

A large debate over tiny creature

Some say 'Pfiesteria' not the true assassin involved in fish kills

By Nicholas Wade
N.Y. Times News Service

Pfiesteria piscicida is a microbe with a Jaws-like reputation. This mini-monster of the ocean is said to exude a toxin that destroys fish and produces neurological problems, even memory loss, in people.

When *pfiesteria* is detected, waters are closed to fishing and swimming, and in the last five years, federal agencies have financed some \$11 million in research on the microbe.

But all this commotion, some scientists now say, may have been generated by a misreading of the microbe's life cycle. According to several recent articles, including two being published this week, *pfiesteria* does not morph into a multitude of toxin-producing forms, as reported, but is an ordinary marine microbe, perhaps toxic neither to fish nor to people.

In the spectacular fish kills that seem tangible proof of *pfiesteria*'s ferocity, the researchers believe, the true assassin is a water-borne fungus known as *Aphanomyces invadans*.

Dr. JoAnn Burkholder, the N.C. State University botanist who named *Pfi-*

AT A GLANCE

DISCOVERY:

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Her experiences were described in the book 'And the Waters Turned to Blood.'

ATTENTION:

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esteria piscicida and first brought it to public attention, maintains as she originally reported that the microbe is toxic and goes through amoeba-like stages in its life cycle. The laboratories with which she has shared her special strain of *pfiesteria* largely agree.

Pfiesteria's toxin has recently been isolated, her supporters say, and her critics will look foolish when the toxin's chemical structure is announced in October.

Dr. John Ramsdell, a toxicologist at the National Ocean Service, said he had isolated and partly characterized a toxin from *pfiesteria* but could not yet assess its potency. Skeptics say a *pfiesteria* toxin has been said to be imminent for years, and they are waiting to see Dr. Ramsdell's published results.

The two warring camps have been unable to resolve their differences, in part because they do not agree they are working with the same strain of *pfiesteria*, a kind of microbe called a dinoflagellate. Dr. Burkholder says her critics possess a nontoxic strain.

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Such scientific disputes are usually resolved by laboratories' exchanging materials and trying to replicate results. But in this case Dr. Burkholder has declined to make her toxic strain of *Pfiesteria* widely available.

Until she does so, her critics say, it is impossible for them to attempt to confirm her work. This impasse has halted resolution of concerns about a presumed public health menace.

Each camp has benefited from the flow of research funds to the field. And each agrees that the issue has usefully drawn attention to an important issue — the dumping of excrement into coastal waters by pig farmers, chicken farmers and municipalities with low-grade sewage plants. Dr. Burkholder asserts that nutrients from the waste help fuel blooms of toxic *Pfiesteria*.

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Several projects financed by the new research have now come to fruition and some, including one conducted by Dr. R. Wayne Litaker of the National Oceanic and Atmospheric Administration, contradict many of Dr. Burkholder's original claims. Dr. Litaker, a molecular biologist, set about developing chemical probes that bound specifically to the genetic material of *Pfiesteria*. For a year and a half, he says, he was puzzled at his failure to observe the 24-stage life cycle reported by Dr. Burkholder.

Eventually, he said, he concluded that the many amoeba-like stages she included in the cycle were contaminant amoebas brought in on the fish on which *Pfiesteria* feed. As tracked by his DNA probes, *Pfiesteria* itself has a much simpler life cycle, without any of the toxic

amoeba-like stages reported by Dr. Burkholder.

Dr. Litaker said he doubted that *Pfiesteria piscicida* was toxic at all. Tanks full of *Pfiesteria* will kill fish, but probably by eating all the cells of their skin, not by toxin, he said, and there are probably never enough microbes in seawater to harm fish. Still, he said: "You have millions of people afraid to go to the beach. It has become part of the mythos of our culture."

Dr. Litaker and his colleagues describe their work in the current *Journal of Phycology*.

Several scientists agree with Dr. Litaker's thesis. Dr. Wolfgang Vogelbein at the Virginia Institute of Marine Science and other colleagues report this week in the journal *Nature* that *Pfiesteria piscicida* does not kill fish unless it comes in direct contact with them, suggesting that it just eats away the fish's skin and allows other organisms to administer the *coup de grace*.

Members of a team led by Dr. Robert Gawley at the University of Miami say they can find no toxin in a closely related microbe, *Pfiesteria shumwayae*. Their report appears today in *The Proceedings of the National Academy of Sciences*.

Dr. Thomas Nerad, an expert on single-celled marine organisms at the American Type Culture Collection, a repository of micro-organisms and molecular biology materials, said he too had concluded that Dr. Burkholder's 24-stage life cycle for *Pfiesteria* was "totally implausible."

"She never did the proper science of picking a single cell and following it through a life cycle," Dr. Nerad said. He described her work as "science fiction."

Dr. Leonard Haas, an ecologist at the Virginia Institute of Marine Science, said he too had failed to observe any amoeba-like forms in the life cycle of *Pfiesteria piscicida* or in a related organism, *Pfiesteria shumwayae*, which Dr. Burkholder also reported to have a 24-stage life cycle.

Dr. Karen Steidinger, a dinoflagellate expert at the Florida Marine Research Institute and a co-author with Dr. Burkholder on the article that described and named *Pfiesteria piscicida*, issued a statement

dissociating herself from the characterization of the amoeba-like and other life cycle stages. This work was done by Dr. Burkholder, Dr. Steidinger said, and she knew of no appropriate photographic documentation of *Pfiesteria*'s life cycle.

Dr. Daniel Baden, an expert on marine toxins at the University of North Carolina, said that in all the years people had worked on *Pfiesteria* no one had yet isolated a specific toxin from it, a task that should not be difficult if its toxin resembled those produced by the six known classes of toxic dinoflagellates.

As to whether the microbe is toxic to people, Dr. Carol Rubin, the leading *Pfiesteria* expert at the Centers for Disease Control and Prevention, said in an e-mail message that despite intensive research, costing "several million dollars annually" in grants to six state health agencies, "it has not been scientifically demonstrated that *Pfiesteria piscicida* produces toxins or that the presence of the organism in water is a human health hazard."

Dr. Burkholder and her allies say the problem is simply that her critics have been using the wrong strain of *Pfiesteria piscicida*. Kept in fish tanks, the organism can be fed on algae or on fish, Dr. Burkholder explained, but it loses its toxicity if fed just on algae; the toxin is induced only when the microbe needs to kill fish. She maintains that *Pfiesteria* is toxic and has amoeba-like stages as she has reported.

Doctors Litaker, Nerad, Haas and Baden all say their laboratories have been unable to obtain cultures from her.

Dr. Burkholder said she had given her toxic strain of *Pfiesteria piscicida* to 17 different laboratories. But she could not give it to Dr. Litaker because his research overlapped that of her students. "I have a responsibility to my graduate students not to provide cultures to people whose research is so similar that my students couldn't get credit," she said.

She also thinks critics like Dr. Litaker will not evaluate her strain fairly. "I do not believe that Litaker or his research team is remotely objective," she said, adding that only "neutral people" should evaluate her research.

Danger of marine microbe up for debate

By Gareth McGrath
Staff Writer

Something smells a little fishy to an environmental watchdog group headed by Robert Kennedy Jr.

Several years ago, federal officials worried about a toxic fish-killing organism becoming a major public health hazard allocated millions of dollars in research grants to study the little-known microbe.

Pfiesteria piscicida, which scientists believe may be living in pockets up and down the East Coast, flourishes in nutrient-rich waters filled with animal waste, human sewage or fertilizers.

Although harmless in most guises, some researchers believe the microbe can sometimes mutate into a toxic-emitting organism that kills fish and has harmful effects on humans.

In North Carolina, *Pfiesteria* outbreaks claimed more than a billion fish last decade. In 1997 a large outbreak in the Chesapeake Bay killed thousands of fish and left fisherman afflicted with lesions and memory problems.

Institutions that received federal funding to study the microbe include the University of North Carolina at Wilmington, UNC-Chapel Hill, N.C. State University and several institutions in Maryland and Virginia.

But recently many of the researchers who received grant funds – totaling about \$12 million of the \$16 million distributed by a slew of federal agencies since the mid-1990s – have said they've been unable to find or grow toxic *Pfiesteria*, leading them to conclude that the organism isn't toxic.

That finding flies in the face of research from other scientists – led by N.C. State's JoAnn Burkholder – who have identified and cultivated the microbe and published more than 50 peer-reviewed articles about toxic *Pfiesteria*.

Worried about research dollars having been wasted or possibly diverted to non-*Pfiesteria*-related studies, the Waterkeeper Alliance has requested documents detailing the research that was done with the grant funds.

"It's kind of hard to believe that people are suddenly saying this toxin doesn't exist," said Robin Greenwald, executive director of the alliance. "These people aren't making up their illnesses, and these fish really did die."

Rick Dove, the alliance's Southeast representative and former Neuse riverkeeper, said he's seen firsthand the damage the microbe can do.

"There is clear and concise evidence that there is a toxin, it's being produced and it's dangerous," he said.

But in a recent *New York Times* story, backers of the non-tox-

TOXIN

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ic theory said *Pfiesteria* doesn't have the exotic life cycle that's been attributed to it. The scientists said although the microbe might weaken fish, another organism actually kills them.

But Dr. Burkholder, who co-discovered *Pfiesteria* in 1988, said their research could have been compromised because those scientists were working with benign *Pfiesteria* strains or incorrectly cultured ones – raising questions about the researchers' competency to do the work in the first place.

In the recent news article, several researchers also criticized Dr. Burkholder for not widely sharing her toxic *Pfiesteria* strain.

Friday she defended the practice, stating that her team didn't have the resources to provide cultures to researchers beyond the small collection of laboratories she is already sharing the toxic strain with.

"We repeatedly told the federal agencies that we don't have the money to train personnel and we're not simply going to give them cultures because this

is a very dangerous organism to work with," Dr. Burkholder said. "That would be irresponsible of us."

Although she's been researching the microbe for over a decade, Dr. Burkholder said her team received very little of the federal money reserved for *Pfiesteria* research.

Figures on how much funding UNCW has received for *Pfiesteria* research since 1997 weren't immediately available late last week. But the Waterkeeper Alliance estimates the amount at about \$1 million.

Mr. Dove said the issue is relatively simple; if certain researchers either couldn't produce toxic *Pfiesteria* or didn't have the expertise to do the research, then why did they keep accepting federal funds earmarked for that purpose?

"If we let this go by, let people who couldn't do it say there's no toxin because we couldn't find it, that will clearly spell the end for *Pfiesteria* research, and we can't let that happen," he said. "We know we've got a bad organism here. The problem is we don't know how bad."

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PFIESTERIA

WHAT THEY THOUGHT: The microbe is said to exude a toxin that destroys fish and produces neurological problems such as memory loss in people.

NEW FINDINGS: *Pfiesteria* may not morph into various toxin-producing forms during its life cycle but could be an ordinary marine microbe, toxic to neither fish nor people.