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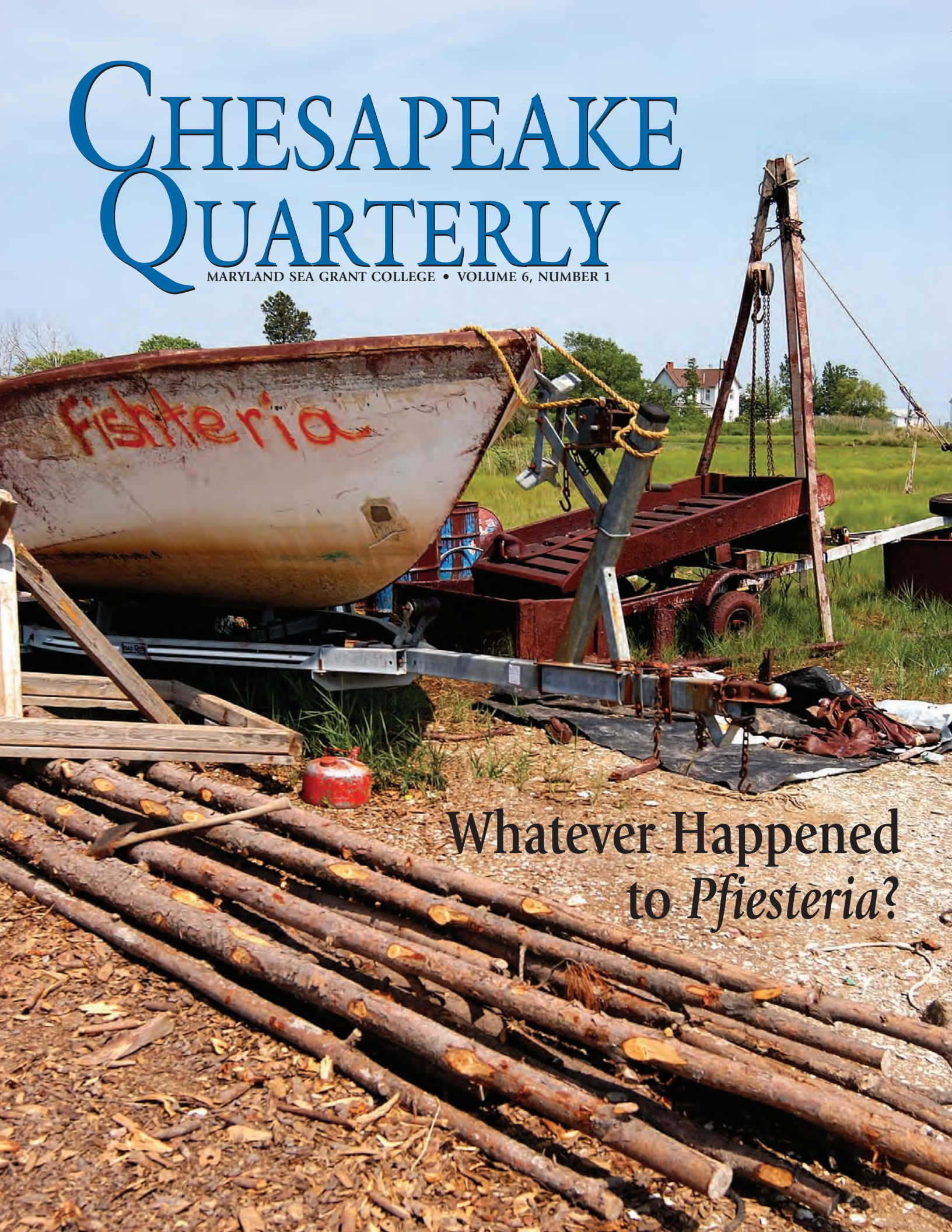

Whatever Happened to *Pfiesteria*?

contents

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Chesapeake Quarterly explores scientific, environmental, and cultural issues relevant to the Chesapeake Bay and its watershed.

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Cover photo: *A hint of irony graces this trailered boat in an Eastern Shore fishing community. The* Pfiesteria *crisis of 1997 put watermen out of work and led to river closures, public panic, and the loss of $40 million in seafood sales in Maryland.* PHOTOGRAPH BY MICHAEL W. FINCHAM.

How Did a Media

Brad Bell had covered environmental stories all over the state of Maryland for years, but he'd never heard of Shelltown.

A reporter with WJLA-TV, Bell was on the road out of Washington in early May 1997, off to report on rumors of sick fish in a faraway river on the other side of the Bay. Pulling out his map he found Shelltown perched on the bottom edge of the Eastern Shore of Maryland, the last stop on a thin blue highway that dead-ended on the lower stretches of the Pocomoke River. It was about as far south as you could drive and still be in Maryland.

Heading across the Bay Bridge, Bell got a call back from a spokesperson at the Maryland Department of Natural Resources (DNR). According to Bell, the DNR message was "Now Brad, as you do this story, don't go saying that it's *Pfiesteria*. We don't want any public panic."

That warning, a DNR official later admitted, was "like waving a red flag in front of a bull." Bell is blond-haired, self-confident to the edge of cocky, and blessed with the kind of strong mid-range baritone that punches across well on television. He resented the "lecture," he admits, and became immediately suspicious that "they (DNR) really didn't want to find out what was going on."

One hundred fifty miles from Washington, Bell found himself driving two-lane blacktops that wound past white churches with country graveyards, open fields with rows of corn and tomatoes, and small farms with long, pencil-straight chicken sheds.

Shelltown itself, he found, was a scattering of houses just past the last bend in the Pocomoke River. One public dock, one boat ramp, and one short sand beach. Across the river was Virginia.

At the end of a long gravel drive, there was also one commercial fishing harbor

Storm Get Started?

The Frenzy over *Pfiesteria*

By Michael W. Fincham

and several angry watermen. Brad Bell had found the story that would put Shelltown on the national media map. Not just Shelltown, but the whole state and the entire Chesapeake Bay.

This year marks the 10th anniversary of the summer of 1997, the summer of *Pfiesteria*, when a tiny microbe became big news, first in the Chesapeake region and then around the world. If you remember *Pfiesteria* at all, you probably remember reading news stories of wounded fish attacked by a mysterious microbe, a dinoflagellate that seemed to be releasing a toxin in the water. Or you remember watching television footage of dead, dying, or dazed fish floating in rivers like the Pocomoke, the Manokin, the Nanticoke, and the Chicamacomico. You knew those were rivers in the Chesapeake, but faraway rivers way down in the distant soggy southern reaches of the lower Eastern Shore. You probably stopped buying fish.

How did *Pfiesteria piscicida*, a single-celled organism, become the center of a media blitz, the magnet that would draw dozens, if not hundreds of reporters to this tiny riverside harbor and drive them to churn out thousands of stories over the next year? In hindsight, it's clear those stories, through their sheer volume, exaggerated the risk posed to fish, people, and the environment. They led to mass panic and major economic loss.

Here's one hypothesis about how that frenzy fired up, call it the conspiracy hypothesis. It's one of several, culled from interviews with reporters, state officials, and scientists who survived the storm over *Pfiesteria*. Together they may explain how *Pfiesteria piscicida* invaded your living room ten years ago. And how it could do so again.

At least since Watergate, a hint of conspiracy has been a red flag for most contemporary reporters. In 1997, Tim Wheeler at *The Baltimore Sun* was talking to watermen and to DNR officials and hearing different stories, and so was Todd Shields at *The Washington Post*. Watermen were saying *Pfiesteria* could be causing sick fish while DNR officials were saying they had no evidence the dinoflagellate was even in the river. They told Wheeler what they told Bell. "We are ninety-nine percent sure that it's not *Pfiesteria*. Be careful what you say."

When Brad Bell arrived in Shelltown in May of 1997, he talked to watermen, and more importantly his cameraman took footage of sick fish with red lesions. Some had tails eaten away as if doused in battery acid. While the words of state officials were reassuring, the images of sick fish were disturbing. Bell had never seen fish looking like that. Nor had the viewers who saw them on Washington television that evening.

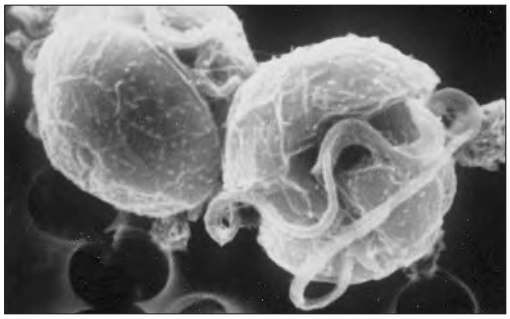
When he saw the viewer response to his sick fish story, Bell decided to follow the *Pfiesteria* trail. Maryland officials were sending their water samples to Florida for analysis by Karen Steidinger, one of the co-discoverers of the dinoflagellate, but Bell decided to get a second opinion. He turned to the other co-discoverer, JoAnn Burkholder, a controversial scientist who had blamed *Pfiesteria* for killing millions of fish in North Carolina rivers — and then accused state officials of ignoring the problem.

In an act of pure enterprise reporting, Bell headed back to the Pocomoke, filled an empty Evian bottle with water from a fish pound, then drove down Interstate 95, and delivered his sample personally to Burkholder's lab in North Carolina. Peering through a light microscope, Burkholder told Bell that the water held cells that looked like *Pfiesteria*. A detailed analysis, including fish bioassays and electron-scanning microscopes, would take a couple weeks, but Bell was not waiting. His report aired that evening.

"It was a breakthrough story," said Wheeler, and it ramped up the conspiracy dynamic. Scooped by Bell, reporters began to dig harder, suspecting a reluctance to reveal bad news about *Pfiesteria*. Now they were hearing yes, there is *Pfiesteria* in the river — but no, it's not connected to the sick fish in the river. More reporters began making the long drive to Shelltown where they heard watermen accuse the state of a coverup.

Here's where the conspiracy hypothesis breaks down, however. Maryland state agencies, in contrast to the Nixon administration, never mounted much of a coverup operation. As more media reports began appearing, the Department of Natural Resources and the Department of the Environment moved quickly towards complete transparency. They set up an interagency task force, put one person, Rob Magnien, in charge of coordinating the state's investigation and began responding quickly to all press inquiries.

Transparency, ironically enough, had its drawbacks — especially when there were so many questions about *Pfiesteria* and not very many answers. State officials were releasing information immediately, even when they didn't know what it meant. "We were scratching our heads," explains Rob Magnien, who had reporters asking questions every day. "We were just sitting there with a bunch of questions, some data, some conflicting opinions," he says. Transparency increased the flow of information through the media, but in the end, it also fed the public panic. The message that came through went something like this: state officials had no clear explanation for what was causing so many sick fish.



The Washington Post

Summer and Fall 1997

Md. Says Microbe May Be To Blame For Ailing Fish

Cell From Hell In Rivers

Fish Kill Prompts Closing Of Part Of Pocomoke River

Three Md. Workers Report Illness After Contact With Pocomoke River

Manure Blamed For Fish Kill

Md. Doctors Say Microbe Has Affected Others

Newspapers went to war *over the* Pfiesteria *story in 1997 when watermen like Jack Howard (first photograph, above) said* Pfiesteria piscicida *could be causing sick fish and the media responded with TV and newspaper reports. Glenn Morris (first photograph, p. 5) led a medical team, including Lynn Grattan (at right, second photograph, p. 5) that tested state workers and watermen for memory loss. Scientists like Ernest Brown (third photograph, p. 5) began using a DNA probe to find* Pfiesteria *all over the Bay.* PHOTOGRAPHS ON PP. 4 AND 5 BY MICHAEL W. FINCHAM, EXCEPT P. 4, MIDDLE BY JOANN BURKHOLDER.

Then there's the media war hypothesis. Who was at war? Newspapers with other newspapers. Television stations with other television stations.

"The *Pfiesteria* story was a huge, competitive news story," explained Wheeler. "It was probably the biggest environmental story we had here. There was a daily drum beat of news coverage. Our major competitor down the road, *The Washington Post*, jumped on and went after it in a big way. So we were in a big horserace."

The Washington Post, the other big horse in the race, competes with *The Baltimore Sun* on many Maryland issues, and they took notice of *The Sun's* heavy coverage. "We read their paper, they read our paper," said Peter Goodman, a *Post* reporter. "They took the story very seriously. It was a front page story in *The Sun* seemingly every day throughout the summer." A slow news season suddenly had a hot story line.

The horserace unleashed another dynamic, a media feedback loop that became a sort of perpetual motion machine. When *The Post* saw *The Sun* giving the story heavy coverage, *The Post* then gave the story heavy coverage, and when *The Sun* saw that, they stepped up their coverage again. Over one year *The Sun* assigned 21 reporters to the story, and *The Post* assigned 24. "There was intense pressure for stories," said Doug Birch, a science writer with *The Sun*. "We had big-league competition. We wanted to shine."

And shine they did. Over a year, *The Post* published some 130 stories focused on *Pfiesteria*, but *The Sun* won, pulling away down the stretch and publishing over 170 stories. "We certainly wrote more than they did," said Wheeler, "by yards of newsprint."

The newspaper war had another unexpected effect. *The Post* is both a local and a national newspaper, somewhat like *The New York Times*, *The L.A. Times* or *USA Today*. When *The Post* kept running stories, hoping to keep up with *The Sun*, they also drew in the national networks. *Pfiesteria* the dinoflagellate made its debut on ABC, NBC, and CBS. "The story just grows and grows," said Goodman. "That's what happened here."

Ironically newspaper reporters never saw themselves competing with television reporters. "I like having TV on the scene because it boosts the story," said Todd Shields of *The Post*. "And if the story is on television, then Gee Whiz, your editor is likely to think it's a major story." As any newspaper reader knows, television news often follows the lead of newspapers. So television was feeding newspapers and newspapers were feeding television. The feedback loop got stronger and faster.

Television, however, does something newspapers don't. "Television is the medium that can terrify people more than print ever can," said Goodman of *The Post*. "Qualifiers tend to get blown away by the sheer power of the medium." Reporters and scientists kept saying that seafood is safe to eat, but daily images of fish with red lesions sent consumers fleeing from the fish markets.

The media war idea, like most hypotheses, has its limits. It explains much — but not everything — about the media frenzy and the panic it produced. Newspapers and television stations, after all, are always competing with each other on big stories. What was it about *Pfiesteria* that made it such a huge story?

Anthropologists think they know. They've weighed in with a different kind of hypothesis, but they don't, unfortunately, have a catchy name for it. They call it the "cultural model" hypothesis. That seems to translate into *Pfiesteria*-as-symbol-of-things-that-scare-us.

The summer of *Pfiesteria* began with sick fish, but it was sick people that made *Pfiesteria* world famous, sick people with strange symptoms. That's when the story got scary. A medical team announced that 13 people, mostly watermen and state workers, now had mental problems, problems like confusion and short-term memory loss, presumably from working around waters with *Pfiesteria* lurking below. At that point press coverage exploded again. And so did readership. Not because 13 people were afflicted — that's not a large number — but because those findings tapped into some large fears.

Call it the *Jaws* factor. Anthropologist Michael Paolisso does. "I think it's real scary to imagine something in the water," says the University of Maryland researcher, "something down there that could come up out of the water and get you." *Pfiesteria*, of course, was only a tiny dinoflagellate, not a great white shark looking for white people to eat off the shores of Martha's Vineyard. But *Pfiesteria* — like the movie shark — was hard to find, it seemed to appear and disappear at will, and it was widely described as an "ambush predator."

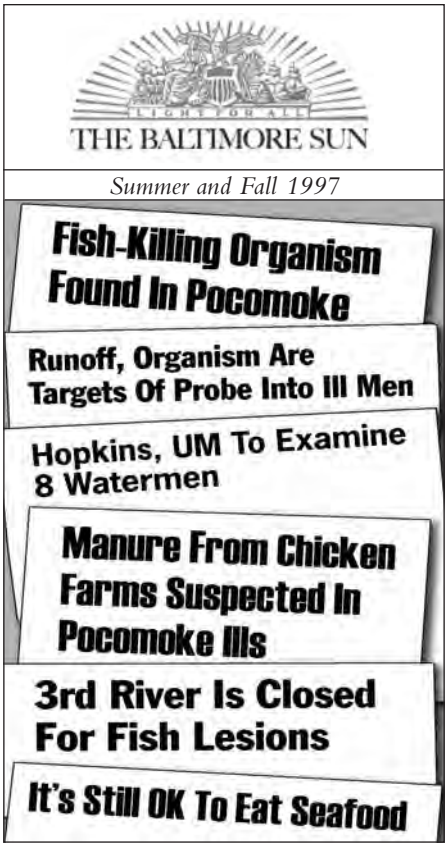
The *Jaws* factor is a good way to get a handle on the concept of "cultural models." According to anthropologists, we carry around ideas, frameworks, and mental models that help us make sense of the world, especially the scary parts of the world. "Predator" is one of those ideas that we use when we think about sharks, grizzlies, wolves, and other animals that sometimes eat people or their pets. "Cultural models" come, in part, from the culture we live in. Think of monster movies, Stephen King novels, doomsday dramas. Think of the energy jolts they give us.

What models were the media tapping into with their *Pfiesteria* stories? According to a survey by two other academics at the University of Delaware, Willett Kempton and James Falk, when people read about *Pfiesteria*, they pulled up five ideas: pollutant, poison, disease, parasite, or predator. None of these sound benign, but some (pollution, poison, predator) sound fairly scary. When press stories described *Pfiesteria* as "phantom-like," "a fish killer," "an ambush predator," and "the cell from hell," they were activating some high-energy ideas bouncing around in the back of our brains.

The sick fish story became a sick people story, and then like the cell from hell, it began to morph again into other shapes. When *Pfiesteria* blooms were blamed on chicken waste running off farms, a sick people story became a sick environment story. When legislators began holding hearings on new regulations for farming, a sick environment story became a political story. The story had to keep moving or it would die. And all these story lines unleashed jolts of fear, anger, and political action.

It was the story that wouldn't stop. Newspapers assigned science writers, environmental writers, agricultural writers, and political writers. Television stations ran pictures of pretty rivers, angry watermen, and ugly fish. Newspapers sent more writers. "Here were all these things coming together," said Rob Magnien, the DNR official who had to deal with the press on a daily basis. "Talk about a perfect storm."

In the eye of the storm, however, sat a tiny, one-celled organism, *Pfiesteria piscicida*, one species among more than 1,000 dinoflagellate species. Now through the media frenzy it had somehow morphed



into something larger. "I always thought *Pfiesteria* was more of a cultural organism than a biological one," says Paolisso. In the end it evolved into a powerful symbol of nature gone awry, of nature striking back at us — like an old movie monster lashing out against the mess that men have made of the natural world.

The storm passed. Watermen went back to work. And the media moved on, leaving scientists to wage their wars about *Pfiesteria* in the pages of technical journals. But the memories linger. The old movies, after the monster died, always left a question hanging in the mind. Could Jaws or Frankenstein or Godzilla come back again someday?

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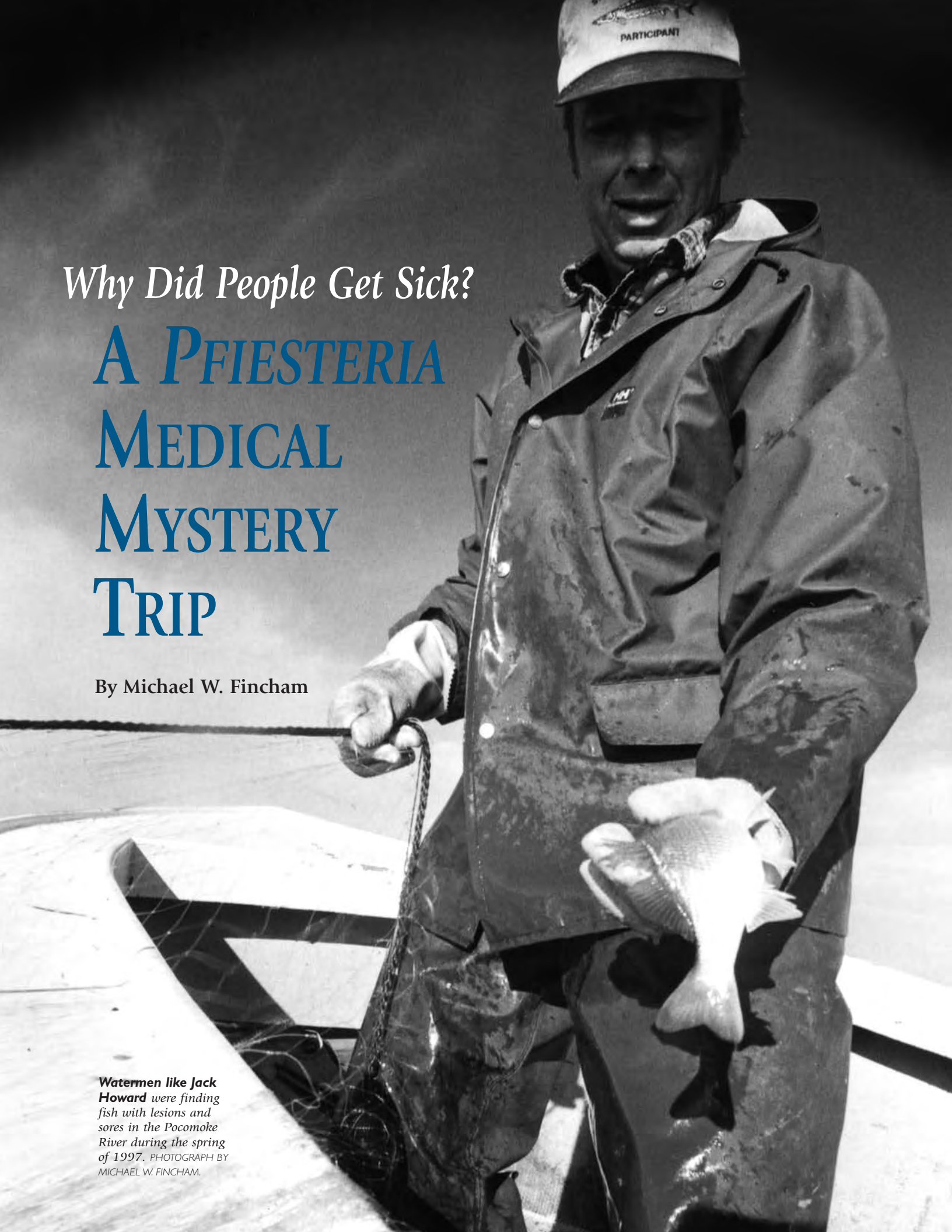
Why Did People Get Sick?

A *Pfiesteria* Medical Mystery Trip

By Michael W. Fincham



Watermen like Jack Howard *were finding fish with lesions and sores in the Pocomoke River during the spring of 1997.* PHOTOGRAPH BY MICHAEL W. FINCHAM.

Remember *Pfiesteria*?

If you do, your memory is probably fine.

Dr. Glenn Morris has no trouble remembering.

On a Friday morning in August 1997 he climbed aboard a large van in Baltimore and got ready to give his first *Pfiesteria* speech. On board with him was a team that he'd recruited from the medical schools at the University of Maryland and Johns Hopkins University. By 7:30 they were threading their way through rush hour streets headed for rural Somerset County, the last county down on the tail end of Maryland's Eastern Shore. Watermen along the Pocomoke River were telling the press they were getting sick, and a local doctor named Ritchie Shoemaker was backing them up. They thought the culprit could be a mysterious microbe in the water named *Pfiesteria piscicida*.

By the time the van hit the Bay Bridge, Morris was deep into his speech. A trim, calm-spoken professional with dark hair and a beard that was going gray, Morris leaned across the seat and gave his team their charge. "The state needs an answer," he told them. "Is there or is there not a human health effect associated with *Pfiesteria*? It's a yes or no sort of thing."

One week later, on the last Friday in August 1997, he announced the team's spectacular findings. Their answer was a Yes: Watermen and state workers exposed to the Pocomoke River had developed "profound" neurocognitive deficits, primarily problems with short-term memory.

The culprit could be *Pfiesteria piscicida*, a dinoflagellate in the river that was suspected of releasing a toxin that could cause sick fish — and now sick fishermen. Exposure to waters with *Pfiesteria* or its unnamed, look-alike cousins could be a health risk to people working, boating, or living around the Bay.

The medical report was a "bombshell" going off in newsrooms, said a reporter for *The Baltimore Sun*. "It was feast time," said a reporter for *The Washington Post*. The press responded with an explosion of coverage that churned out thousands of newspaper and television stories over the next year. The media barrage led to public confusion, political controversy, and economic loss. Charter boat captains lost customers. Tourists stayed home. Swimmers stayed off the beaches. Many anglers and boaters stayed off the Bay, as did some water-skiers and windsurfers and sailors. Sales of Maryland seafood plummeted, causing an estimated $40 million in lost income.

Michael W. Fincham

Back in 1997, Glenn Morris had three days *to recruit a team of doctors and one neuropsychologist from the medical schools of the University of Maryland and Johns Hopkins University. Their job: examine watermen and state workers suffering odd symptoms from, they thought, exposure to the Pocomoke River. Was there a health effect caused by* Pfiesteria *or some other biotoxin lurking in the river?*

Michael W. Fincham

Tommy East motored out of a small harbor in Shelltown in an open boat and headed down the Pocomoke River in the pre-dawn twilight. His early morning commute — in good weather, at least — was enviable. Under a brightening sky, he glided past woodlands, scattered homes and farms, and then broad stretches of tidal marsh.

Three miles downstream, where the river widens into Pocomoke Sound, East went to work as the sun came up, wrestling with a dip net to pull fish out of large, roped-off fish pounds. He usually dumped shad and perch into his boat along with croaker and stripers. Ten years ago he kept finding fish — a lot of fish — with ugly red lesions. He would drop to his knees in the bottom of the boat and start throwing sick fish overboard.

Last year, in 2006, after a decade of follow-up research, Morris announced another spectacular finding. But now his answer was a No. "The bottom line was we could find no evidence that having *Pfiesteria* in the water was having any effect on health," said Morris. Exposure to waters with *Pfiesteria* — in the Pocomoke or any other river in the Chesapeake — was probably not a threat to people who live or work around the water.

This kind of "good news" medical report was not a bombshell in any newsroom. *The Baltimore Sun*, which once carried over 170 *Pfiesteria* stories in one year, now gave the new report one short article. *The Washington Post*, which carried over 130 stories in one year, competing neck and neck with the *Sun*, decided to give the report one (even shorter) story. Reporters and editors who once feasted on the "bad news" about *Pfiesteria* seemed to have a long-term memory deficit.

The new medical finding, though barely mentioned in the press, begs this question: How did a Yes turn into a No?

And this question also: If *Pfiesteria* is not a serious threat, then what really happened ten years ago on the Pocomoke River?

The Road to Shelltown

As the big state van carried his medical team south down the Eastern Shore, Glenn Morris looked out the window and saw a television truck also headed for Somerset County. The press had immediately played up the idea of a medical dream team from the big city invading rural Maryland, ready to give locals what one reporter called "the mother of all medical exams." The exam would answer the question: Was something in the river making people sick?

The Pfiesteria Files

For much of the spring Tom East kept throwing away 30 to 40 percent of his catch. And he kept getting sick. A waterman with pale red hair, a healthy build, and a history of hard work, East found himself struggling with headaches, a crampy stomach, and breathing troubles. The watermen working with him, could see he was losing weight.

He was also losing his memory. He could remember jobs from 10 years ago, but he couldn't remember conversations from 10 minutes ago. In the middle of a job — cleaning a boat or repairing an engine or patching a net — he would pull up short like a boxer taking a rib shot and suddenly go blank. He'd stand there wondering what he was doing. He'd never been like that before.

Morris thought he already knew the answer — and it was No. As chairman of the Department of Epidemiology at the University of Maryland School of Medicine, he had studied the existing evidence about *Pfiesteria piscicida*, a dinoflagellate so small it could only be seen under a microscope. In North Carolina, the microbe had been blamed for killing fish and for making scientists sick during laboratory experiments. It had been found several times in Maryland waters, but only once around fish nets with sick-looking, lesioned fish. Watermen, prodded by the press, said *Pfiesteria* might also be making them sick. Morris, however, thought the health problem was probably an episode of mass panic. This might simply be "*Pfiesteria* hysteria," he told his van load of doctors.

Pfiesteria and other Hysterias

"*Pfiesteria* hysteria" became a popular catch phrase in the summer of 1997, both with the press and the general public, often as a way of dismissing watermen's worries as simple mass hysteria. In the growing medical literature, however, mass hysteria is far from simple, either as a definition or a diagnosis. It's usually called "mass psychogenic illness," a term that describes episodes where groups of people develop the same physical symptoms — but where medical exams reveal no organic causes for those symptoms.

When professionals talk of "mass psychogenic illness," they are never being dismissive. "It is very difficult to explain to people affected by mass psychogenic illness that they aren't crazy, that they aren't imagining their symptoms," says Tim Jones, an epidemiologist and author of a report in the *New England Journal of Medicine*. "These are real symptoms," he adds. "They are not malingering or making this stuff up to get out of work." Pains with no physical cause are still pains. Disabilities are still disabilities.

The history of mass psychogenic illness stretches back to the Middle Ages with hundreds of accounts of demonic possession, often striking nuns in convents or suspected witches living in villages like Salem, Massachusetts. The preferred treatments in the past were exorcism or execution. In recent centuries, mass episodes have been reported in factories and cotton mills and more frequently in schools — many of them girls' schools — in Europe, America, Asia, and the Middle East.

Ancient ideas about demons have now given way to fears about toxics in our food, our water, and our air, fears often triggered by a strange odor. Since the gas warfare of the First World War, fears of toxic gasses — rather than any actual gasses — have been implicated in a number of prominent mass illnesses. After the anthrax episodes that followed 9/11, an epidemic of mysterious rashes showed up in elementary schools in half a dozen states around this country.

As a working hypothesis for the complaints along the Pocomoke, "*Pfiesteria* hysteria" made a certain amount of sense. The illness usually takes one of two forms, according to Simon Wessley, a psychiatrist who has written widely on the disease. "Motor hysteria" includes twitches and spasms and strange speech, the kind of symptoms once found among nuns and witches. A second form called "anxiety hysteria" includes headaches, dizziness, nausea, shortness of breath, and general weakness — and nearly all these symptoms were cited by watermen exposed to the Pocomoke River.

The medical expedition that Glenn Morris was leading down to Somerset

County was, in many ways, a classic contemporary response to a potential mass hysteria event. According to Tim Jones, writing in the *American Family Physician*, the best ways to halt mass psychogenic illness are to perform medical exams, provide credible reassurances from respected authorities, and spread the word there is no health problem. "Our major objectives," Morris told his team during the bus ride, "are to try to relieve anxiety and reassure everybody that everything is fine."

When Glenn Morris and his team arrived at the Somerset County Health Department, nearly twenty television trucks were in the parking lot pointing satellite dishes at the skies, and reporters and cameramen outnumbered patients nearly four to one. Morris began a day of medical exams by putting on his white doctor's coat and holding a press conference.

He told the press throng that the doctors came from both the University of Maryland and Johns Hopkins University (only one physician represented Hopkins). The two-school strategy was the idea of Marty Wasserman, then Secretary for Health and Mental Hygiene, who said he didn't want one Maryland medical school criticizing the other in the press. Often blamed in the medical literature for extending mass hysteria, the press could now perhaps help in spreading the word. A clean bill of health from a two-school team — widely reported in the press — could help calm the mounting anger and panic along the Pocomoke.

By the time he'd talked to five patents, Morris began to change his mind about his hysteria hypothesis. Medical care begins with narrative, with the patient's oral history of his problems, and all the examining doctors kept hearing story after story about forgetfulness and mental confusion.

The medical team examined 13 people that first day in Somerset County: seven watermen, the wife of a waterman, four state workers and one water-skier — and nearly all of them reported the same odd symptoms. There's something going on here, thought Morris.

The Pfiesteria Files

John Rafter put his kayak in the water and paddled out into the Pocomoke River. Rafter was not a waterman, but a field technician with Maryland's Department of the Environment, and one of his jobs was to take water samples near the sewage outflows for Pocomoke City. The river is narrower there, miles upriver from Shelltown. Gliding out to the middle, Rafter put his paddle down, picked up a sampling bottle and slid his bare arm down into the water up to his elbow. During lunch time at the* Pizza Hut *in town, he suddenly went blank. He walked out of the rest room, then simply froze. He had no clue, no memory of where he was.

When he went to bed that night, he suddenly felt like his head was in free fall, sinking down through the pillow, the mattress, the floor. The next morning when he woke up he was totally disoriented. His girlfriend drove him to an emergency room. He thought he had a brain tumor.

The Case for Yes

Lynn Grattan climbed on the van that August morning, carrying briefcases full of mind games. A neuropsychologist with the University of Maryland School of Medicine, she would be screening for any brain misfunctions that might lie behind all those odd stories of memory lapses. Her job was to use science to investigate storytelling — and she had 14 tests ready.

Grattan culled these tests from the array of psychometric tests used to uncover unusual brain pathologies. Some screens could assess language and visual/spatial abilities. Others could test cross-brain connections and problems with concentration and divided attention. Several could probe memory and mood, including anxiety and depression. Many of the tests had questions designed to dig out symptom faking and exaggeration.

As she worked through the day Grattan was not listening closely to the stories her patients told. She spent her time setting up tests, asking questions, recording answers, measuring response times — in short, taking data. She wouldn't make much sense of that data until she could go back and score all the tests.

By the time she climbed on the van for the ride home, all the doctors were buzzing with talk about the stories they'd heard. Like university researchers everywhere they were jazzed by the idea of a new discovery, a new syndrome for the medical literature. It's one of the reasons you do science, Morris liked to say, finding something new. They kept the lights on in the bus, tabulating data all the way back on the long drive up the Eastern Shore and across the Bay Bridge.

The only one who wasn't talking much was Grattan. As the neuropsychologist for the group, she didn't want to say anything until she could work through all her data.

Lynn Grattan sat down at her kitchen table at 5 a.m. the next morning, a Saturday, and went to work scoring her tests. By early afternoon she was able to call Morris, the team leader, with some disturbing findings. Out of 14 screening tests, nearly everyone was performing poorly on the same three tests.

The Stroop Color-Word Test was one. It starts with a list of words, each naming a color, but then adds a twist: The word "red" might be printed in the color green, while the word "green" might be printed as purple. "What's that color? she would ask, pointing at the word "green." The correct answer, of course, is purple, but it's easy to feel a disconnect, to hesitate. If you hesitate a lot, you could have a

slow connection between the right brain (which processes color) and the left brain (which handles words). You could have a problem with divided attention.

A second test — called the Grooved Peg Board Test — also measured cross brain connections, concentration, analytical skills as well as manual dexterity — all on a timed trial.

The strongest findings came from the simplest test. With Rey's Auditory Learning Test, Grattan would show her patients simple, unrelated words (BELT, RAIL, FORK, INCH) one at a time — then ask them to recall as many words as they could. Later in the evaluation, she would suddenly ask her subjects to recall the first list again.

The scores on this widely used test were startling. When she checked the normative data base for this test, a collection of scores from hundreds of thousands of people of similar age and occupation who had answered the same questions, she found nearly everybody scored better — much better than the people from the Pocomoke. Those with moderate exposure to the Pocomoke were scoring near the bottom eight percent on word recall. Those with high, nearly daily exposure to fishing areas along the Pocomoke were scoring in the lowest two percent for their age and occupation.

Grattan told Morris she was seeing evidence of a clear, consistent pattern of cognitive deficits. The Pocomoke patients had problems with divided attention, response inhibition, and especially short-term memory. For most of us, short-term memory is the engine for new learning, the mental machinery that takes in new information — whether from a conversation, a phone call, a newspaper. She thought the results were frightening.

If it was "a Yes or No sort of question," then the answer was a Yes: There did seem to be a health effect from *Pfiesteria* or some other toxin in the water.

The Decision Dilemma

When Glenn Morris hung up the phone, he knew he was facing a classic public health dilemma.

Michael W. Fincham

Tim Murray was diving in the Wicomico three rivers north of the Pocomoke. Crew cut, stocky, and well-muscled, Murray was replacing X beams on piers at a yacht club. Working in a dive suit, he wore a heavy helmet attached to an air hose, but he kept losing his breath — not just under the water but up on land. In the middle of eating a sandwich, he'd have to stop to catch his breath. He also began losing weight.

His girlfriend told him he was also losing his memory. He finally noticed his memory problem when it slowed down his work. Looking for a tool, he would climb out of the water, wrestle his massive diving helmet off and clump over to his step van. Standing there, staring at his tool racks, he would completely blank out. He thought he was losing his mind.

Do you take action before you know everything about the threat? Or do you wait for more evidence, at the risk of exposing more people to the threat? If you take action, do you cause other, greater problems?

Morris solved his quandary with a question: Would I let my daughter go swimming in the Pocomoke? When he realized the answer was No, he gave Wasserman and other state officials the answer nobody wanted to hear: Exposure to river waters associated with sick fish and *Pfiesteria*-like organisms was somehow causing short-term memory loss. The press and the public and the politicians could drop the mass hysteria hypothesis.

Faced with this kind of finding from this kind of medical team, Maryland Governor Parris N. Glendening had little choice. On the Friday before Labor Day weekend, he called a major press conference in Annapolis. "The public must be informed of this connection to human health," he announced, and then — because of the health threat — he closed seven miles of the Pocomoke River. "There is," he added, "no reason to panic."

What followed looked a lot like a low-level panic. Newspaper and television reporters now drove down by the dozens from Washington and Baltimore and began wandering the backcountry roads around Shelltown and Nanticoke looking for locals who might be suffering memory loss. All those reporters were quickly turning *Pfiesteria* in the Pocomoke into a national story. More reporters then flew in from New York, Atlanta, Europe, and Asia, turning *Pfiesteria* into an international story.

The dream team strategy had backfired. Alerted by the press, a second wave of potential patients called in to hot lines set up by the State Department of Health and Mental Hygiene and state officials were soon listing 31 diagnosed cases.

On The Road Again

For David Oldach, crossing the Bay Bridge was usually a homecoming — but not today. He grew up in the Eastern Shore town of Salisbury, but today he was driving down to West Ocean City and Chincoteague, oceanside fishing ports where he used to work on scallop boats and ocean clam boats. Slim, articulate, and confident, Oldach was an infectious disease doctor who had talked his way onto the *Pfiesteria* medical team, and today he had Lynn Grattan riding shotgun in his Jeep Cherokee hauling her briefcase of tests. Oldach knew there were holes in the medical team's findings and he was hoping they could patch one of them.

Thirteen people showing memory problems on three tests taken on one day. Could these medical findings be a fluke? Was this enough evidence to close down a river, kick off a media blitz, and perhaps

cause a panic? Could the science case hold up?

Here was one hole in their science: The findings about Pocomoke watermen had no control group study to back it. Perhaps the memory problems among watermen were not caused by a biotoxin. Perhaps commercial fishermen everywhere — not just watermen on the Pocomoke — were more likely to score poorly on tests for memory and mental agility. Perhaps they were affected by antifouling paints or diesel fumes or long days on the water. Perhaps they were simply poor test takers.

Oldach was headed for West Ocean City because he thought they could find the perfect control group. Dozens of commercial fishermen motored out everyday headed out for fishing waters well beyond the beaches and boardwalks and high-rise hotels of Ocean City. He knew from experience they worked as hard as watermen on the Pocomoke — but had no exposure to the Pocomoke or other estuarine waters with *Pfiesteria*-like dinoflagellates that might cause memory problems. Screening a group of these fishermen could verify (or falsify) their findings about a *Pfiesteria*-connected health problem.

The Pfiesteria Files

The Pfiesteria Files

Responding to the threat to human health *during the* Pfiesteria *crisis, David Oldach (above) developed a gene probe for quickly identifying* Pfiesteria *DNA. Working with team members like Holly Bowers (below), they found* Pfiesteria *in water and sediment in many Chesapeake Bay rivers.*

To test fishermen, they first had to find them. When they drove up to the work docks at West Ocean City, Grattan, at least, was still nervous. They had no hotel rooms booked and no testing appointments lined up. What they had was Oldach's optimism and chutzpah. When the boats pulled up, he began walking the docks asking fishermen, most of them back from a long work day, if they could hang around for another hour or more. He wanted them to sit for a medical interview evaluating their medical status and then take neuropsychological tests measuring their mental status.

His straightforward approach worked. None of the fishermen were happy about the *Pfiesteria* controversy, but nearly all of them agreed to testing. Grattan was able to give eight ocean fishermen the same screens and mind games she gave the Pocomoke watermen. While the tests were the same, the results were different. The ocean fishermen scored remarkably well on the same tests that befuddled the Bayside watermen. Something peculiar to the Pocomoke must be causing memory loss among watermen and state workers.

With findings from the control study, the case for Yes, the science case for a *Pfiesteria* health syndrome, got a little stronger.

Pfiesteria in the Bay

There were other holes in the science case: Where in the Bay was *Pfiesteria* living? What kind of toxin, if any, did it make? What was the route of exposure? Were there other toxic-forming dinoflagellates out there? Elsewhere in the country, other dinoflagellates were known to cause devastating diseases like Paralytic Shellfish Poisoning, Neurotoxic Shellfish Poisoning and, more recently, Amnesiac Shellfish Poisoning. Was *Pfiesteria* the new toxic threat?

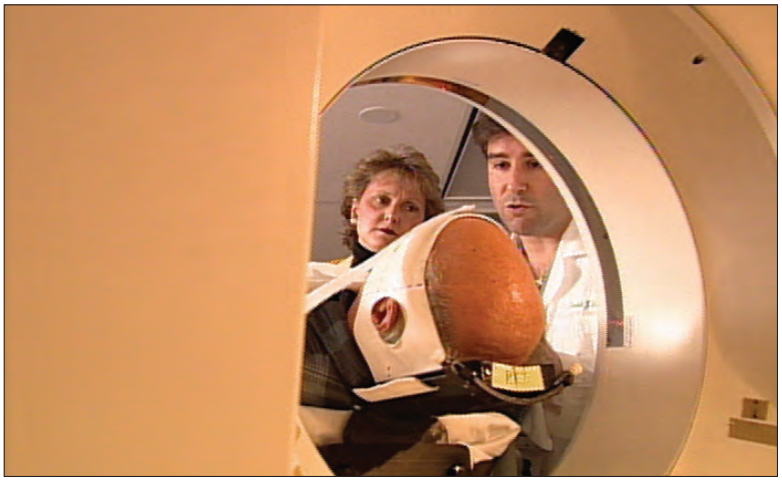
Flush with that first excitement of a new discovery, the medical team went after the illness question with high hopes and research smarts. Led by Morris, Oldach, and Grattan, the team went into the medical tool box of the time and pulled out a number of techniques, some classic, some cutting edge. In addition to control studies, they tried brain scans, rat studies, cohort studies, and a new, high-tech gene probe. Not every question, they found, had an answer.

Development of the gene probe by Oldach was an early breakthrough. His technique could find *Pfiesteria* and find it fast, primarily by detecting its DNA fingerprint in water and sediment. As a medical researcher at the Institute of Human Virology, a high-tech department of the University of Maryland Biotechnology Institute, Oldach was familiar with new techniques that doctors were using to track fast-changing viruses in the human body. By collaborating with aquatic biolo-

gists in Maryland, North Carolina, and Norway, he was able to adapt these medical techniques and create a probe that marine scientists could use to track dinoflagellates in water bodies like the Chesapeake Bay.

The probe produced an immediate and practical payoff: *Pfiesteria* could now be identified in hours instead of weeks. When state workers began using it, however, they came up with an unwelcome payoff. They began detecting the DNA of *Pfiesteria* nearly all over the Bay.

That discovery raised the stakes for everybody. *Pfiesteria* was not some rare, perhaps alien species that somehow snuck into the Pocomoke and a few distant Eastern Shore rivers. The organism was clearly at home all around the Chesapeake. What showed up in the water down in the Pocomoke could show up in Baltimore Harbor.



The Pfiesteria Files

Looking for evidence of how a biotoxin, *possibly from* Pfiesteria*, could affect the human brain, Lynn Grattan, a neuropsychologist, supervises a PET scan, a picture of the brain that uses Positron Emission Tomography. The PET scans held clues but no conclusions that could be published in the scientific literature. "The more we looked at them, the less sure we were about what they meant," says Glenn Morris, leader of the medical team.*

Pfiesteria on the Brain

When Glenn Morris and his medical team climbed aboard the van that muggy August morning, they were embarking unknowingly on a 10-year road trip full of discoveries, unexpected detours, and more than a few dead ends. The destination seemed clear enough at first — figure out what was making people sick — but over the next decade the goal seemed to retreat down the road before them like a water mirage disappearing in the distance.

Some holes in their science case are still there. What happened, for example, in the brains of people exposed to a toxin from the Pocomoke, a toxin that chemists struggled for over a decade to identify. Without a toxin sample to work with, the medical team tried techniques like high-tech scans of human brains and lab studies of rat brains, but the best they got from all this work were hints and hypotheses for further testing.

From the brain scans came hints that the Pocomoke toxin may slow down brain areas like the hippocampus, one of the sites for short-term memory. From the rat brain studies came evidence the toxin may turn off a neuroreceptor (the NMDA receptor) that receives signals aimed at the hippocampus and other memory structures. Short-term memory loss in that case could come from poor communication at the cellular level in the brain, according to Morris. New information comes in but new memory is never laid down. As far as your brain is concerned, the job you just started, the conversation you just had, this article you've been reading — all those events never happened.

Short-term memory loss was, luckily, not a long-term event for people along the Pocomoke. When they were retested, three months after their exposures, most of the Pocomoke patients were again scoring within normal range on their memory tests, and at six months all the patients had improved enough to bring their scores within national norms for their age and gender. Whether they all came all the way back, however, remains another unanswered question.

The Best Laid Plans

Did *Pfiesteria* pose a major public health threat? With *Pfiesteria* DNA turning up all around the Bay, the Morris-led team pinned their highest hopes for a clear answer on a classic research tool called a "cohort study," an epidemiological study designed to give a definitive "Yes or No" sort of answer.

The basic idea was a before-and-after study tracking a group of watermen, a "cohort" that was likely to be exposed to water with fish and *Pfiesteria*. Over four years, they would test watermen at the beginning of each work season, creating a baseline on their medical status, much as your family doctor does with your annual physical. By retesting at the end of the season — and during the season if anyone was encountering toxic blooms or developing symptoms — the team could see any changes pointing to a *Pfiesteria*-like health effect. The expected results should be the cleanest data yet on how *Pfiesteria* affected anybody working the Bay.

The unexpected result, however, was four years with no documented toxic *Pfiesteria* blooms, no large fish kill episodes, no reports of new episodes of neurocognitive deficits. Fish kills occurred in the Bay, but none of them broke out near the watermen in the study group. *Pfiesteria* might be all over the Bay, but it seemed to have stopped forming toxic blooms.

That was bad news for the researchers, but good news for the Bay's watermen, state workers, and waterskiers. No new cases came from this cohort group, not even from all those watermen clocking heavy work weeks on the water. The study began with 123 test-case watermen and finished up with 88 who hung in for four years of twice-yearly testing and biweekly reporting. During those years

state officials and watermen took 3,500 samples from the waters they were working, and *Pfiesteria* showed up in 110 or 3.2 percent of the samples.

Those are samples large enough for strong conclusions, and the strongest, according to Morris, is that *Pfiesteria* is not a health threat to working watermen — or probably to anybody else. "For the average waterman or Sunday boater," says Morris, "I would say with a high degree of confidence, there is no risk."

That "no risk" finding — based on 88 people — has drawn criticism, especially from Ritchie Shoemaker, the doctor from Pocomoke City who first began diagnosing a *Pfiesteria*-like syndrome back in 1997. Six dropouts from the cohort study came to see Shoemaker, claiming they were sick with classic symptoms of toxic exposure. "Seven percent of the people they followed got sick," says Shoemaker. The cohort study, he also points out, failed to track recreational boaters and waterside residents. Shoemaker estimates 20 people have come to him over the last six years with symptoms from low-level exposures to toxins in the water.

Over the last decade, however, the estimate of cases from Maryland waters remained low. The question that Morris and his team first set out to answer — Is there a major public health risk? — seems to have a new answer. Nine years after 1997, his Yes answer had become a No.

The End of the Road

That answer leaves a large question: whatever happened to the watermen and state workers on the Pocomoke River?

"*Pfiesteria* ten years ago still remains an intriguing and puzzling mystery." says Morris, sitting in his office in Baltimore still looking trim and calm, if slightly grayer, his high hopes dwindling into some humility in the face of the unexplained. "It is very frustrating in today's world with the sophistication of science today not to be able to explain what happened."

A lot of science happened over the last decade, he points out, and some of it pro-

Continued

How Many People Got Sick?

Illness Is Real, But Rare

The syndrome never had a name that stuck for long. It's been called "the *Pfiesteria* thing," the Pocomoke syndrome, the Estuarine-Associated Syndrome, and finally the Possible Estuary-Associated Syndrome, or PEAS.

It started with 13 cases from the Pocomoke River, a number that would never grow much larger over the years, in part because the definition of the disease was never very clear. Ten years later, it's not even clear how many people in Maryland really got sick.

In August 1997, eight watermen, four state workers, and a housewife from the Pocomoke River were all diagnosed with odd mental symptoms including concentration problems and loss of short-term memory. The diagnosis came from a medical team recruited from the University of Maryland and Johns Hopkins University and led by Dr. Glenn Morris.

Something in the water seemed to be making them sick, and the suspected cause was exposure to waters with sick fish and with some kind of toxin — perhaps released by *Pfiesteria* or by other dinoflagellates that could be releasing a toxin in the water.

Scientists, however, were unable to identify a toxin, so officials with the U.S. Centers for Disease Control (CDC) chose not to label this as a *Pfiesteria*-related syndrome. They first defined these Pocomoke cases as Estuary-Associated Syndrome (EAS) and then as Possible Estuary-Associated Syndrome (PEAS). They also asked states along the coast to expand their existing surveillance systems and to report back on people affected by PEAS-like symptoms.

So how many people over 10 years have gotten sick with this syndrome? Neither state nor federal agencies have an official count. "The surveillance system for PEAS kind of fell apart," says Lorraine Backer, of the CDC. "There wasn't a lot going on, and the states just stopped reporting to us," she says. "So we don't really have a good handle on what the final vetted numbers were for Maryland."

The unofficial numbers seem to be: not very many. Current estimates come from two Maryland doctors who first diagnosed sick watermen in 1997 and now disagree about the nature of the diagnosis and the number of people affected.

If you listen to Ritchie Shoemaker, the outspoken family practice physician from Pocomoke City, you get a number somewhere north of 50 cases. He lists 37 cases he diagnosed for a 2001 research study, and he estimates he has since then treated an additional 20 PEAS patients. That brings his total to 57 cases, not a large number spread over 10 years.

If you listen to Glenn Morris, the leader of the university-based research team, you get a lower estimate, somewhere south of 30. In 1998, the university team published a study listing only 19 cases from exposures in Maryland waters. Since then, the team has diagnosed more cases, but Morris now has his doubts that all those new cases came from exposure to a toxic algal bloom. "What I would be most comfortable saying is that there were less than a dozen (new cases)," he now says. "And it could be zero."

So whom do you listen to? The small-town doctor or the university researcher? It was Ritchie Shoemaker, ironically enough, who first pushed the medical establishment to examine those watermen who said the river was making them sick. A decade later, Shoemaker is still pushing to publicize the syndrome, but now he is pushing against Morris and the university team that originally confirmed his claims.

Their disagreements center on disease definition and diagnostic technique. For his diagnosis Shoemaker relies on an interview, a series of lab tests, and an eye test called Visual Contrast Sensitivity (VCS). Morris prefers neurocognitve testing, but he has his doubts about any diagnostic tool, including VCS. Until a toxin is purified and tested, there will be no gold standard for identifying its presence in the region's rivers or in a person's bloodstream. "There isn't a definitive diagnostic test," says Morris. "It's not like you can draw a blood sample and say, 'Yep, he's got it.' "

Absence of evidence, of course, is not evidence of absence. Toxic blooms do break out and people can get sick. According to Morris, the university researcher, the Pocomoke syndrome — whatever the cause — is real, but rare. According to Shoemaker, the small-town researcher, even a single case of illness is worrisome: it means others can become ill also and never realize the river could be making them sick.

— M.W.F.

Comparison of Diseases in Maryland, 1997-2006

Disease	# Cases Reported
Legionellosis (caused by aquatic bacterium)	671
Lyme Disease	7692
Rabies	4090
PEAS (Possible Estuarine-Associated Syndrome)	<30 to 57?

Source: Legionellosis, Lyme Disease and Rabies, tallied from cases reported by the Centers for Disease Control (CDC); Possible Estuarine-Associated Syndrome (PEAS), estimated from cases cited in articles published in Lancet *and Environmental Health Perspectives and on interviews with Glenn Morris, University of Maryland School of Medicine and Ritchie Shoemaker, family practice physician, Pocomoke City.*

Does Pfiesteria Produce a Toxin?

Researchers Track Elusive Chemical

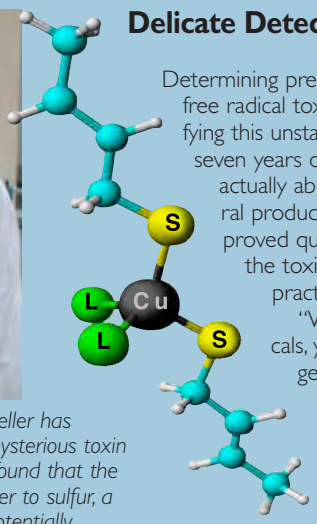
By Erica Goldman

Scientists know that *Pfiesteria* feeds voraciously on blood and tissue — not to mention other algae — but is it toxic? Does it produce a toxin that it releases in the water, a poison that not only stuns microscopic prey but that might kill fish and possibly sicken people?

Showing whether *Pfiesteria* produces a toxin has proved no simple matter. Time and time again over the years, different labs reported finding toxic activity in the water of their *Pfiesteria* cultures. But the substance would seemingly vanish before chemists could lay their hands on it. Frustratingly elusive, the suspected toxin appeared either nonexistent or a master of deception.

Although they still don't know what role, if any, it played in specific fish kills, scientists have now caught a toxic culprit — at least part of it. Earlier this year, a team led by natural products chemist Peter Moeller of the National Oceanic and Atmospheric Administration's (NOAA) National Ocean Service in Charleston, South Carolina identified a compound with toxic activity produced by *Pfiesteria*. They published their findings in the January 2007 issue of *Environmental Science & Technology*.

Peter Moeller



Over seven years, *NOAA's Peter Moeller has worked hard to pin down* Pfiesteria's *mysterious toxin (molecule pictured at right). His team found that the toxin forms when* Pfiesteria *binds copper to sulfur, a pathway that unleashes a fierce and potentially destructive free radical cascade in the process.*

Moeller's team found that the toxic compound made by *Pfiesteria* contains copper and sulfur and that it produces free radicals, highly reactive and unstable atoms or groups of atoms sometimes described as a sort of molecular welding torch. In animal tissues, free radicals can wreak havoc on cells and may speed the course of cancer, cardiovascular disease, and age-related diseases.

The newly identified compound produced by *Pfiesteria* represents the first time a free radical mechanism of toxicity has been identified in the marine environment. In the terrestrial world, Moeller notes, there are several examples. Scientists have linked a food contaminant called ochratoxin, a free radical compound produced by a few species of fungi, to kidney damage, birth defects, and cancer. Researchers have also discovered that bleomycin, a compound with free radical properties produced by the bacterium *Streptomyces verticillus*, can be used as a powerful chemotherapy agent.

How does *Pfiesteria* produce this free radical toxin? And why? Moeller explains that copper could hold the key to both the "how" and the "why." Copper is toxic to phytoplankton, even in small amounts. *Pfiesteria* binds copper tightly to sulfur, locking it up, but in doing so produces a toxic chemical reaction (see "The Copper Connection," p. 18).

Moeller suspects that the *Pfiesteria*'s toxic compound forms as a direct response to copper in the environment and may have evolved to protect the dinoflagellate from cellular harm.

To determine why any given harmful algal species produces a toxin is quite tricky, says Alan Lewitus, a long-time *Pfiesteria* researcher and now a program manager for NOAA who helps to administer the Ecology and Oceanography of Harmful Algal Blooms (ECOHAB) grant program. According to Lewitus and others, while *Pfiesteria* may have evolved the ability to make this chemical in order to remove metals from its environment, the resulting toxin may also play an important role in inhibiting competitors or predators. This would make it a trait that evolution would select for in a seemingly purposeful manner.

"It's funny how nature and evolution work," Lewitus says.

Delicate Detective Work

Determining precisely how *Pfiesteria* produces this free radical toxin is still a long way off. Just identifying this unstable compound took Moeller's lab seven years of painstaking work. Seven years is actually about average for a discovery in natural products chemistry, but the work itself proved quite tricky, Moeller explains, because the toxin degraded so dynamically — practically in real time.

"When you're dealing with free radicals, you're dealing with a moving target," says Moeller. "I like to jokingly invoke the Heisenberg uncertainty principle — once you pin down one aspect of it, the rest has changed. That is what free radicals do."

Moeller's team worked under red light to slow the toxin's disappearing act long enough to study it. Moeller knew from previous work with free radicals that white light makes them even less stable. By removing most of the wavelengths that make up white light, the researchers gained a little more time, an extra day or two, to prepare the samples for the Nuclear Magnetic Resonance (NMR) spectrometer, an instrument that analyzes a molecule's structure using a strong magnetic field.

Early in the game the team suspected that a metal such as copper was involved in the molecule's structure. Mass spectral analysis, which identifies molecular structure based on mass and charge, kept showing a substance that was many times heavier than what the NMR data predicted. Moeller implicated metals early on because the substance appeared very water soluble and not lipophilic (fat-loving), both properties of metals. In addition, the mass of the compound fluctuated, a characteristic common to metal complexes.

But the larger community of harmful algal bloom (HAB) researchers was skeptical. "They kept telling me, 'No, no we don't have metals. Those things don't exist. They are not important,' " Moeller says. "So I let it go for a couple of years, looking for different things. But we kept isolating the same substances. Every time."

So the team moved forward and performed a process called sublimation. Taking samples from a *Pfiesteria* bloom in the Neuse River in North Carolina, they removed the water and under a vacuum evaporated the salts that were left. What they found was more copper than could be accounted for in natural seawater. That gave a pretty good clue that they were looking for copper in the structure of the toxin.

To actually characterize the structure of this mystery molecule, Moeller grew *Pfiesteria* in mass culture to provide toxic extracts for further analyses. To start this culture he took Neuse River *Pfiesteria* cells and cultured them in seawater from the open ocean, rather than from shallow estuarine water where the dinoflagellate is typically active. Why? Because ocean water is less complicated to analyze chemically. The open ocean of the Gulf Stream is not as rich in organic and other contaminating matter, making it easier and faster to purify the compound of interest.

But in the end, choosing to culture *Pfiesteria* from the open ocean may have made things more complicated for Moeller. In Moeller's proposed structure, he suggests that the molecule contains ligands — atoms or smaller organic molecules that act like counterweights to stabilize the copper atom. These ballast-like units are small in Moeller's pro-

posed structure, but in the organic soup of an estuary, one might suspect that they would be much bigger and more varied.

Moeller has taken criticism on this point and he admits that the exact nature of these molecular counterweights remains unknown. In his view, if they turn out to be longer than he's predicting — as might be the case in estuarine waters — the toxin might prove even more potent. This is because longer ligands could stabilize free radicals better, and the more time these free radicals hang around the more toxic they could become. He's careful to add that "we still don't know what this will mean in the wild."

Fueling Debate

The discovery by Moeller and his team answers one important question about arguably the world's most notorious dinoflagellate. *Pfiesteria* can produce a compound that exhibits toxic activity.

Other questions still remain. Has the burden of proof been met to definitively identify the *Pfiesteria* toxin? Could *Pfiesteria* produce this toxin in sufficient quantities — and would the toxin linger long enough — to cause fish kills? Do other marine organisms make free radical toxins?

Robert Gawley for one, a prominent organic chemist from the University of Arkansas in Fayetteville who has also studied *Pfiesteria*, believes that a new toxin has not yet been identified. "The commonly accepted criteria for chemical characterization of new compounds have not been met by Moeller's evidence," he writes in an email.

Moeller knows that there is still work to be done and a lot of questions to be answered. Free radical toxins are not common and they are new to the community of harmful algal bloom (HAB) researchers. But, he says, "I believe that we've stumbled upon a very real and very important mechanism."

NOAA's Lewitus agrees. "I think this work by Moeller puts to rest the question about whether *Pfiesteria* can potentially produce a toxin. I think that this is a discovery that will allow the field to move forward," he says. Until now, the toxin question had created a research bottleneck, Lewitus says, and this discovery will free up researchers to move on to the question of what environmental relevance this toxin might have.

Lewitus does not know yet whether the discovery will pave the way for a spate of research proposals on the environmental significance of this *Pfiesteria* toxin. He'll be one of the first to find out, though, because in his role at ECOHAB he'll see many of the proposals on harmful algae as they start to come in during the next grant competition.

Other researchers remain skeptical of the whole concept of toxic *Pfiesteria*. Al Place, a biologist at the University of Maryland Biotechnology Institute in Baltimore who studies the toxic dinoflagellate *Karlodinium*, raises the point that until this new compound can be identified in nature and until a dose-response curve for it has been developed in the laboratory — an experimentally verified relationship that shows an increasing response to increasing amounts of the chemical — we won't know what significance this molecule may have. Place also doesn't think that the free radical toxin could have remained in the water long enough to have killed fish. Instead, he suspects that *Karlodinium*, which co-occurred with *Pfiesteria* during the blooms, was more likely the culprit (see "The Case for a Toxic Culprit," p. 16).

As for Moeller, he hopes that other scientists will soon explore whether *Pfiesteria*'s free radical toxin could have played a role in large-scale fish kills. Free radical mechanisms of toxicity are new to the marine environment, and research will likely require a departure from more conventional approaches, like dose-response curves, he explains. And Moeller recognizes that working with this highly unstable chemical system will be quite difficult. He himself plans to explore whether other marine organisms, in addition to *Pfiesteria*, employ metals to produce free radical toxins. He suspects that this may be a common mechanism that dinoflagellates use to protect themselves from metals in their environment. But he hopes to move out of the *Pfiesteria* business for good. He says, "I've only got so much blood to give."

— email the author, goldman@mdsg.umd.edu

Medical Mystery Trip, *continued*

duced useful findings. The cohort study came up with a "good news" finding that is important for policy makers as well as for watermen and waterskiers and boaters. Rat studies showed evidence that *Pfiesteria* diminishes new learning, at least among rats running a maze, and they turned up clues about how toxins affect neuroreceptors.

That's good progress, but the sick-people question remains unanswered. "I have to agree with Glenn. We just don't know," says Oldach, whose DNA probe put a new technique in the tool box of marine biologists and field workers.

Both will tell you the Pocomoke syndrome was not "*Pfiesteria* hysteria," their original hypothesis. "There was no mass hysteria," says Morris. This wasn't the kind of population likely to experience mass psychogenic illness, he explains. The similar stories, the testing, the screening — all this evidence convinces him. The finding was too specific, too focused for people to fake. "There was a real event on the Pocomoke," he says, "but whether it was related to *Pfiesteria*, I have no idea."

Both scientists agree that another toxic event could happen, either in the Pocomoke or elsewhere, even though it hasn't happened again in the last 10 years. "Perhaps we have to think of *Pfiesteria* events like this as a 25-year storm," Oldach says. "Or a 100-year storm."

The *Pfiesteria* next time will probably not bring on another Pocomoke syndrome. "The Pocomoke was a unique natural experiment," says Morris. "People kept being told, 'It's okay. There's no problem. Go back. Keep working.' So we had people who were constantly being exposed to the point that there were profound deficits."

Thanks to fast findings from new DNA probes, state officials can now close a river before people suffer repeat exposures. "Ethically," says Morris, "we can never let that happen again." The uncontrolled experiment is over.

At the end of the research road then a classic Catch 22 was lying in wait for Morris and his medical team. Thanks to the progress of science, there may be no more progress on the science question that started them down the road.

Scientists may not have many more subjects to study, or more natural experiments that might help them answer the question that first took them into the field.

"I can't tell you what happened in the Pocomoke in 1997," says Morris, "And I'm not sure I'm ever going to be able to tell you."

— email the author, fincham@mdsg.umd.edu

A note on sources: Descriptions of events and reactions were drawn from personal reporting and multiple interviews conducted with participants and experts between 1997 and 2007. For a list of articles consulted, go to www.mdsg.umd.edu/Pfiesteria.

Was There Another
The

There is another scenario for the fish kills blamed on *Pfiesteria* in the Pocomoke River and other parts of the Chesapeake in the late 1990s — and for the fish kills that have happened since.

That alternate narrative emerges from the work of professor Allen Place and others at the Center of Marine Biotechnology (COMB), part of the University of Maryland Biotechnology Institute. Here is what Place thinks happened. In August 1997 an algal bloom formed at the mouth of the Pocomoke River, common in the nutrient-rich waters of the Chesapeake Bay. Most likely this crowd of algae contained what scientists call cryptophytes. Cryptophytes are not toxic. They are, in fact, a major food source for algae eaters, like menhaden.

When menhaden gathered to feed on the bloom, dinoflagellates also showed up. Not unusual. Dinoflagellates are whip-tailed single-celled organisms that may use photosynthesis to process energy from the sun, like plants, or they may feed on other organisms — or they may do both. For many feeding dinoflagellates, cryptophytes are a favorite prey.

As the menhaden fed on algae, these dinoflagellates passed through their gills. A toxin — likely evolved by dinoflagellates to help them catch their food — began to attack the gill tissue of the fish. With oxygen levels in the shallow river mouth already low, the menhaden struggled to breathe.

Then the algal bloom crashed and dissolved oxygen plummeted, consumed

Tracking sample after sample, *Allen Place turned up a toxic dinoflagellate in his laboratory that now shows up at fish kills all along the Mid-Atlantic and beyond.* PHOTOGRAPH BY MICHAEL W. FINCHAM.

Fish Killer?

Case for a Toxic Culprit

By Jack Greer

in the breakdown of dead algal cells. With their gills compromised and oxygen now scarce, the fish began to die in droves.

Dead and dying fish drew dormant *Pfiesteria* out of their cysts in the sediment and they began to feed — a process well described by scientists like JoAnn Burkholder at North Carolina State University. Living up to their reputation as the "cell from hell," *Pfiesteria* gorged on this banquet of fish tissue.

While this scenario does not differ widely from what many surmised before, in Place's version *Pfiesteria* did not kill the fish. He believes the toxin that damaged the gills of menhaden came from another dinoflagellate, one that shares *Pfiesteria's* diminutive 10-micron size, one that now goes by the name of *Karlodinium veneficum*.

Veneficum is Latin for poisonous, or in our vernacular, toxic.

Flash back to 1996, when Maryland scientists first picked up the trail of this Chesapeake Bay fish killer. The earliest clue came with a phone call to Dan Terlizzi at his home near Westminster, Maryland. Terlizzi is a Sea Grant Extension specialist and an expert on algae, water quality, and aquaculture. The phone call came from Tony Mazzaccaro, the owner of HyRock fish farm in Somerset County.

Mazzaccaro had invested heavily in a system of ponds and in a large stock of hybrid striped bass. He had had good luck in finding a market for his fish, but he was having problems with water he drew from the Manokin, a river located just north of the Pocomoke on Maryland's Eastern Shore. His stripers had gone off their feed and were swimming at the surface. It didn't sound good.

Terlizzi made the 170-mile drive down to Somerset County where he found a fish pond gone reddish brown, the color of what many call a mahogany tide. Under the simple microscope they had at HyRock he could see a dense bloom, with several species of dinoflagellates swimming around in their characteristic swirl. And the fish were suffering.

Terlizzi advised treating the pond with a pesticide, and Mazzaccaro decided to use copper sulfate, a compound he'd used successfully before. Terlizzi nodded and drove home.

By the next morning the dinoflagellates were dead. So were the fish — some 15,000 almost-market-sized hybrid striped bass. Terlizzi and Mazzaccaro needed to know what had killed them (see "The Copper Connection," p. 18).

Many of the dinoflagellates swarming under HyRock's microscope were about 10 microns or so, the size of a droplet of mist and tiny even for microscopic phytoplankton. Dinos this small look pretty much alike under a conventional microscope, so they sent samples to Wayne Coats at the Smithsonian Environmental Research Center (SERC) for further analysis. Coats identified several small dinoflagellates, including one that looked like *Gyrodinium estuariale*, originally discovered in Woods Hole, Massachusetts in the 1950s and widely assumed to be nontoxic.

Coats then forwarded the samples to a leading expert on identifying and categorizing marine dinoflagellates, Karen Steidinger at the Florida Fish and Wildlife Conservation Commission. Using an electron microscope, Steidinger detailed the physical structure of these tiny dinos, and reported that she found several species. One of them was "armored" with cellulose plates and positively identified as *Pfiesteria*, a species she had helped to describe with JoAnn Burkholder and her colleagues in North Carolina. Her micrographs also revealed a "naked" species without plates, *Gyrodinium galatheanum*, the species we now call *Karlodinium*.

While the spotlight at that time fixed on *Pfiesteria*, Terlizzi and others noted that in the HyRock fish kill cells of the species now known as *Karlodinium* outnumbered those of *Pfiesteria* a hundred to one. In a paper delivered at a conference in Vigo, Spain in June 1997 Terlizzi detailed the toxic algal bloom at HyRock. He described the presence of *Pfiesteria*, but questioned whether it had caused the fish kill. After all, the bloom had been dominated by this other species. While others remained focused on *Pfiesteria*, Terlizzi began to suspect that the other species — *Karlodinium* — might have been to blame.

Public concern about sick fish and sick people (see "A Medical Mystery Trip," p. 6) soon brought more focus and more funds to the world of dinoflagellate research. By 1998 Place and others had won a five-year grant to study toxic algae from the National Ocean and Atmospheric Administration (NOAA) and the National Institute of Environmental Health Sciences (NIEHS).

When Place put out a call for graduate students to help with the research, Jonathan Deeds saw the ad. Deeds worked at the Aberdeen Proving Grounds on microbes and water quality issues and had been contemplating a return to graduate school. He had followed the news about *Pfiesteria* and saw a

chance to serve on the front lines of scientific research. He took it. Place interviewed him and a week later hired him.

Deeds hit the ground running, but he and Place faced a serious roadblock. Though they had cultures of *Pfiesteria*, they could not readily get hold of *Pfiesteria* cultures that had been certified as toxic. What they did get were cultures of a co-occurring dinoflagellate, one quite similar in size to *Pfiesteria* that would make a good control. For them, as it turns out, this proved a remarkable piece of scientific luck and an important turning point in their story.

The dinoflagellate in question was the same one found at HyRock — *Karlodinium*. It came from Aishao Li, a graduate student at the University of Maryland Center of Environmental Science (UMCES) who worked with Diane Stoecker, a leading dinoflagellate expert. Li sent them two samples of *Karlodinium*: One came from the Chesapeake Bay and one from the deadly HyRock bloom.

When Place and Deeds set about growing and testing cultures of *Pfiesteria* and *Karlodinium*, they soon found that the apparently non-toxic cultures of *Pfiesteria* did not kill fish at normal environmental densities. But *Karlodinium*, the co-occurring dinoflagellate, did — again and again. When Deeds ran one *Karlodinium* culture lightly through a centrifuge to get a better concentrate, the resulting fluid not only killed fish larvae, it virtually "dissolved" them.

Both *Karlodinium* cultures — the one gathered from the Bay and the one taken from HyRock — proved toxic. Getting the toxin from this single-celled dino was relatively easy, since *Karlodinium* is a "naked" dinoflagellate, without cellulose plates like those of *Pfiesteria* and other "armored" species. The toxin oozes out when the cell passes through a filter or through the gills of a fish.

Place and his team now turned their attention away from *Pfiesteria* and focused on *Karlodinium*. Could this be the fish killer? They ran more experiments, using fish blood (hemolytic) assays, which proved to be very sensitive to the toxin. To dissect the purified compound, they used precise instruments that analyze the mass and color spectrum of molecules.

Place and Deeds established a dose-response curve for karlotoxin, an essential step in documenting the toxicity of any compound. They know how much toxin it takes to kill a fish, what tissues are targeted (such as gill tissue) and they can demonstrate this lethality in a matter of minutes, showing characteristic tissue damage. Their results match well the early descriptions of the toxin and its lethal effects first provided in the 1950s by British researchers Mary Parke, Dorothy Ballantine, and B.C. Abbott. These early researchers found that the toxin would kill mice in two to four minutes and a two-foot dogfish in three hours. It also caused respiratory problems for one asthmatic worker.

Fifty years ago, researchers did not have the tools now available to characterize the toxin's molecular structure. That classification depends on the sophisticated techniques of modern analytical chemistry and it requires a lot of purified toxin to work with. Place and his team would spend a year producing two milligrams, holding nearly four billion cells of *Karlodinium veneficum*. And then they would spend a second year purifying the sample.

Place eventually sent samples to two different chemists to determine the structure of karlotoxin. One sample, sent to Jeffrey Wright at the University of North Carolina at Wilmington, came from the Chesapeake Bay. The second sample, sent to Mark Hamann at the University of Mississippi, came from waters south of the Bay. The toxin in both samples had a

The Copper Connection

What causes fish kills? Lack of dissolved oxygen in the water can be one cause. Toxins emitted by certain algal blooms another. But the actual mechanism of how toxins and environmental factors like oxygen levels and water quality combine to create trouble can be complicated — and surprising.

One surprise came when fish farmer Tony Mazzaccaro added copper sulfate to control a toxic dinoflagellate bloom in his ponds. The copper, which he had used successfully before, killed his fish en masse. Why? Maryland Sea Grant aquaculture specialist Dan Terlizzi believes that the copper split open (lysed) *Karlodinium* cells that were present and released their toxin into the pond. Al Place at the UMBI Center of Marine Biotechnology agrees. They both believe that the combination of copper and a toxic *Karlodinium* bloom caused the sudden die-off.

But another surprise came when NOAA researcher Peter Moeller found that toxic events associated with *Pfiesteria* have occurred only in waters rich in metals like copper and high in sulfur. "That has held true so far in all examples I'm aware of to date," he says.

Moeller believes that *Pfiesteria* removes copper from the environment by binding it in a manner that produces an unstable "free radical" compound that's toxic to many organisms. In Moeller's view, copper sparks *Pfiesteria's* destructive chemical cascade, another scenario for what might have happened at Mazzaccaro's fish farm.

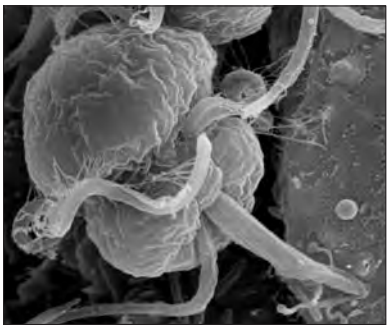
That copper has shown up at the scene of fish kills would not surprise Ritchie Shoemaker, a family practice doctor in Pocomoke City, Maryland. Since 1998, Shoemaker has argued that copper might hold the key. He noted that copper showed up in the Pocomoke and Neuse rivers, both scenes of fish kills and both agricultural watersheds. In each place farm workers applied copper to tomato plants as a fungicide or to hog and chicken feed to prevent spoilage. Rains likely washed that copper into the rivers. Maryland researchers registered 15 parts per million of copper on the Pocomoke River in 1997, says Shoemaker, an amount considered toxic.

Could the combination of copper and the two dinoflagellates, *Pfiesteria* and *Karlodinium*, create a noxious cocktail?

Moeller has considered the possibility that the two dinoflagellates might make each other more lethal when they appear together. He suspects that like *Pfiesteria*, *Karlodinium* may be sensitive to copper, making its toxin even more powerful. Place agrees that he'd like to test this possibility.

"To be honest," Place says, "I haven't tested whether the addition of copper to karlotoxin makes it more potent. I need to do that."

— J.G. and E.G



Michael W. Fincham

Focused first on Pfiesteria, *Allen Place soon identified* Karlodinium *as the producer of a potent toxin. Called a "naked" dinoflagellate,* Karlodinium *(top left) lacks the armored cellulose shell and feeding tube (peduncle) of* Pfiesteria *(lower left). Both single-celled organisms are about the size of a droplet of mist (10 microns).* MICROGRAPHS OF KARLODINIUM AND PFIESTERIA BY VINCE LOVKO, VIRGINIA INSTITUTE OF MARINE SCIENCE.

structure resembling another class of marine toxins called Amphidinols, metabolites produced by a marine dinoflagellate of the genus *Amphidinium* — a group previously associated with fish kills.

Place believes he can now describe how the toxin works at the cellular level. Think of a cell as a tiny battery, he says, with its electrical charge isolated from its surroundings by a membrane (the cell wall). Karlotoxin destabilizes the cell's ionic balance by puncturing the membrane, a strategy similar to what white blood cells in humans do when they attack a virus or other invader.

Place theorizes that karlotoxin lands on a cell, chemically penetrates the membrane, and then draws sterols (like cholesterol) to itself to form a molecular barrel-like pore through the cell wall. Once the pore penetrates the cell wall, ions and water flow in and out of the cell, and the battery is discharged. The cells literally explode.

Can karlotoxin be found in nature — specifically at the site of fish kills? For Place, answering that question was as important as identifying the structure and behavior of the toxin. After monitoring karlotoxin levels in the Chesapeake Bay for the last three years, he thinks the answer is yes. Every year since 1998 there have been fish kills attributed to *Karlodinium* along the Atlantic coast, with karlotoxins found in samples taken from Delaware, Maryland, North Carolina, South Carolina, Georgia, and Florida. Deeds and Place note that today's more accurate methodologies have turned up both *Karlodinium* and karlotoxins at virtually every dinoflagellate-related fish kill in mid-Atlantic estuaries over the past seven or eight years — including the June 2007 fish kill in Weems Creek, Annapolis. *Pfiesteria*, on the other hand, has appeared less often and in far fewer numbers.

Asks Deeds, "Is this a coincidence?"

But what about the fish kills in the Pocomoke in 1997? Does Deeds believe that *Karlodinium* killed those fish? There's no way to know for sure, he says. We just didn't collect enough information at the time. Molecular probes that provide more definitive identification did not come until later — in 1998 for *Pfiesteria* and in 2000 for *Karlodinium*. He notes that no toxins had even been identified in what was called "*Gyrodinium galatheanum*" back then.

Wayne Coats at the Smithsonian agrees that reconstructing what happened in 1997 may prove impossible. Coats feels that research has pointed to at least two toxic dinoflagellates at the site of Pocomoke fish kills, *Pfiesteria* and *Karlodinium*, and maybe that's all we need to know.

Place has heard this argument but remains convinced that *Karlodinium* is the real culprit responsible for fish kills. He notes that researcher Holly Bowers, now also at COMB, has run molecular probes on archived DNA samples taken near fish kills back in 1998 and she's found evidence of *Karlodinium* there. Questions remain about the roles of *Karlodinium*, *Pfiesteria*, and other co-occurring microbes, including bacteria. But there is one organism, Place argues, that repeatedly turns up at estuarine fish kills worldwide, with a toxin that we can reliably measure, identify, and verify in the laboratory. That organism is the toxic marine dinoflagellate first found in the 1950s. The one we now call *Karlodinium veneficum*.

— email the author, greer@mdsg.umd.edu

Pfiesteria, Karlodinium and Other Harmful Dinoflagellates

When *Pfiesteria piscicida* roared into the public consciousness in 1997, harmful blooms became dinnertime conversation in households across the country — practically overnight. But *Pfiesteria*'s place in the spotlight often overshadows the reality that harmful algae pose a much bigger problem than this one, enigmatic, dinoflagellate.

Harmful algal blooms (HABs) of certain dinoflagellates, diatoms, and cyanobacteria can cause a host of environmental and human health problems, from fish kills to toxic seafood. Eating shellfish and fish contaminated by toxic dinoflagellates or diatoms can cause a number of serious conditions which can produce symptoms that range from mild cramps, vomiting, and diarrhea to moderate or severe neurological effects and even death. So far none of these toxic organisms has caused big problems in the Chesapeake Bay.

Other algal blooms are considered harmful, but not toxic. These organisms can cause brown tides and fish kills, but generally do not affect human health directly.

Federal and state agencies maintain forecasting systems that rely on satellite imagery, field observations, and buoy data to map bloom location and movement. This helps managers provide timely information to the public and mitigate the effects of HAB events.

For More Information

Background

Woods Hole Harmful Algae Page
www.whoi.edu/redtide

Toxic & Harmful Algal Blooms
www.bigelow.org/hab

Harmful Algal Blooms in Maryland
www.dnr.state.md.us/Bay/hab

Extreme Natural Events: Harmful Algal Blooms
www.cop.noaa.gov/stressors/extremeevents/hab

Pfiesteria: A Selected Bibliography
www.mdsg.umd.edu/Pfiesteria

Forecasting

HAB Forecasting
www.csc.noaa.gov/crs/habf

The *Pfiesteria* Story

For an in-depth look at what happened in the Pocomoke ten years ago, order a copy of our Emmy-Award Winning film, *The Pfiesteria Files*, just released on DVD. The first glimpse that scientists had of this infamous organism set off alarms among fishermen and scientists alike and caused an outbreak of competitive media coverage by newspapers and television reporters that helped add to a mounting public hysteria. *The Pfiesteria Files* offers a fascinating look at a complex ecological problem, a biological mystery story whose ending has yet to be told.

You can order a copy of the DVD ($29.95) through our online store, www.mdsg.umd.edu/store (credit cards accepted), or by phoning 301.405.6376.

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