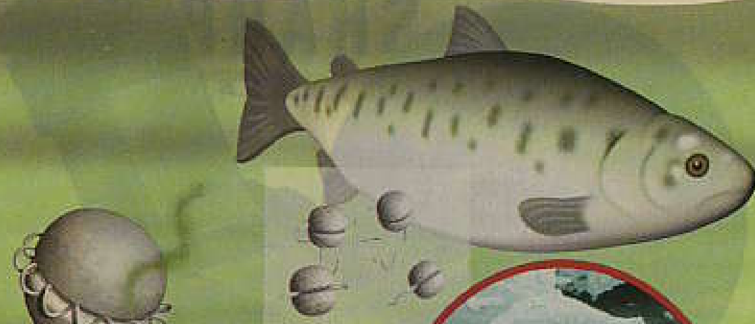
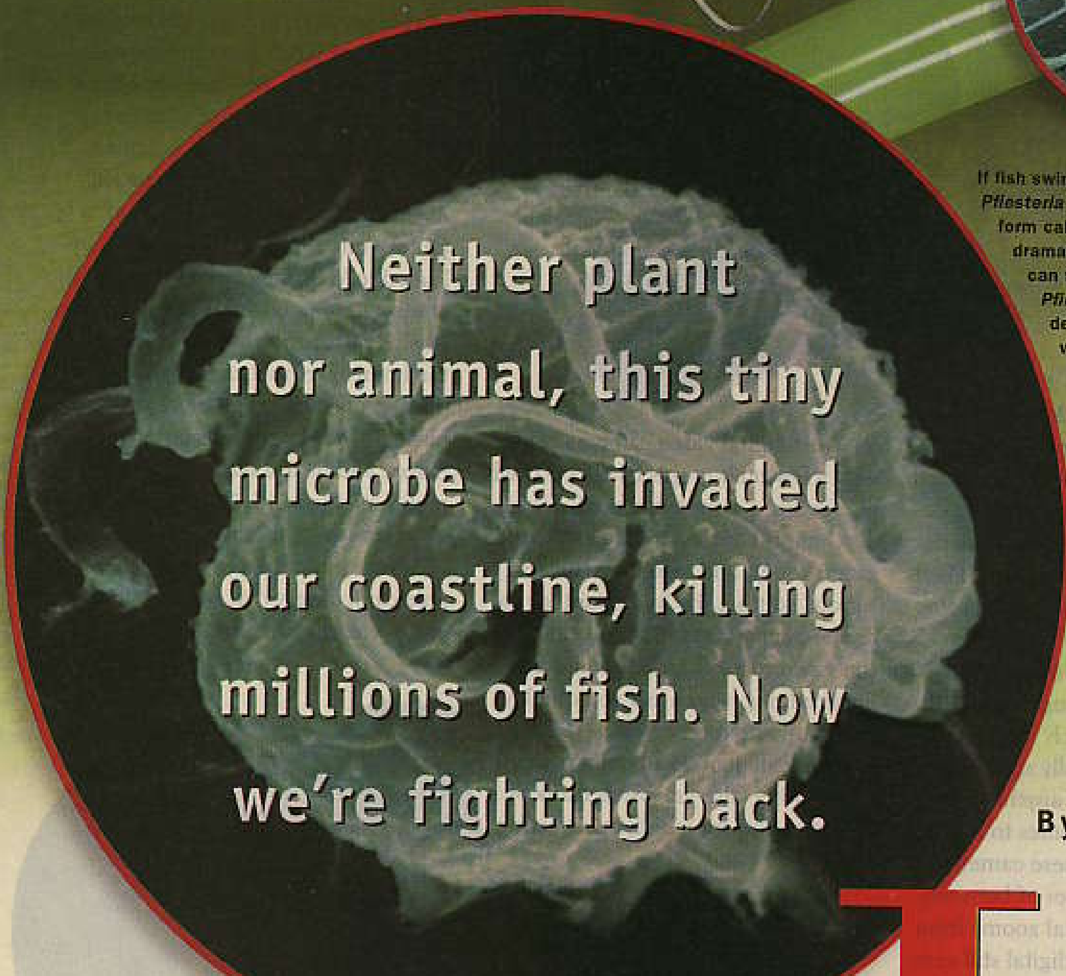
Different Forms for Different Foods

THE UNIQUE toxic terrors called *Pfiesteria piscicida* are microorganisms that can morph into 24 different body forms, depending on their dinner plans. Linked to massive fish kills along the East Coast, the animal-like microbes can at times behave like a plant. In an activity known as kleptochloroplast, *Pfiesteria* "steal" chloroplasts, the parts of plants that produce energy from sunlight in photosynthesis, and use them to supplement their diet for weeks or months.



Not to scale: Image has been magnified to show detail.

If fish swim by, microscopic *Pfiesteria* morph into a toxic form called a zoospore. The dramatic metamorphosis can take mere minutes. *Pfiesteria* release a deadly toxin into the water, which paralyzes fish and dissolves their flesh.

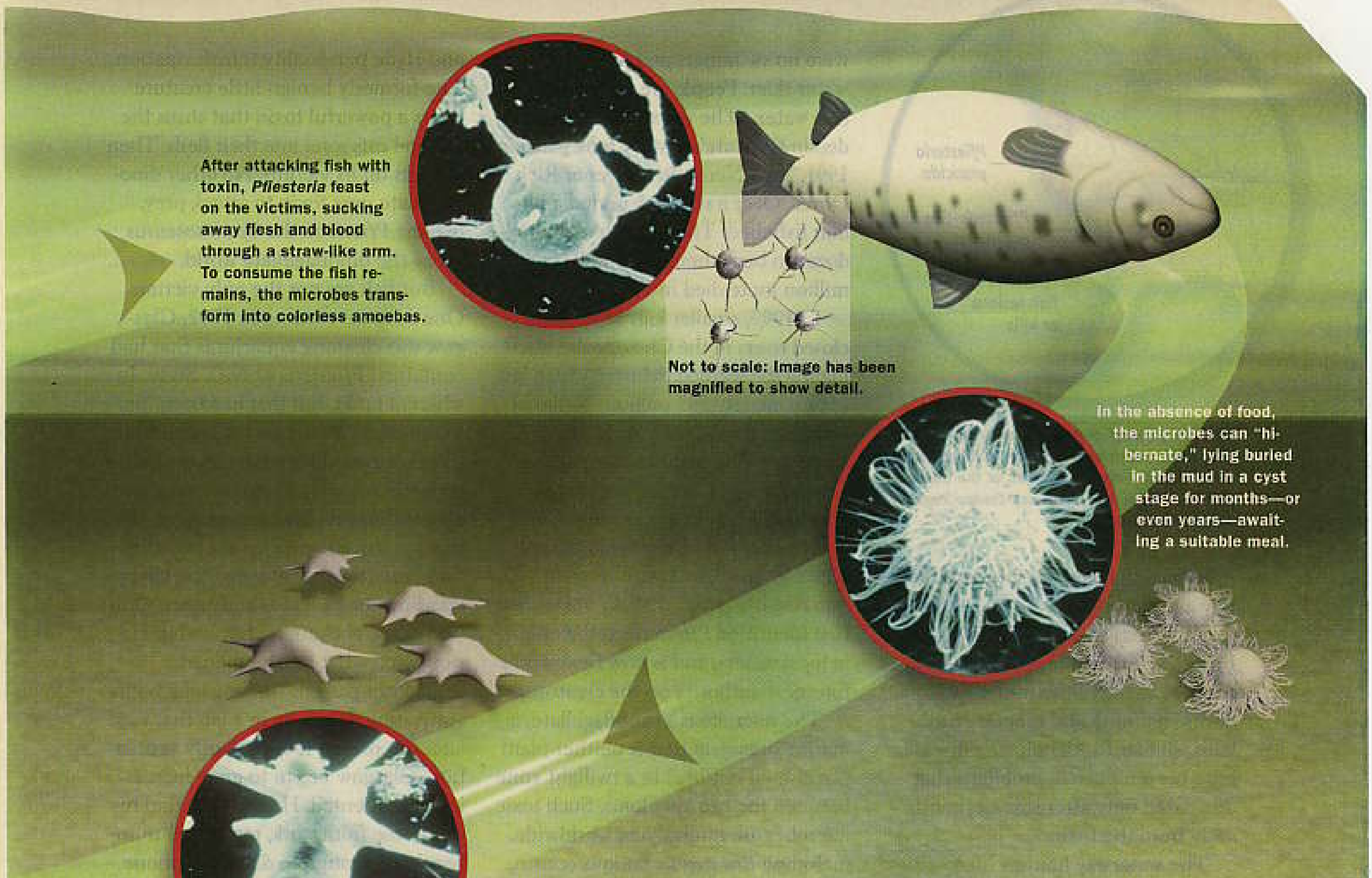


Neither plant nor animal, this tiny microbe has invaded our coastline, killing millions of fish. Now we're fighting back.

By Sharon Guynup

The Cell from He

INSET IMAGES: NCSU AQUATIC BOTANY LABORATORY (8)



After attacking fish with toxin, *Pfiesteria* feast on the victims, sucking away flesh and blood through a straw-like arm. To consume the fish remains, the microbes transform into colorless amoebas.

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In the absence of food, the microbes can "hibernate," lying buried in the mud in a cyst stage for months—or even years—awaiting a suitable meal.

On a river bottom, *Pfiesteria* may take the shape of large amoebas, engulfing small prey such as other dinoflagellates, algae, and other microbes like a blanket.

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RESEARCHER HOWARD GLASGOW pricks my finger and drips my blood onto a glass slide. He places it under a powerful microscope connected to a television monitor. A legion of tiny microbes called *Pfiesteria piscicida* swims, frenzied, back and forth across the screen, maneuvering through a logjam of my blood cells. I watch in fascination and horror as one of them "acquires" a cell. It spins around in a macabre, dizzying dance as it sucks out the contents. The microbes continue feasting on my blood, here in the North Carolina State University Aquatic Botany lab, until they are so engorged they can no longer move. Like an alien from the X-Files, this recently discovered toxic microbe, dubbed the "cell from hell" by researchers, has invaded coastal waters from Delaware to Alabama.

Dozens of scientists have been working to unravel the many mysteries surrounding this dangerous and amazing creature: its behavior, the chemical identity and effects of its toxin, the short- and long-term effects on human health and the environment—and what has made it grow out of control. Now researchers are at last putting together some of the pieces of the *Pfiesteria* puzzle.

It all started in the mid-1980s, when mysterious gaping, bloody sores the size of quarters began appearing on fish hit by *Pfiesteria* in North Carolina's Pamlico Sound region, the wetlands cradled by the Outer Banks. Soon after, massive fish kills began, periodically carpeting rivers and bays with silvery carcasses as the

ILLUSTRATIONS BY JOHN MACNEILL

With 75 percent of Americans living—and producing nourishing sewage, fertilizers, and other runoff—within 50 miles of the coasts, problems from *Pfiesteria* and similar organisms are on the rise.

Pfiesteria attacked the Chesapeake Bay and beyond.

Hundreds of people believe that *Pfiesteria* has also made them sick, some by merely breathing its vapors. Severe learning and memory problems, similar to Alzheimer's disease, have been reported, problems that dissipated only after several months away from the toxin.

The waterway hardest hit by *Pfiesteria* is the Neuse River, which flows 250 miles from Durham to the coast through North Carolina's most heavily populated watershed. Much of the Neuse appears pristine, banked by cypress trees overhung with Spanish moss. On a steamy Saturday last August, boats plied the river, but there were no swimmers and just one lonely water-skier. People are afraid to go in this water. "The worst kill ever recorded in the state's history took place in 1991," says Neuse Riverkeeper Rick Dove. "Over a six-week period a billion fish died. They had to use a bulldozer to bury them. And well over 10 million more died in 1995."

In 1997, smaller kills repeatedly closed rivers in the Chesapeake, decimating the seafood industry, which lost approximately $43 million. National headlines screamed about "*Pfiesteria* hysteria." The outbreaks prompted Congress to release $15 million in emergency funding for research.

Chief among Pfiesteria researchers is JoAnn Burkholder, an aquatic biologist at N.C. State. She first identified *Pfiesteria* as the culprit in local waters, and is now the world's foremost authority on the creature.

The microbe is a dinoflagellate, a marine organism that is neither plant nor animal but lives in a twilight zone between the two kingdoms. Such toxic microbes are multiplying worldwide, including *Pfiesteria*'s famous cousin, red tide. But unlike any other creature on Earth, *Pfiesteria* has the ability to morph into 24 different forms, depending on its dinner plans, either grazing on algae or devouring other dinoflagellates, sewage, or fish.

When fish swim by, the microbe undergoes what Burkholder calls a Jekyll-and-Hyde personality transformation. The formerly benign little creature emits a powerful toxin that stuns the fish and eats sores into their flesh. Then it feasts on its victims. No other dinoflagellate uses toxin to attack prey, making *Pfiesteria* the *Tyrannosaurus rex* of the dinoflagellate world.

But fish are not the only victims. One autumn evening in 1992, Glasgow was cleaning aquariums that had contained *Pfiesteria* at N.C. State. In adjacent tanks, fish that had been "fed" to the organism were writhing in death throes, covered with open sores. After about 20 minutes, Glasgow began gasping for breath, his eyes burning. His legs went numb and he lost coordination. Vomiting, he escaped the lab by crawling out on hands and knees. Outside in the cool air, he felt better. He wondered what had happened.

Months passed. Working long hours cultivating *Pfiesteria* in a lab that was later discovered to have faulty ventilation, Glasgow began to grow increasingly disoriented. He couldn't find his way home from work, remember numbers long enough to dial a telephone, or even read. Others in the lab got sick, including Burkholder. "We didn't know enough about it to know how concerned we should be [about adequate protection]," Glasgow recalls.

Many of their acute symptoms, including memory loss, skin sores that resembled acid burns, and Multiple

Guilty: Pigs and People

AFTER FINGERING *Pfiesteria* as the culprit in huge fish kills in 1991, JoAnn Burkholder, an aquatic biologist at North Carolina State University, wondered why it was infesting the state's coastal waterways. With 75 percent of Americans living within 50 miles of a coastline, Burkholder suspected that *Pfiesteria* was merely a symptom of larger ecological problems.

North Carolina's placid, warm coastal rivers act like lakes, making them vulnerable to buildup of nutrient-rich fertilizers, sewage, and other pollutants. Burkholder's experiments show that *Pfiesteria* reproduces rapidly in these waters.

Where toxic outbreaks have occurred, huge livestock operations—without effective waste treatment—dominate the landscape. North Carolina raised 10 million hogs in 1997; the Chesapeake Bay's eastern shores produced 620 million chickens. "Hogs in North Carolina produce the same amount of feces and urine as is produced by all the people in the states of New York and California," says Rick Dove, riverkeeper of the Neuse, the waterway hardest hit by *Pfiesteria*.

Hundreds of thousands of tons of manure from these animals are sprayed on crop fields as fertilizer. "They basically spread it on like peanut butter," says Chad Smith, a conservation policy analyst for American Rivers. "The soil can't absorb all that. As soon as you get rain, the manure goes right into a tributary or a river."

Pfiesteria's invasion has sparked EPA investigations into the impact of livestock waste. In April 1998, Maryland passed the nation's first laws regulating this waste. "I would like similar legislation to be passed in my own state," says Burkholder.—*S.G.*

Sclerosis-like coordination problems, disappeared after a few months away from the toxin. But some still suffer from blinding headaches, recurrent infections, and respiratory difficulties. "We've had lingering problems for eight years now," says Burkholder. Doctors have advised them never to handle the toxin again.

Their colleagues who handle the toxic *Pfiesteria* now suit up in protective gear. They work in a brand-new, Biohazard Level 3 facility—on par with those used for AIDS and one step below Ebola—that is complete with air locks and decontamination chambers.

Having been sickened by the toxins herself and seeing the people around her also become ill has turned the petite Burkholder into a dynamo, her intensity evident in her piercing blue eyes. She works relentlessly, giving 300 lectures a year, attending public meetings, writing, publishing—and studying *Pfiesteria*. As a child growing up in the Midwest, Burkholder was given a keen appreciation of nature from her father.

"Learning about staggering water problems in the Great Lakes, I resolved to do something to help." She became an aquatic biologist, and her work is now paving the way to understanding *Pfiesteria*'s habits and why it has invaded coastal waterways.

No one knows yet what the long-term health effects of exposure to the toxin may be. Both health and environmental research have been handcuffed because a major piece of the *Pfiesteria* puzzle is still missing: the chemical identity of the toxin. From 1992 to 1997, researchers put toxin analysis on the back burner, not believing *Pfiesteria* was truly dangerous. That changed when Peter Moeller at the National Ocean Service got involved. The chemist exudes an energy that would seem more suited to a field biologist.

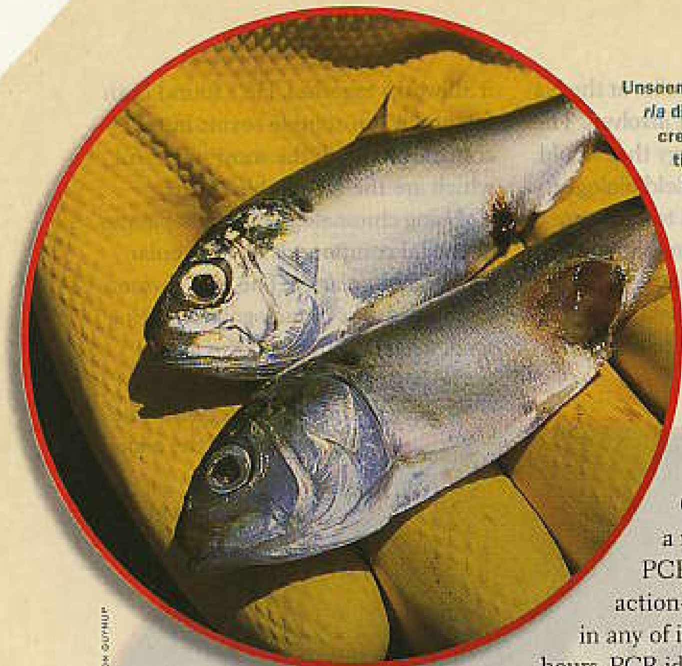
Dinoflagellates tend to produce "suites" of toxins—a whole soup of them that are often "chemical cousins," closely related with small differences. Fishing out toxin from amongst sea salt and thousands of other compounds is difficult. Moeller has thrown his entire chemist's arsenal at the problem. After much trial and error, he has found substances that extract active compounds from *Pfiesteria* cells; the compounds are then purified using specific solvents. With a complex soup of toxins, Moeller first sought to first determine each component in order to understand how it all works together. He's found both fat- and water-soluble toxins but is concentrating on the most powerful, which are the fat-soluble toxins.

Using chromatography, he separates chemical compounds by molecular weight and polarity. The way chromatography works is analogous to sifting sand, where a sifter removes all but the large grit. Moeller also maps the 3-D molecular structures of the com-

Leading the battle against toxic *Pfiesteria* is JoAnn Burkholder, who co-discovered the microbes and linked them to once-mysterious fish kills—and later was sickened by the tiny organisms herself.

JOHN B. CARNETT

The microbe can morph into 24 different forms. When fish swim by, "it undergoes a Jekyll-and-Hyde transformation," ***says biologist JoAnn Burkholder.***



SHARON GUYNUP

Unseen killer: Toxins from *Pfiesteria* dissolve fish skin, sometimes creating gaping sores, and the tiny organisms then feast on their victims.

pounds with NMR, nuclear magnetic resonance spectroscopy, the equivalent of MRI for molecules.

The toxins that Moeller has purified kill fish within minutes. "These toxins tend to be very toxic at very low levels," says Moeller. "We usually tend to work on a milligrams [thousandths of a gram] scale. Here, we're working with nanograms [billionths]."

Hellish cell roundup www.popsci.com

Burkholder's laboratory provides essential support, acting as a veritable toxin factory to produce samples for analysis. Her lab is the only one that can successfully culture *Pfiesteria*—and that is equipped with the proper biohazard facilities to do so.

Another key to protecting humans from *Pfiesteria* is developing fast-working tests to detect toxic outbreaks. Last year, states from Delaware to Florida set up rapid-response teams that continually monitor affected waters. But the only available test cost $1,500 and took two to five weeks to provide results, too long to wait when people are in danger. At N.C. State, for instance, researchers confirmed the presence of *Pfiesteria* by exposing live fish to water samples. If the fish eventually died—it could take as long as 2 to 5 weeks for any *Pfiesteria* present to go toxic—they'd found their perpetrator.

Now come diagnostic tools that detect the organism whether it's in its toxic form or not. Parke Rublee, a biologist at the University of North Carolina Greensboro has created a molecular probe using PCR—polymerase chain reaction—that detects *Pfiesteria* in any of its 24 life forms in only 8 hours. PCR identifies an organism by replicating a sequence of DNA unique to that species; an exact match is positive identification.

With his colleague Dave Oldach at the University of Maryland Medical School, he is also creating a "genetic fingerprint" for *Pfiesteria*. Each organism has a collection of DNA patterns that separate it from all others, which in combination form its "fingerprint." PCR offers easy, rapid screening and can be used in the field; the "genetic fingerprint" is a more sophisticated technique to identify *Pfiesteria* and closely related organisms in laboratory cultures.

To test for toxic outbreaks, Moeller's colleague, John Ramsdell, developed a $50, 12-minute "reporter gene assay," which creates a kind of cellular Frankenstein that is the key to detection of the *Pfiesteria* toxin. A luciferase gene, which creates the enzyme that causes fireflies to light up, is attached to a human gene and inserted into a rat pituitary cell flooded with toxin. A water sample "hot" with toxin glows brightly under a luminometer, just like a lightning bug on a summer night. In field tests conducted during last July's fish kill on the Neuse River, both assays proved effective; after fine-tuning for sensitivity, Rublee's test is now ready for use this season.

Meanwhile, fish stocks in hard-hit areas have dropped dramatically. Swimming through mildly toxic waters does not kill fish, but weakens their immunity. "*Pfiesteria* toxins suppress their white blood cell count down to 20 percent of normal," says Burkholder—a condition that has been likened to "fish AIDS." This has occurred, for instance, in the Pamlico—particularly worrisome since it's America's second-largest estuary (after the Chesapeake), providing half of all fish nursery grounds from Maine to Florida. Young fish are especially vulnerable to *Pfiesteria* toxin, and fish eggs don't hatch when the toxin is in the water.

Burkholder is investigating the cumulative, long-term impact on fish. Casualties from fish-kill areas and from Burkholder's lab will undergo "autopsies" at the N.C. State Veterinary School to observe what biological havoc these toxins wreak on tissue and organs. An important next step will be to establish how much toxin is too much—for both fish and people. In other experiments, Burkholder is seeking to discover how the presence of fish transforms the cell into a toxic killer, and trying to understand the cell's shape-shifting abilities—activities that are related to eating. She's performing lab and field taste-tests, offering the microbe a wide cuisine, from algae and other "dinos" to fish flesh, and both fish and human blood. She will also serve *Pfiesteria* a smorgasbord of pollutants to pinpoint its preferences, findings that could affect future environmental regulations. This is perhaps the most important research for affecting legislative decisions on pollution and agricultural waste that might ease the *Pfiesteria* problem.

"The story of *Pfiesteria* lets folks know that human health and fish health are very strongly linked," says Burkholder. Although researchers are studying *Pfiesteria*'s effects on human health, and early-warning tests have been developed to keep people out of harm's way, the goal is to identify the root of the problem—and address it.

"The only way we're going to fix this is to let Mother Nature get back her balance," says Dove. "*Pfiesteria* is only a symptom. The real problem is the pollution in our waters."