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Scientists track the 'phantom'

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What happened to Howard? The questions circled through JoAnn Burkholder's mind, like the sweep hand of a clock. During long walks in the woods, during the nights she couldn't fall asleep. *Was he careless?* Not Howard. He was one of the best scientists she had ever worked with. *What did I miss?*

What is happening to my husband? Aileen Glasgow was asking her own questions. Howard was a whiz at math but now he couldn't add two plus two. *Is it stress?* One night he got lost driving home from work.

And those rages?

They were getting worse. And they were over trivial things. One time he burst into the bedroom at 2 a.m. screaming about how the vacuum cleaner cord wasn't coiled. The next morning he didn't even remember his outburst.

Could it have something to do with his work?

For the past few years, Glasgow had been consumed by his research. He and a handful of scientists at N.C. State University in Raleigh were studying a newly discovered microbe called **Pfiesteria** piscicida, nicknamed "the phantom."

Usually found in shallow estuaries, **pfiesteria** has the ability to change into at least 24 different forms, from an amoeba with Gumby-like appendages, to a stealthy round cyst that hides in sediment, to a ferocious two-tailed killer that drugs schools of fish and sucks off their skin, sometimes leaving behind millions of carcasses with blood-red sores and holes the size of half dollars.

This strange critter was responsible for about half the fish kills in North Carolina in the early 1990s, including one on the Neuse River that wiped out more than a billion fish.

It has been found near Charleston, close to an aquaculture operation. While it hasn't caused any major fish kills here, scientists are keeping an eye out for it. It has been found as far south as the Gulf of Mexico and as far north as Delaware. It is perhaps the world's most flamboyant and stealthy algae. And a source of real concern.

Scientists like Burkholder say that in the past two decades, there has been an alarming increase in the growth of toxic algae like **pfiesteria**. These organisms pose a danger not only to fish but also to people. Many scientists fear the increase of these nearly invisible creatures is yet another signal that pollution and development have thrown the earth's environment out of whack.

Like a horror film

In 1993, the year Howard Glasgow "went down," as his colleagues say, he and Burkholder knew as much as anyone about **pfiesteria** (pronounced fee-steer-ee-uh).

In fact, several years before, it was Burkholder and researchers at N.C. State's veterinary school who discovered it. Ed Noga, a fish pathologist, and a grad student, Steve Smith, walked into their lab one morning and found the fish belly up. Noga put more fish in, but they died too.

The only clue was a single-celled organism with two tails that appeared when the fish were dying and then seemed to vanish. Noga asked Burkholder to look into the matter.

Burkholder, a self-described workaholic with curly brown hair and a quick laugh, was an aquatic ecologist in the school's Department of Botany. As she examined the aquariums in Noga's lab, she found what looked like harmless ball-shaped cysts in the sediment.

Hour after hour, she and other researchers watched these cysts under the microscope. Eventually, they learned that in the presence of fish, these cysts would change form, Burkholder says, "like Dr. Jekyll and Mr. Hyde."

The cysts transform into a microbe with sperm-like tails. The new organism discharges a poison that drugs the fish and causes their skin to slough off.

While the fish gasp for air, swim upside down or in circles, the **pfiesteria** swim toward the peeling fish and feed on them, sucking up bits through a straw-like arm. The **pfiesteria** reproduce while they're eating. When they're sated, they turn into harmless, colorless amoebas or into cysts that sink back to the sediment, safe and secure.

Burkholder learned that Noga had gotten water for his tanks from the Pamlico-Albemarle Sound in North Carolina. And it was there, on May 23, 1991, she was able to confirm her discovery in the field. During a massive fish kill, a state chemist noticed a coffee-colored slime among the carcasses. He immediately shipped samples to Burkholder. They contained the same creature found in Noga's aquariums.

"Something like this for a scientist comes only once in a lifetime," Glasgow said. "We discovered something fascinating and mysterious."

"It's really a triumph of evolution, so versatile," Burkholder added. In one of its forms, **pfiesteria** steals the green coloration from algae, allowing it to ambush fish while masquerading as a microscopic plant. And, "the first time we saw this thing transform in three minutes and gain a 20-fold increase in size, we had to pick our jaws up. We were elbowing each other out of the way from the microscope."

Sometimes, because of the phantom microbe's bizarre behavior, it was tempting to give it human characteristics.

"When an amoeba was in the petri dishes and we jiggled it inadvertently, it looked like it would hold on with one arm and wave with the other," Burkholder recalls with a smile.

But there is something sinister about this organism. For instance, it loves human blood.

One day Glasgow pricked his finger to see what the **pfiesteria** would do. He mixed a few drops of blood and **pfiesteria** and put the sample under a microscope. The microbe went into a feeding frenzy, sucking the blood cells dry, turning red in the process.

And like Jason in those Friday the 13th movies, **pfiesteria** was really tough to kill. Douse it with sulfuric acid. Pour bleach onto it. Dry it out. It would still come back.

The more they learned about the phantom organism, the more concerned they became about its effect on the environment. Burkholder's experiments showed that **pfiesteria** thrived in sewage and waters polluted by agricultural runoff, especially runoff from the state's massive hog farms.

"I think **pfiesteria** has always been here, but we've been adding tons of nutrients to our estuaries, and we've slowly tipped things in favor of it. **Pfiesteria** is a sign of an estuary that's out of balance."

When she told North Carolina officials of her concerns in 1993, a top health official said he didn't believe **pfiesteria** killed fish.

"We just can't go on such a small amount of data. You've only got two years worth of data," he told her.

Burkholder couldn't believe what she was hearing.

"We just had major fish kills. We're talking a billion fish. The fish had to be bulldozed from the beach. I had two years of fish kill data and 400 confirming bioassays." She recalls the official saying that she was irresponsible to suggest that hog farms might be causing problems.

"I'll never forget the wording of what he said next. He said, 'Come back in 10 years. When you have 10 years of data, we will be willing to consider that **pfiesteria** might be killing fish."

Microbe's bizarre effects

Burkholder was furious, in part because she suspected **pfiesteria** was not only dangerous to fish, but also to humans. She knew this from experience.

In January 1993, she poured samples into beakers the size of an egg shell. It was a tricky operation. "I was pouring it right up against my face. I was doing that for about four hours. I can tell you that I was very lucid when I walked in the lab, but I don't remember much for about eight days afterward."

The fragments of memory she has left of that period are incomplete, like a puzzle with its pieces thrown about. "I would have lucid moments and then fade back out. I would stare at a computer screen and I could recognize words but I could not write a sentence.

"It was a frightening feeling. I had to hide. I didn't talk to anyone. I couldn't have a telephone conversation because I literally couldn't remember what the person said at the beginning of their sentence when they got to the end."

After eight days, she felt better and went back to work determined to upgrade her laboratory.

"The trouble was that there were no federal regulations or guidelines on working with toxic phytoplankton," Burkholder said.

"So we called industries and asked them what they did around neuro-toxic substances. I talked with a culturist at the FDA who told me he kept his stuff in big open vats. 'Don't worry about it. Wear a lab coat and gloves,' he told me."

But by the summer of 1993 and after \$40,000 in renovations and safety equipment, she had her own small building on a hill away from campus. The building had three main rooms. Near the entrance was a small alcove and office area - the "cold room." Next door were two other rooms where the **pfisteria** were kept in small tanks. These were the "hot rooms." Respirators were required as well as gloves and special booties. No one could work in the hot rooms for more than two hours. A ventilation system changed the room's air once every five minutes. "It was supposed to be a great facility," Burkholder said.

A scientist's collapse

That summer Howard Glasgow's mind began to scramble. He had been working 80 to 100 hours a week, much of it in the trailer's safe "cold room," planning experiments, writing reports.

But as the weeks went by, he missed appointments, complained about headaches, had trouble sleeping and remembering phone numbers. His wife, Aileen, who worked in a doctor's office, became more and more concerned. "I saw him change from a fun-loving, easygoing person - very laid back - to someone who was irritable and forgetful."

At first, she chalked it up to stress. After all, Howard was about to make an important presentation in France about **pfisteria**. "He had the hardest time putting that speech together," she said. Glasgow gave an excellent presentation, however, although afterward he couldn't remember questions people asked him.

When he got back to Raleigh, he continued to unravel. "He would get mad over very insignificant things, like if I asked him to go pick up some coffee," Aileen said.

At work, he was sullen and sarcastic. "Here was someone who respected you and now seems like he doesn't want to work with you," Burkholder said. "We didn't know what to think." Burkholder also thought it might be stress, or maybe some problems at home.

"I did a little test. I asked him something about the lab and he told me about it. A few minutes later I asked him about it and he couldn't remember. The hair on my arm stood up."

One day he stomped into Burkholder's office. "He said he was sick and tired of me saying I hadn't told him things, and he stormed out, tossing out lots of profanity. If he had been anyone else, I would have fired him, but all we wanted was our old Howard back."

A few days after Thanksgiving, Glasgow was putting water into an aquarium when he splashed water on his arm. Within 30 minutes, he had a big lesion with red streaks. A day or two later, he got lost driving home. "He came home absolutely distraught," Aileen said. "I said, 'you probably were thinking of something else.' He said, 'No, I really got lost.'" He had tried to call home but couldn't remember the phone number.

Enough was enough. Fearing he might have a brain tumor or some other disease, Aileen said they were going to the hospital. He insisted on driving. Even though the hospital was just a few miles from their home, he drove to a strip mall instead.

Appointments with specialists were made, but his condition worsened through the week. Glasgow has degrees in biology and chemistry, but his reading level plunged to the level of his 6th-grade daughter. He couldn't add or subtract. All the while, he never suspected anything was wrong.

"If someone thought I had a problem, I'd say, 'It's your problem. Not mine.' My peers and colleagues said I would go into rages but I have no memory of that."

Eventually, Aileen said, "It got to the point where all he could do was stutter. We were frantic."

After extensive tests at Duke Medical School, doctors found bruises in his brain in the area that controls memory. They found high levels of enzymes in his liver. His blood pressure was erratic and his kidneys were secreting phosphates. All this suggested that his body was trying to detoxify itself. He was told to stay away from the lab.

Solving the mystery

While Glasgow recovered, Burkholder was obsessed with what happened. As lab director, she felt professional responsibility. As a friend, she felt tremendous guilt. "Howard was like a brother to me. Here we are, we're all excited about the research, and you know you've done some good detective work, but someone is collapsing in front of you, and you don't see it."

She began to talk to fishermen. They were nervous at first. "No one wants their waters to be known to have problems because no one will buy their fish."

But some eventually told her and other researchers stories of how they had sores. A shrimper described how he went shrimping in 1992 in the Pamlico estuary with a history of fish kills. He felt nauseated and developed sores later that day. A mental fog enveloped him - so thick he said he wasn't able to drive for two days. Commercial fishermen who worked in the Pamlico spoke of "fish poisoning" and how they routinely rinsed their hands with bleach after sorting fish.

Still, Glasgow's illness didn't make sense. He and everyone else in the lab had taken so many precautions. Howard himself had overseen much of the lab's construction. If *pfiesteria* was to blame, how had he been exposed?

"I'd dream about it, the way he looked in the office when he exploded," Burkholder said. "I had nightmares about the fact that he could hardly talk. I had visions of his face with a dull look, his muscles fixated like a person with Parkinson's. I wanted to know what happened. I had to know what happened."

Suddenly one night she woke up. She knew.

She called the university's public safety people to test the ventilation. They did a smoke test to trace where the air went. It was obvious what had happened. Because of a contractor's mistake, air was piped from the hot room into the cold room through a vent over Glasgow's desk. As soon as she saw the smoke entering Glasgow's office, Burkholder phoned Aileen.

"I remember being so enormously relieved that we knew what happened, almost overjoyed," Aileen said. "It make perfect sense."

After several months at home, Glasgow's condition improved. Doctors say he can't go near the **pfisteria** when it's in its toxic stage. They fear that, like someone who is hypersensitive to bee stings, Glasgow could walk in a lab with **pfisteria** and never walk out.

Slow to respond

The summer of 1995 was a bad one on the Neuse River. Week after week, it seemed, thousands of dead fish would wash up along its shores. At first people thought it was because the river lacked dissolved oxygen, a common cause of fish kills.

But something else was at work here. There was plenty of dissolved oxygen, and the dead fish had big red sores. There was that familiar coffee-colored foam and sweet smell Burkholder was so familiar with. It was the phantom at work.

North Carolina was slow to come to grips with with the fish kills, some say.

"We had a lot of trouble trying to get the health department to respond to this thing," said Rick Dove, Neuse river keeper. Sponsored by the Neuse River Foundation, Dove monitors the river and finds ways to improve it. "I was calling them on the phone to get down here. We've got fish down here, and people are eating them. A lot of them took the fish home and cut the sores off.

During these fish kills, some people who were in the water developed health problems.

Joe Lopes put in a piling for a dock and spent hours in the water. He cut his foot. Not long after that he was hospitalized with fever and sores all over his body. Doctors thought he might have chigger bites, but then he started vomiting every 15 minutes. He collapsed in the hospital.

"His white blood count was sky high," his wife, Yvonne, said. "They still don't know what's eating his system." She learned later from Burkholder that **pfisteria** was active in the area her husband had been working. She doesn't let her son go near the water now. And she wonders why it took the state so long to warn people about the river.

Bill Lotz and three other divers for the N.C. Department of Transportation were examining bridges during the fish kills.

"Out of my crew three were affected. Three had skin lesions. One had memory loss," he said. One time he almost walked off a 10-foot culvert. "I was almost totally unaware of the surroundings."

Last October, the state finally closed the river to fishing.

Sometimes, however, Burkholder gets the feeling North Carolina's power brokers don't want to find out how bad the **pfisteria** problem is. After Burkholder took water samples during a 26-million gallon hog waste spill last summer, the university started getting calls from the agriculture industry. "They asked what business I had on the river and if I could be encouraged to stop."

Anonymous callers have threatened her unless she dropped her research. She has had difficulty getting funding. Research money has gone to other less experienced researchers, including one scientist who learned how to identify **pfisteria** by reading Burkholder's articles. "You've got a lot of moneyed interests involved and the good old boy network, and she doesn't play that game," Glasgow said. "She'll win out. She'll be stepped on, but she'll win out in the end."

'We can't give up'

Glasgow is back at work now. Gone are the headaches and memory problems, although it took months for many symptoms to disappear. He remembers a bizarre episode a few months after he got back. He was in Burkholder's office when the palm of his hand suddenly began to drip with sweat. The rest of his body was perfectly dry.

Burkholder continues to have health problems she believes are related to *pfiesteria*. Like clockwork, whenever she runs hard, she gets pneumonia. Her doctors have speculated that whenever she burns fat cells, they release the *pfiesteria* toxin. Since 1993, she's had pneumonia seven times and bronchitis once.

Burkholder and Glasgow say despite what happened, they won't stop their research. They know *pfiesteria* emits a toxin, but they haven't identified it. It's as if they had a venomous snake but not the venom. If they can identify the toxin, scientists might someday be able to control *pfiesteria* outbreaks.

They know there will be more *pfiesteria* fish kills. So far this summer, cool weather has helped keep the microbe at bay. But researchers already have noticed many fish have sores.

And worldwide, scientists say there has been an alarming increase in toxic algae and dinoflagellates, often called red tides. A red tide has destroyed reefs throughout the Florida Keys. Another toxic dinoflagellate decimated endangered manatees this year.

Dinoflagellates similar to *pfiesteria* produce among the most toxic substances known, in some cases a thousand times more potent than cyanide. A pinhead-sized quantity can easily kill a person. Several years ago, three people died and hundreds were poisoned in Canada after a toxic algae bloom. One person chewed and digested most of his lower lip before he died. Some surviving patients have permanent short-term memory loss.

"It's not a vendetta, but we have a strong vested interest in learning more about *pfiesteria*," Burkholder said. "We've lost all the time, the health problems. We have invested so much. We can't give up now."