a cannon flash from a long-forgotten battle.

Could a "ghost fleet" of crumbling warships have anything to do with a disease that has ravaged native oysters for nearly half a century?

INVASION of Chesapeake Bay

By Michael W. Fincham



Running close along the line of ships, Burreson starts to rouse his crew. "I'm worried about the Coast Guard coming out. They only arrest the chief scientist," he says. The low-key joke, delivered in his bottom-key voice, seems to work. His lab assistants, three young women in sunglasses, sunblock, and baseball caps, start pulling out dredging gear. It's time to go to work.

There's a long-standing mystery about the oyster reefs along the James River. The mother lode is still there, but most of the oysters hauled out of these waters never make it to market. When these upriver oysters are replanted downriver they are supposed to grow fat and salty. Now, however, they shrivel up and die, many of them victims of a parasite called MSX.

The James River Reserve Fleet *(shown above), often called the Ghost Fleet, dates back to 1925. By 1950, it held 800 ships, many of them reactivated for the Korean, Vietnam, and first Gulf wars. Like the oyster, the fleet has dwindled — only 57 now remain.* PHOTOGRAPH BY MICHAEL W. FINCHAM.

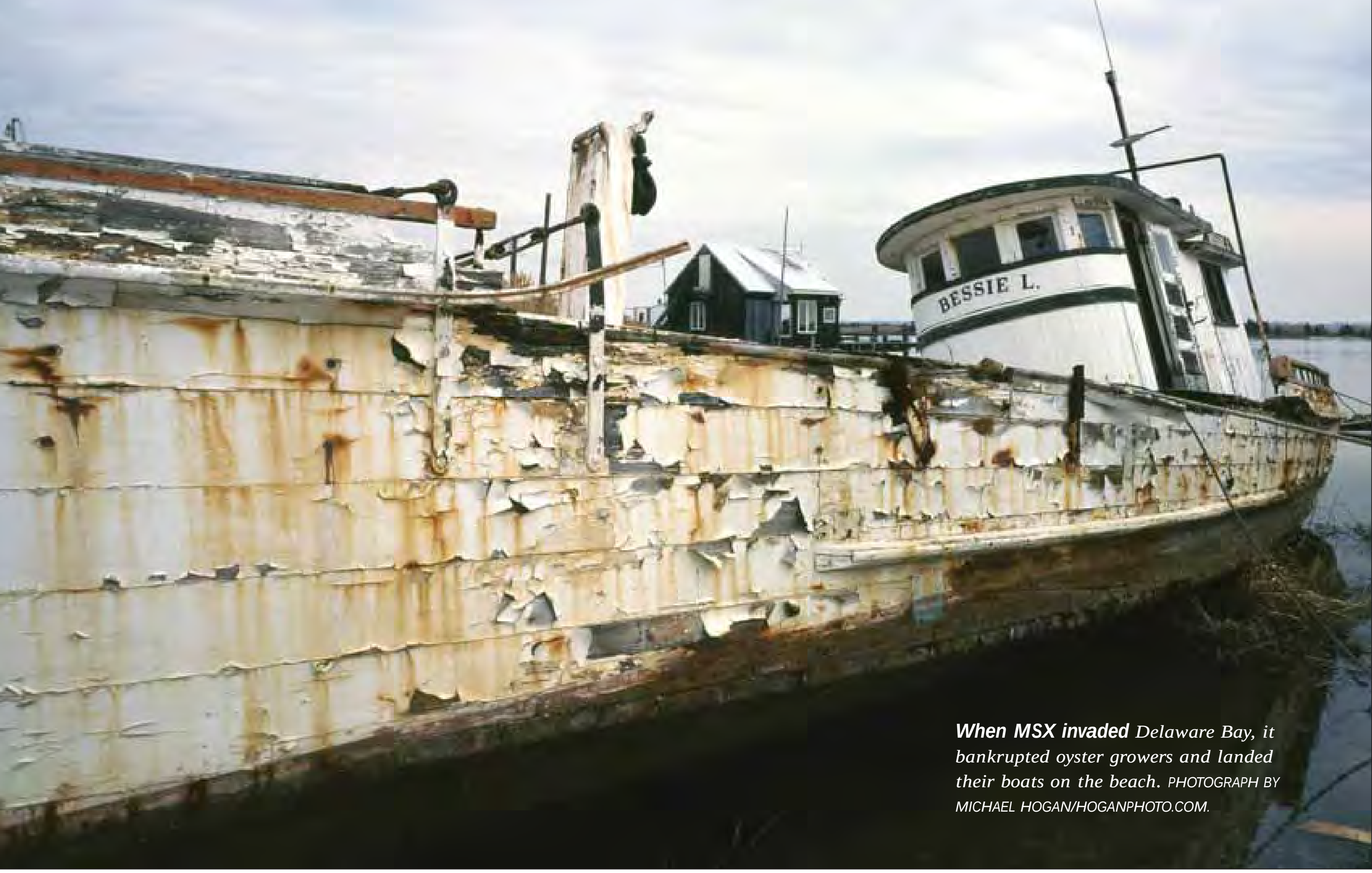
The dying started about 50 years ago. And it started suddenly and massively over most of the southern Chesapeake. That's one part of the mystery. The dieoffs eventually spread north, reaching from the James River all the way up to the Bay Bridge.

And no one could figure out where this MSX parasite came from. That's another part of the mystery. That's one of the questions that first brought Burreson out to these reefs.

But it would be years before he guessed how the Ghost Fleet could be part of the answer.

IT BEGAN AS A PARTY ON DELAWARE BAY, a holiday picnic on one of the big commercial oyster boats, and two scientists — one at the beginning of his career, one at the end — got to come along for the ride. The holiday was Labor Day. The year was 1958.

To celebrate the holiday, Norm Jeffries, an oyster grower out



When MSX invaded *Delaware Bay, it bankrupted oyster growers and landed their boats on the beach.* PHOTOGRAPH BY MICHAEL HOGAN/HOGANPHOTO.COM.

of Port Norris, New Jersey, would load his best-looking boat with wife, family, and friends and motor them out for a floating party and dress-up preview of this year's harvest. It was a celebration of sorts, this day on the water, an annual "trying of the grounds" before the opening of the oyster season.

Like farmers, oyster growers are gamblers. Jeffries, the son of a Port Norris oysterman, had borrowed heavily to plant oyster seed across hundreds of acres of leased bottom. He brought scientists to the party because he needed to know whether his bet had paid off.

The scientists on board often worked at the Rutgers shellfish laboratory at nearby Bivalve. One was Walt Canzonier, a young graduate student. The other was Thurlow Christian Nelson, a famous biologist with a long list of honorary degrees and awards to his name, as well as more than 125 publications, most of them about *Crassostrea virginica*, the native oyster that supported profitable fisheries in Delaware and Chesapeake bays.

Now 67, retired and ailing, Nelson took a seat on a trunk cabin as the boat glided south past the grassy wetlands lining the Maurice River and out onto the broad flat reaches of the mainstem Delaware. From his perch on the cabin, Nelson could give orders and keep the young Canzonier busy.

The grad student's job was to fill a bushel basket with oysters, then empty it oyster by oyster, counting the living and the dead. With oysters, the dead come in two forms: boxes and gapers. When you crack open an oyster and find it empty of meat, you call it a box. When you find the oyster hanging open with the meat dead or decayed, you call it a gaper.

When the first oyster dredge hit the deck, it was Canzonier who was down on his hands and knees culling through oysters. Counting through the first bushel, he began calling out boxes and gapers at a rate that stunned Nelson and every one else on board. Nearly all the oysters were dead. "That can't be right," Nelson snapped. "Count them again." Young

The party was over for the entire Delaware Bay oyster industry. A devastating disease was sweeping through oyster beds all around the estuary.

Canzonier started over, counting out one dead oyster after another.

Perhaps this was just a bad patch, an odd local dieoff. Jeffries pulled his dredge and motored away to try another oyster ground. When he dumped the dredge on deck, Canzonier sank to his knees again and again began calling out boxes and gapers. Jeffries headed for another oyster planting. Then another. And another. In disbelief, Nelson kept barking orders, calling for recounts.

Norm Jeffries just watched, shaking his head, glancing at his wife. An oyster grower born into the business, he didn't need numbers to tell him they were in deep trouble. Jeffries and his wife had built one of the most successful oyster operations in the region, largely by floating huge loans, first to expand their plantings and later to build a shucking house. Planting oysters, he liked to say, was like putting money in the bank. The more money he put in the bank, the larger the loan he could take out. There was a catch, of course. He had to pay all those deckhands, all those shuckers, all those loans. He had to harvest a lot of oysters.

Finally he steamed across to the eastern side of Delaware Bay, to his prized Cape Shore grounds where he had made his largest, most expensive planting yet. The Cape Shore grounds had paid off well in the past — but not this year. The dredge came splashing up, Canzonier dropped to his knees, and Jeffries discovered there would be no money going in the bank this year. The dying was general all over Delaware Bay.

The party was over — not just for Jeffries, but for the entire Delaware Bay oyster industry. A devastating disease was sweeping through oyster beds all around the estuary. Within two years the commercial harvests were cut by over 90 percent.

Norman Jeffries, the 61-year old oyster grower, lost everything. "He went into bankruptcy," says Canzonier. He sold off the business, then the shucking house, then the boats. "The creditors took everything he owned — except for one little boat that was listed in his wife's name," says Canzonier. All along the north shore of Delaware Bay, in little towns like Bivalve and Shellpile and Port Norris, local growers and watermen by the dozens lost their boats to the banks and went out of business.

Chesapeake Bay was next. A year later the same disease swept through the lower Bay, killing millions of oysters in Mobjack Bay and in the lower reaches of the York and the James and the mainstem estuary. Within three years oyster harvests in Virginia dropped by 75 percent and oyster growers were getting out of the business.

Out on his holiday boat ride Walt Canzonier had become an accidental witness to a turning point in ecological history. As he knelt on deck counting all those boxes and gapers he was documenting, unknowingly, the first, sudden sign of an oncoming ecosystem decline.

These oyster dieoffs would do more than put a lot of oystermen out of business in Delaware, Maryland, and Virginia: they would change the ecology of Chesapeake Bay.

THURLOW NELSON WAS seeing jello-like blobs, dozens of them, each one stuffed with dark, round dots.

Sitting at his microscope, he was squinting at oyster tissue riddled with some kind of unnamed parasite. The blobs, roundish and irregular, seemed to be single-cell plasmodia, and the dots seemed to be nuclei, but Nelson could not identify the mystery killer, and neither could Harold Haskin, his protégé and now the director of the New Jersey oyster laboratories.

The mystery only deepened when they called in other experts. Leslie Stauber from Rutgers was a leading authority on parasites, and John Mackin from Texas A&M had looked at more oyster slides than anyone else in the world. Both pored over the new slides and paged through the research literature, pulling out photographs and drawings, hunting for a match. Both searched their memories, and in the end both made the same report: they had never seen this organism before.

What they could see was the route of infection. The parasites invade through the gills, sucked in as the oyster feeds. Once in the gills, they divide and multiply rapidly, eventually breaking through to the circulatory system and moving easily throughout the body. With its tissues overwhelmed and its nutrients absorbed by the parasite, the oyster soon shrinks and dies.

Even Walt Canzonier, the grad student, got his turn at the scope. "The plasmodium was spherical, and it was full of nuclei," says Canzonier. "We were sitting around looking at this parasite. And we didn't know what it was."

Since the new parasite needed a name, lab director Haskin gave it one. "Well, it's obvious," he told the scientists gathered around the microscope.

"This is multi-nucleated sphere X. Or MSX." The X stood for origin unknown, and the name stuck because the mystery stuck.

Gene Burreson swings a small oyster dredge over the side of the research boat and splashes it down towards the bottom of the James River. The research boat guns forward, the line starts vibrating, and the dredge digs into the unseen oyster reef below.

The biologist and his crew are now working the Horsehead oyster grounds just downstream from the Ghost Fleet. Scientists like Burreson have been coming back to these James River reefs every year for nearly half a century now, hoping to find evidence the dying is over.

Burreson is one of a second generation of oyster scientists to tackle the mysteries of MSX, and his assistants will be part of a third generation. Early researchers at the Rutgers shellfish laboratories were able to classify the parasite as *Haplosporidium nelsoni*, a protozoan with at least two life history stages: spores and plasmodia. Over the decades they would document how warm winters, drought years, and high salinities helped spread MSX and how cold winters, rainy years, and low salinities helped slow it down. By selecting and breeding MSX survivors, scientists even made good progress in developing disease-tolerant oysters in the lab.

Certain tough questions, however, remain unanswered, passed down to Burreson's generation. What are the other life stages and where are they? Scientists think there is more to MSX than spores and plasmodia, the two life stages they can see in oysters. Those other life stages probably occur in another organism, an alternate host that carries MSX around and releases it where it can infect oysters. Could it be carried by copepods floating in the plankton, worms in the sediment, even fish or crabs? There are dozens of possibilities and no

Special Collections & Archives, Rutgers University Libraries

Special Collections & Archives, Rutgers University Libraries

***Thurlow C. Nelson** (left) was the famous son of a famous scientist, Julius Nelson, who pioneered the study of oysters in New Jersey. The son extended his father's work, with new findings that proved crucial to the oyster growers of Delaware Bay and led to the creation of two shellfish laboratories. Harold Haskin (right), a protégé of Thurlow Nelson, gave the MSX parasite its popular name and organized long-term studies on the biology and ecology of the organism.*

answers to the question (see The Missing Link, p. 14).

While researchers have wrestled with these questions over the last half century, MSX has been depopulating the oyster grounds of Chesapeake Bay, especially during drought years. In the last decade and a half, a second disease called Dermo — caused by another protozoan parasite, *Perkinsus marinus* — also exploded through the Bay's dwindling oyster populations (see The Culture of Disease, below). The Chesapeake lost bottom reefs, a major habitat for biodiversity, as well as millions of filter-feeding oysters, a major force for water clarity. By the early 21st century, 99 percent of the oysters were gone and the ecology of the Bay had changed dramatically.

As the small dredge rises, dripping, out of the river, Burreson and a young, red-haired lab assistant swing it on board and dump its contents. Like rocks hitting a boardwalk, dozens of oysters thud across the culling board. Burreson and his crew tug on gloves and start digging through the pile with knives and hammers.

Cracking apart a clump of oysters, Burreson holds open two interior shells, each covered with a brown, scum-like biofilm. He points to several barnacles growing inside the shell. "This is an oyster that's probably been dead for six months," he says, flipping it overboard. "Maybe a year or more."

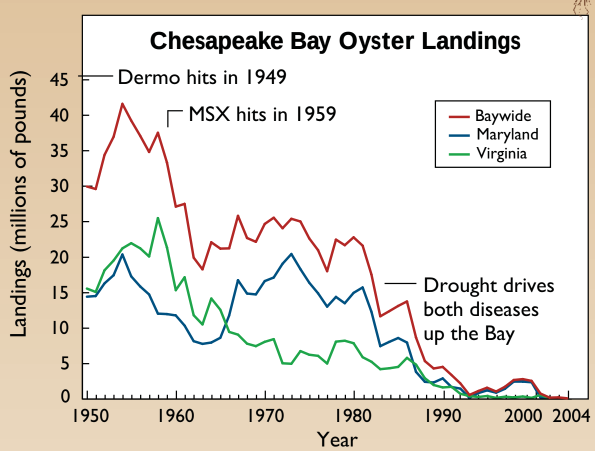
He taps an oyster with his knife. "It looks perfectly healthy," he says, but the hollow-sounding knock tells him otherwise. Cranking it open, he finds no meat and no biofilm, just two shiny, pearl-white shells, "which means this is a real recent mortality, probably within the last week or so. It's an indication that there is a mortal-

The Culture of Disease

Dermo, not MSX, is now the dominant oyster killer in Chesapeake Bay. A protozoan parasite, Dermo (known to scientists as *Perkinsus marinus*) was first documented at low levels in the Bay in 1949, a decade before MSX showed up. It may have been here forever, scientists say, or it may have been carried in from southern U.S. waters where it had been killing oysters for years.

In the mid-1980s, a series of warm winters and dry summers unleashed a Dermo epidemic in Chesapeake Bay, and by 1990, Dermo had exploded in Delaware Bay also. A remnant population left over in low levels from the 1950s had apparently endured, moving from oyster to oyster and surviving cold winters in small numbers, suggests Susan Ford, a researcher at Rutgers University's Haskin Shellfish Laboratory in Bivalve, New Jersey. In Delaware Bay, Dermo has so displaced MSX as Parasite Enemy Number One, that in Ford's words, "If we didn't have Dermo we wouldn't have a disease problem."

In Virginia, VIMS researcher Gene Burreson notes that when Dermo moved into the rich seed areas of the James River it brought the oyster industry to its knees. Back in the 1950s,



***Dermo arrived early in the Chesapeake,** but its effect in Maryland wasn't fully felt until years after MSX hit, when drought caused both diseases, which thrive in higher salinities, to spread. Together, these diseases decimated what was once the Chesapeake's most valuable fishery. SOURCES: GRAPH, NOAA FISHERIES ANNUAL COMMERCIAL LANDINGS STATISTICS DATABASE. ZOOSPORE DRAWING,* THE EASTERN OYSTER: CRASSOSTREA VIRGINICA, *BY KENNEDY, NEWELL, AND EBLE.*

Dermo, biflagellated zoospore stage

he says, some 75 percent of the harvest came from leased bottom stocked with James River seed oysters — 3 to 4 million bushels a year. First MSX arrived, then after the 1980s drought, Dermo followed saltier water up into the rich seedbeds and hit the oysters — and the oystermen — hard.

"They just lost everything," he says.

What have we learned about this other oyster killer? How does it survive? How does it grow? Does it have an Achilles Heel?

To attack these questions, scientists sought to study the parasite under controlled conditions in the laboratory, but as the 1980s gave way to the 1990s, they did not yet know how to culture Dermo and had to rely on samples gathered from the wild.

Culturing an organism in the laboratory is a key step in biological research. Cultures provide controlled populations for ongoing studies of all kinds. But coaxing a microorganism to grow outside of ity going on up here at Horsehead," he says. "The salinity is just high enough that it could be from MSX." Dead oysters have a tale to tell, and the moral of this tale is no surprise: after nearly 50 years MSX is still killing oysters in Chesapeake Bay.

As Burreson scrabbles through his piles of oysters, he keeps looking for live adults. Gapers and boxes and small oysters go back overboard, but his keepers go into a small bucket for the boat ride back to the lab. Adults usually carry heavy loads of parasites, and Burreson has been pioneering a technique for analyzing the MSX samples, a technique never dreamt of back in 1957 when MSX first showed up.

Live oysters, it turns out, also have some tales to tell — and one of the tales comes with a surprise ending, an answer to the enduring mystery: where did this killer parasite come from?

It took a technique never dreamt of back in the 1950s, when MSX first showed up, to solve the mystery of the parasite's origin.

BURRESON'S FIRST CLUES came from scientists studying oysters from Korea and Japan. In 1971 Fred Kern was analyzing oysters at the Bureau of Commercial Fisheries laboratory in Oxford, Maryland when he saw parasites in Korean oysters that looked like MSX. The clue was a similarity based on a shape, a shape seen through a microscope, something akin to a witness watching a police lineup full of suspects who are all about the same size — but are all wearing masks.

In 1991, Carolyn Friedman had the same reaction while she was checking Japanese oysters with the California Department of Fish and Game. In both cases the MSX-like parasite turned up in *Crassostrea gigas*, a fast-growing Japanese oyster that has been profitably grown since the 1930s in California, Oregon, and Washington State. By the mid 1990s, the question of MSX's origins had been in the cold case files for nearly four decades. Scientists had clues, but no way to follow up, no way to unmask any suspects.

A breakthrough was coming, however, and it began late at night in 1983 on a lonely road in the hills of northern California. Kary Mullis, a lab technician who dreamed of being a novelist, was driving to his cabin in the woods, mulling over ways to analyze mutations in DNA when — in a flash of insight — he saw a new way of making copies of DNA. As he drove he became so excited, he later wrote, that he woke up his girlfriend to tell her. He also began dreaming about a Nobel Prize.

His insight led eventually to an inventive technique called PCR, short for polymerase chain reaction, a technique that allows scientists to multiply one small piece of DNA into millions of pieces. PCR became the hottest tool in molecular biology, opening the door to all kinds of new experiments and applications and investigations. Doctors could use PCR for diagnosing diseases, chemists for creating

---

its native habitat can be tricky — after a half-century of research, researchers still cannot culture the elusive parasite MSX.

The effort to develop a reliable method for culturing Dermo became priority number one for researchers like Gerardo Vasta at the University of Maryland's Center of Marine Biotechnology (COMB) in Baltimore.

Vasta was well equipped for the task. He had two Ph.D.'s — one in biochemistry and one in zoology, both from his home country of Argentina. Vasta had come to the U.S. in 1979 on an international scholarship, and he soon discovered that oysters and their immune systems provided excellent biochemical models.

In 1989, only four years after the creation of COMB, Vasta joined the new faculty. Right away he focused on the oyster's defense mechanisms, especially its defenses against Dermo. The lack of a reliable means to culture Dermo in the laboratory proved a fundamental barrier in pursuing this research, and so he set his sights on that.

Vasta and his colleague Julie Gauthier proceeded to test one growth medium after another, Vasta drawing on his extensive knowledge of mammalian systems and applying it to this marine organism. During 1992 and 1993, their breakthrough came, and they announced their development of a novel means for culturing this parasite that was ravaging the Chesapeake's oyster bars.

It was a spectacular moment for oyster research. With federal funds and scientific competitiveness at a peak, other researchers also devised methods for culturing Dermo at about the same time. Jerome La Peyre at VIMS and Stephen J. Kleinschuster at the Haskin Shellfish Laboratory at Rutgers University used different methods, but all three teams soon reported their findings in the scientific literature.

The breakthroughs did not stop there. Vasta's lab went on to produce molecular probes for Dermo, with work done by Adam Marsh, who later joined the faculty at the University of Delaware. Now, within a matter of months, Vasta says that his lab, working with the Institute for Genomic Research (TIGR), will complete the sequencing of Dermo's entire genome.

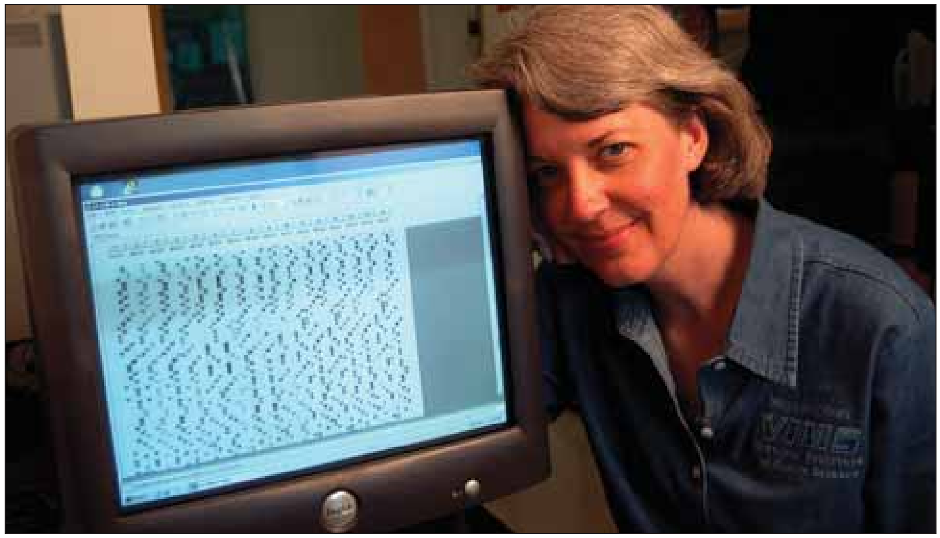
According to Kennedy Paynter, a longtime oyster researcher at the University of Maryland College Park and the UM Center for Environmental Science, the ability to culture Dermo has allowed researchers to ask and answer important questions, to test the parasite's response to salinity and temperature ranges. "We can see which conditions spur the parasite's growth and which hinder it," he says.

The molecular probe has also allowed scientists and research managers to pinpoint the presence of Dermo, whether in an oyster or in the environment.

Surveys conducted by Vasta and his colleagues from New England to the Chesapeake turned up Dermo everywhere. It used to be that oysters from Maine were uninfected, he says, but in a recent batch of Maine oysters Vasta found that 30 percent were infected with the disease.

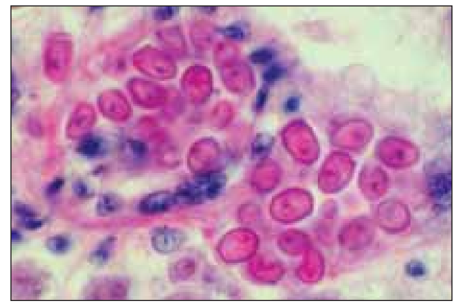
The future will not be easy for oysters facing this persistent parasite, but Vasta remains optimistic. He envisions finding Dermo's weak spots — in its need for iron to fuel its metabolism, for example — and using selective breeding or genetic manipulation to tilt this tough biological battle in favor of the native oyster.

— Jack Greer and Michael W. Fincham



Photograph at left and on opposite page by Michael W. Fincham

Blobs full of nuclei *(right) are what researchers see today — just as they did 50 years ago — when they look at MSX under the microscope. To finally unravel the mystery of those blobs, Gene Burreson and Nancy Stokes (above and opposite page) used new technologies in the 1990s to show the organism's DNA sequence on X-ray film (opposite page). Today they use laser scans and computer programs to read its DNA sequence digitally (above).*



Gene Burreson

new drugs, police for doing DNA fingerprinting. It was a dream come true for biologists — and for Kary Mullis: in 1993, at age 49, he won his Nobel Prize.

For Burreson, the biologist-turned-detective, it was the dream tool for cracking the long-standing mystery of MSX. His first step was to hire Nancy Stokes, a scientist trained not in marine science but in the new DNA-based tools of molecular biology. With the PCR technique they could finally do more than peer at parasites through a microscope: they could try unmasking them by examining their DNA.

Their approach was elegant in concept: compare the DNA of the Chesapeake parasite against the DNA of the Japanese parasite. Was the killer in the Chesapeake a parasite from Japan?

Sitting down at his high, black lab bench, Burreson picks up an unshucked oyster and inserts a long, thin needle through the shell, slowly plunging it into the adductor muscle. Much like a doctor during an office visit, he's taking a blood sample from a sick oyster, one of his James River oysters infected with MSX. The oyster, a perfect patient, doesn't flinch. The blood comes up the needle as a clear liquid.

This is the first step in getting a pure DNA sample of MSX. Floating in the colorless blood are blob-like plasmodia. When Burreson squirts his blood sample into a petri dish holding a saline solution, the plasmodia continue to float while the blood cells settle out and stick to the glass. He pours his solution into another dish, and the blood cells settle out again. By pouring and settling several times, he gradually discards most of the oyster cells and concentrates the MSX cells. "That was the hardest part," says Burreson, "getting the pure MSX DNA without a lot of oyster DNA contaminating it."

The rest was hardly easy. With these samples Burreson and Stokes first had to create a DNA fingerprint for MSX. And PCR was their key tool because it gave them a lot of DNA to work with.

Using cycles of heating and cooling plus a key enzyme, PCR can split a DNA strand in two and then create two separate copies. By running the process multiple times — the heating, the cooling, the enzymes — scientists are, in effect, setting off a chain reaction. They are copying the copies, then copying the copies of the copies until they have quickly "amplified" one piece of MSX DNA into millions of pieces.

With all those DNA copies, Burreson and Stokes went looking for some of the signature genes that define MSX as MSX. After picking one sector of one key gene, they were able to outline a 564 base pair sequence that was unique to the parasite, as unique as a fingerprint to a criminal.

Their work, for the first time, unmasked MSX, the Chesapeake oyster killer. It identified the parasite not by its body shape as seen under a microscope, but by a diagram of its underlying DNA, its genetic fingerprint.

Like any detective worth his badge, the biologist took his new tools and started rounding up the usual suspects and fingerprinting them.

In this case the suspects were Japanese oysters that came from the far side of the country and the other side of the world. Burreson gathered tissue slides or spat or living oysters that originated in Matushima Bay in Japan, in Geoje Bay in Korea, and in Drakes Estero in Marin County, California.

Thanks to his work with PCR, Burreson now had a molecular probe that would seek out and stick itself to any DNA from MSX in any samples of oyster tissue. When his probe turned up MSX in the tissue of all his non-native suspects, the last mask was lifted: MSX, the killer parasite, was carried in oysters from Japan, Korea, and California.

For a final proof, Burreson and Stokes sequenced a section of DNA from the parasite in Japanese oysters. When they compared it with their earlier DNA fingerprint from the Chesapeake parasite, they found a near-perfect match, a 99.8 percent match that would convict a culprit in any court. "That is conclusive evidence that the parasite in *Crassostrea gigas* is, in


VIMS
VIRGINIA INSTITUTE
OF MARINE SCIENCE

Michael W. Fincham

Gene Burreson *draws a blood sample from a sick oyster, the first step toward creating a DNA fingerprint of the MSX parasite.*

fact, MSX," says Burreson. "They are the same organism."

His final verdict: the Japanese oyster was the culprit. It sometimes carried MSX, a parasite that seldom sickened *gigas* but was lethal to oysters in both Delaware and Chesapeake bays. "MSX was like smallpox coming in with the Europeans," says Burreson, "and the native Americans were wiped out, because they were naïve to it. They hadn't seen it."

An answer like this only leads, of course, to a new question: who brought Japanese oysters — and MSX — into the Chesapeake?

THE FIRST PERSON TO plant Japanese oysters in East Coast waters was probably a scientist. Sometime in the early 1930s, a researcher drove down to the New Jersey shore, carrying a bushel of Japanese oysters and planted them in Barnegat Bay, a narrow estuary behind the state's barrier islands. The scientist was none other than Thurlow C. Nelson, already chairman of the Zoology Department at Rutgers and director of New Jersey's two shellfish laboratories.

His Japanese oysters grew quickly at first, raising his hopes that *gigas* oysters might revive Barnegat Bay's struggling oyster industry. After two weeks, the oysters stopped growing and gradually died out, perhaps from low salinity and low oxygen. But Nelson's interest was piqued. If he wasn't the first person to ever plant *Crassostrea gigas* in East Coast waters, he was certainly the first to talk about it.

In the spring of 1946, shortly after the end of World War II, Nelson strode to the podium at the New Yorker Hotel and told the annual convention of the National Shellfisheries Association that it was time to try planting oysters from Japan in East Coast waters like Chesapeake and Delaware bays.

It was a powerful message to an important audience. Nelson was a well-established scientist, and his audience that day included members of the Oyster Growers and Dealers Association of North America. In the decade before World War II, growers along the West Coast had done very well importing, planting, and harvesting a Japanese oyster called *Crassostrea gigas* — so well that East Coast growers began worrying about losing the post-war market to the faster-growing Japanese oyster. World War II was now over, seed oysters from Japan were becoming available again, and a post-war economic boom seemed to be gearing up.

It was a propitious moment for optimism and Nelson was nothing if not optimistic about the potential of the Japanese oyster. He extolled its fast growth in high-salinity waters and passed along reports of four-foot oysters once found in Japan. "If it were possible to obtain in our Eastern oyster the rapid growth of the Japanese oyster," he argued, "it would revolutionize our industry." He called for Japanese oysters to be "promptly shipped" to shellfish laboratories on the East Coast. To an audience of oyster growers he also suggested that "test plantings be made on a small commercial scale under natural conditions."

GENE BURRESON FOUND a copy of Nelson's 1946 speech when he was sleuthing through the historical literature, looking for evidence of early introductions of Japanese oysters. From historical records like this he arrived at an uncomfortable scenario: scientists like Nelson and growers like those in his audience may have brought in MSX.

If one exotic oyster species brought disease, can we depend on another species of non-native oyster to restore the Chesapeake?

At first only a few people followed Nelson's advice about testing Japanese oysters, at least according to the published reports that Burreson found. In 1949 a shellfish manager on Cape Cod planted six bushels of spat into Barnstable Harbor. That same year wildlife officials in Maine tried a small planting of Japanese and European oysters in a pond, and Victor Loosanoff, director of a federal marine laboratory, planted European oysters in the waters of Maine and Connecticut. According to a 1950 report, there were also "numerous attempts" at introducing Japanese oysters in Long Island Sound.

In Nelson's speech, in hindsight, are some of history's terrible ironies — and perhaps some scientific hubris. The siren call of fatter, faster-growing oysters apparently led some scientists and growers to try planting local waters with non-native oysters — and all their hidden hitchhikers. In popularizing the potential of Japanese oysters, Nelson may have unwittingly helped call down the destruction of the native oyster species he spent his life studying.

For Burreson, the reports and the anecdotes are evidence of a trend, a readiness on the part of scientists and growers along the East Coast to experiment in a casual fashion with Japanese oysters. "It was brought in a lot by scientists," says Burreson. "They would bring them in and just put them off the dock or put them in trays to see how they would grow. It was done by industry members [oyster growers] as well, probably a lot, but not nearly so well documented."

It's not surprising that Burreson's claims have stirred some dissent, especially from scientists who worked at Nelson's old lab at Bivalve, New Jersey. "Some people would like to say Doc Nelson brought *gigas* into Delaware Bay." says Walt Canzonier, the former grad student who worked with Nelson nearly 50 years ago. "I don't see anything in the record to indicate that. He may have had *gigas* here to work with, but not in very large numbers, that's for sure."

Despite his claims for Japanese oysters, Nelson had little reason and probably no funding for launching a major research effort or planting program in Delaware Bay, says Susan Ford, another scientist with the Rutgers Bivalve Lab. "In the early 50s, oysters were not in short supply," argues Ford, "so there would not have been any emphasis to do anything serious." Growers elsewhere along the coast may have been planting *gigas*, as the historical records suggest, but there is no evidence, says Ford, that anybody tested *gigas* in Delaware or Chesapeake bay — at least in the years before MSX appeared.

After MSX struck, everything changed. New test plantings, most of them small scale, were reported in Delaware, Maine, Massachusetts, Maryland, and Virginia. And always there were anecdotes of unreported plantings. One of Burreson's favorites is the story of the man who brought Japanese oysters home from the Seattle World's Fair of 1962 and planted them off his dock. An ambitious seafood entrepreneur admitted planting *gigas* in Maryland waters in the 1970s, and a Virginia grower announced at a science conference that he made large plantings during the 1980s.

By the 1990s, the Virginia Seafood Council, in hopes of saving a declining seafood industry, was asking scientists to find an alternative oyster to replace the rapidly disappearing native oyster. The first strong candidate that scientists put forward for planting in the Chesapeake was, ironically enough, the Japanese oyster, *Crassostrea gigas*. And one of the scientists who went to work researching the potential for *gigas* in Chesapeake waters was, ironically enough, Gene Burreson.

THERE'S A SECOND UNSETTLING scenario for the MSX invasion.

In 1946 when Nelson was calling for the planting of Japanese oysters, the old Ghost Fleet of mothballed Navy ships was bringing more ships into the James River every week. With the end of World War II, hundreds of ships were arriving in Chesapeake Bay and steaming upriver for long-term anchorage. They were sailing back from Europe — and from Japan.

In 1950, the James River Reserve Fleet hit its historic high with 800 ships lashed together in groups of 12 to 30 ships, a flotilla that stretched miles up the river. Between 1950 and 1954, many of those ships were reactivated to carry troops off to the Korean War — and bring them home again. By 1957 MSX began showing up in oysters.

The timing of all that shipping traffic and the sudden onslaught of MSX a few years later is, at the least, suggestive. Ships can carry MSX in various ways. Oysters can attach to their hulls, says Ford, and MSX and perhaps an alternative host could be hitchhiking here in ballast water. If ships dump their ballast water or their bilge water in the Bay, they could be releasing those MSX or its hosts into the Bay.

"Let's look at this scenario," says Canzonier who finds this option more plausible. "Suppose we had MSX in the Chesapeake Bay for a number of years being introduced by the Naval fleet coming from Korea. And it just never flared up because conditions were not quite right." MSX may have invaded the Chesapeake first and then shipped north to Delaware with James River seed oysters, says Canzonier, now president of the New Jersey Aquaculture Association.

"Perhaps in the mid-1950s, the environmental conditions were more appropriate in Delaware Bay or the alternate host was present in sufficient number," he says Canzonier. "And it caught fire here before it caught fire in Chesapeake Bay."

The Missing Link

MSX Middleman Remains Elusive

Measures to control malaria improved nearly overnight after the 1897 discovery that mosquitoes play a role in the life cycle of the malaria parasite, *Plasmodium* spp. This ancient disease, according to recent research, probably played a part in the downfall of the Roman Empire in the 5th century and nearly stopped the building of the Panama Canal in the 20th century. When simple pest control measures were put in place around the Panama Canal dig, the number of deaths from malaria dropped nearly eight-fold in the span of just three years. Even in the 21st century, reducing mosquito abundance remains a reliable control strategy for harnessing the spread of this global disease.

What if the mosquito's role as intermediate host in the life cycle of the malaria parasite had remained stubbornly hidden? It's hard to guess at the impact. But the prolonged mystery of the missing host for the MSX parasite has no doubt hastened the decline and fall of the oyster empire of the Chesapeake Bay.

Since the 1960s, generations of scientists studying MSX have searched for answers to the transmission question. They've found that native oysters never "catch" an MSX infection in the lab from infected oysters placed next to them. But when scientists place healthy oysters in a Bay area empty of oysters, the clean oysters "catch" a heavy MSX infection — and very quickly. Since other oysters apparently do not release infective particles, some other animal — the alternate host — must be releasing MSX.

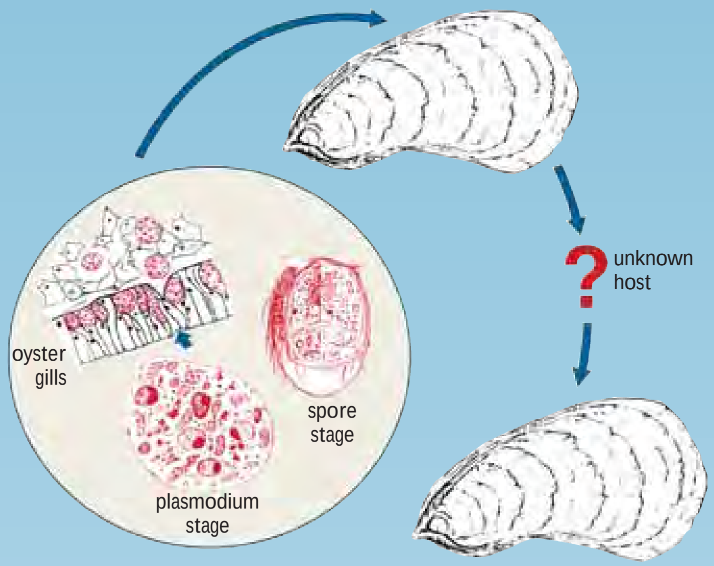
What is this alternate host? A crab, a copepod, a worm? In 2006, scientists still don't have the answer — but not for lack of trying. Every two weeks, over a three-year period that started in 1999, scientists sampled an array of organisms for MSX, using both conventional and molecular techniques. "We sampled literally every organism we could get our hands on," says VIMS oyster biologist Gene Burreson. "As you can imagine, the number of possible organisms is huge," says Rutgers biologist Susan Ford, who worked with Burreson on this study.

Burreson and Ford no longer have targeted funding to pursue the search for MSX's alternate host but they haven't given up. They are still sampling. "We hope we are going to get lucky," Burreson says.

Burreson feels strongly that MSX's alternate host must be something that eats the spores that form as one stage of the parasite's life cycle. But the spores are tiny, just 7 microns, and almost anything could eat them. "If we had narrowed it down and could say, 'look, it's in worms,' that's one thing, but we can't say anything still," he says.

Part of the problem, explains mathematical biologist Marjorie Wonham, is that disease cycles in marine systems in general are much less well-known than those in terrestrial systems. "When you are living in the water, you are in a soup of organisms that range from the nano to microscales. There are a lot more options available as potential intermediate hosts."

MSX Life Cycle



A lingering mystery, *the suspected alternate host for MSX remains unknown to scientists despite years of dedicated effort. Knowledge of the parasite's complete life history could provide clues for controlling the spread of the disease. DRAWINGS ADAPTED FROM* THE EASTERN OYSTER: CRASSOSTREA VIRGINICA *BY KENNEDY, NEWELL, AND EBLE.*

And water is so much harder to search, Wonham continues. "We don't live in the ocean. We don't have the same ease of access and intuition about marine systems as we have with terrestrial systems," she says. Wonham, who works at the Center for Mathematical Biology at the University of Alberta in Edmonton, Canada develops models to predict outbreaks of West Nile Virus and invasive species introductions through ballast water exchange in the Great Lakes.

Until they unveil MSX's intermediate host, scientists will lack the ability to control the transmission of MSX. Generally speaking, "if you don't understand the life cycle [of a parasite], you have no hope," says ecologist Andrew Dobson of Princeton University, who studies the role that parasites play in the population ecology of infectious disease.

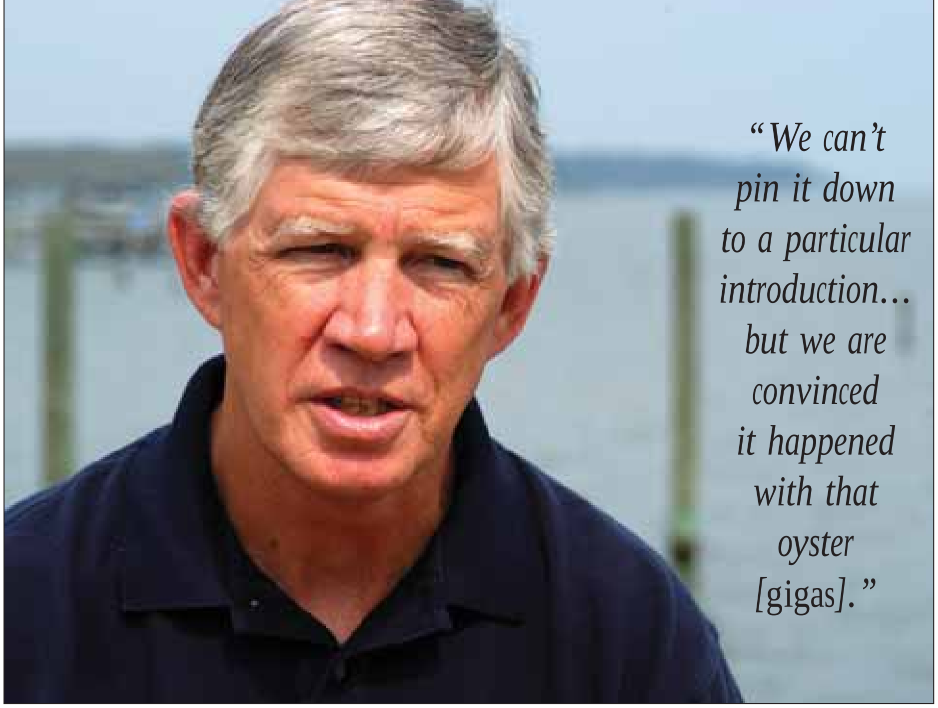
In the case of malaria, Dobson explains, once British medical officer Ronald Ross discovered that mosquitoes enter into the parasite's life cycle twice, once to transmit the disease to humans and once to receive the parasite back from human hosts, he rapidly concluded that controlling mosquito abundance would be key. "Targeting the right point of the parasite's life cycle gives you a lot of insight," Dobson says.

But in the case of MSX, just knowing the alternate host likely would not be enough. "It might help," says Ford, "but how would you control copepods or worms? Remember, we do know how *Perkinsus marinus* [Dermo] is transmitted and it continues to kill oysters in large numbers."

The unknown intermediate for MSX also clouds the question of the parasite's introduction to Chesapeake Bay. It is possible that MSX came to the Chesapeake, not with Japanese oyster (*Crassostrea gigas*), but inside this hypothetical intermediate host by some yet uncharted route.

Solving the mystery of MSX's middleman would open new doors in understanding the disease. But detectives Burreson and Ford may be up against a funding roadblock in continuing their search. "You have to sample a lot of organisms and that's been the problem. It's risky research for people willing to give you money," says Burreson. "If you do solve it, then I think the payoff is certainly worth it."

— Erica Goldman, with reporting by Michael W. Fincham



Michael W. Fincham

Working just south of the Ghost Fleet, Burreson and his crew finish culling through their last dredge load of James River oysters. The biologist leans over and peers at his one bucket of live oysters, then with a sweep of his arm shoves all the shells and dead oysters overboard.

"It's possible that MSX came in ballast water," says Burreson, glancing at the ships to his north. "Some of these ships here were probably involved in moving troops from Korea. It could have happened during that period. And there are some cases where ships came from Korea into Delaware Bay and dumped ballast water there."

Naval ships, of course, were only part of the shipping traffic that was steaming through the Chesapeake and Delaware during the 1950s carrying cargo and ballast water and perhaps MSX from Korea and Japan. As the size and speed of commercial tankers and freighters have increased in recent years, ballast water has emerged as a major research focus for invasion ecologists, who suspect numerous organisms are offloading in our coastal waters. The ballast water research team at the Smithsonian Estuarine Research Center estimates more than 150 invasive species are now surviving in the Chesapeake. The Chinese mitten crab may be the latest arrival.

WHO STARTED THE MSX invasion of the Chesapeake?

The interim verdict: the MSX invasion may have begun with an experiment by an oyster scientist, with a planting by a grower, with a ballast water release by a ship — or with all of the above in several places on several dates. The jury is still out, the history still open to debate. "We can't pin it down to a particular introduction in a particular place," says Burreson, "but we are convinced it happened with that oyster [*gigas*]."

The future is also open to debate. With native oysters now depleted, thanks in part to a Japanese oyster parasite, some scientists and growers and politicians want to rebuild the commercial seafood industry and perhaps restore the Bay's ecology by planting Chinese oysters, *Crassostrea ariakensis*, in the Chesapeake. It's a prospect that stirs considerable debate in the scientific community, yet a prospect that Burreson, surprisingly enough, does not oppose.

There may be lessons in his research, but keeping out *ariakensis* is not one of them, at least for Burreson. "People are now using this [finding] as an argument against introducing *ariakensis*," says Burreson before offering a different, more upbeat reading of his own research. "It shows what can go wrong," he says, "when you don't do things right."

Contemporary scientists, in his opinion, are doing things right: they are keeping *ariakensis* oysters isolated in hatcheries, then spawning sterile offspring, and only then trying test plantings in the Bay. If this approach had been followed with Japanese oysters, he says, MSX would never have invaded these waters.

Burreson, like Thurlow Nelson 50 years ago, is optimistic that science may yet find or build a better oyster for the Bay. Through patient crossbreeding, researchers at Rutgers and VIMS have developed several strains of disease-resistant native oysters for use by the aquaculture industry. And Mother Nature all by herself, through natural selection, may build a native, disease-tolerant oyster for the Bay. Oysters that survive MSX tend to pass on their resistance to their offspring until, over the long generations, an MSX-tolerant oyster emerges. That process has already worked in Delaware Bay, according to Susan Ford, and it may one day work in the Chesapeake.

Some mysteries remain — and some ironies. Burreson's work with MSX continues because, even after half a century, key questions about the disease have never been solved. The other life stages of MSX remain unknown, as does the alternate host. Burreson and his team keep searching for the DNA of MSX in dozens of species, hoping to find the host species which carries MSX around the Bay. "It's like searching for a needle in a haystack," he says. "I hope somebody cracks this before I die."

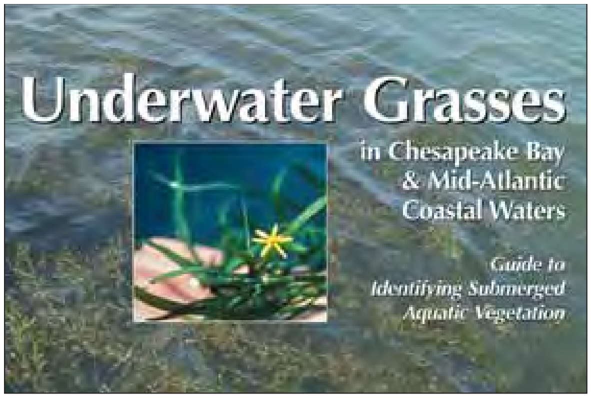
Thurlow Nelson, the man with high hopes for Japanese oysters, died long before any MSX mysteries were solved. On September 12, 1960, he left his summer cottage near Cape May as Hurricane Donna swept up the coast. Hoping to tie up his rowboat, he waded out into Delaware Bay and drowned there in the rough waters of the rising storm.

The biologists he mentored decided to honor his memory in the name of a new species they called *Minchinia nelsoni* — and, later, *Haplosporidium nelsoni* — the scientific term for the parasite Nelson knew only as MSX.

— *E-mail the author at* fincham@mdsg.umd.edu

New Guide to Underwater Grasses

Underwater grasses, or submerged aquatic vegetation (SAV), play a key role in the ecology of coastal waters, and especially in Chesapeake Bay — the nation's largest estuary. These grasses provide habitat for blue crabs and fish, and food for waterfowl. They help keep waters clear and offer shelter and living space for many aquatic species.



Over the past several decades, clouded waters — a result of too many nutrients and too much sediment — have blocked sunlight and smothered many SAV beds. Efforts are currently underway to help map, study, and reestablish underwater grasses in the Chesapeake and other coastal waters.

To help citizen volunteers, students, and others interested in learning more about these plants, Maryland Sea Grant has produced a new guide to underwater grasses in collaboration with the National Oceanic and Atmospheric Administration's Chesapeake Bay Office, the Alliance for the Chesapeake Bay, and the Maryland Department of Natural Resources.

This 80-page guide, *Underwater Grasses in Chesapeake Bay & Mid-Atlantic Coastal Waters*, is 8.5" wide and 5.5" tall and features more than 100 color photographs, 55 line drawings and helpful descriptions of 20 of the most common SAV species, along with other aquatic species you might see. The spiral-bound book is printed on heavy, waterproof stock, similar to the marine mammal guides published by Alaska Sea Grant and Rhode Island Sea Grant. The guide also includes ways to distinguish between similar plants, as well as additional information about floating aquatic vegetation and algae, including algal blooms that can impact water quality. Charts and maps detail the salinity range of each SAV species, including expected salinity ranges in Chesapeake Bay during wet and dry years. Especially useful is an identification key with details about leaves, stems, and other characteristics to help the user identify the plant in hand.

The guide costs $29.95 plus $2.00 shipping and $1.50 tax (Maryland residents only). To place an order and pay by check,visit the web at www.mdsg.umd.edu/SAV, e-mail Maryland Sea Grant at connors@mdsg. umd.edu, call 301.405.6376, or fax 301.314.5780. To pay by credit card, order through Maryland Sea Grant's distributor Cornell Maritime Press at http://cmptp.com/6647.htm; phone: 1.800.638.7641; fax: 410.758.6849.

Send us your comments — visit *Chesapeake Quarterly Online* at www.mdsg.umd.edu/CQ

Maryland Sea Grant College
4321 Hartwick Road, Suite 300
University System of Maryland
College Park, Maryland 20740

Address Service Requested

Non-Profit Org.
U.S.Postage
PAID
Permit No. 04386
College Park, MD

Recycled Paper
PRINTED WITH SOY INK

Chesapeake Quarterly is printed on recycled paper, processed chlorine free, with soy-based inks