The Fish Kill Mystery

by
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It was a hot North Carolina July morning and Susan and her friends were headed to the beach. Everyone had the day off from work and it seemed a wonderful opportunity to cool down and have a good time. Susan figured she could get to the beach in two hours from her house in Rocky Mount. After putting on her swimsuit and grabbing her sunscreen, she got into the car. Twenty minutes later she had picked up Kathy and Linda and they were off to the coast!

After driving over a bridge that crossed the Pamlico Estuary, a strong odor began to permeate the car. Kathy covered her nose with her hand. Linda, who hadn't eaten breakfast, felt her stomach turn over and thought to herself that the day was going to be spoiled. Susan, however, was curious and suggested they explore. "What do you think that smell is, anyway?" she said as she pulled the car over and jumped out, leaving her friends no choice but to follow her.

As they got closer to the estuary, the source of the odor became clear when they saw hundreds of dead fish floating on the water and washed up on the beach. When Kathy caught up to Susan and Linda, she gasped, "I've never seen such a sight—how horrible!"

A group of people were gathered on the shore. Some were pulling dead fish out of the estuary while others were sampling the water using various pieces of equipment. The three women walked over and learned that the group was made up of volunteers who were working to clean up the area as well as several biologists gathering data. Kathy, Susan, and Linda offered to help.

Susan was working with one of the biologists, Dr. Edwin Trout, and his graduate student from the local university. She liked Dr. Trout. He appeared focused on his work, but was friendly at the same time. His graduate student, Mark Cooper, was also nice and seemed very passionate about collecting data. Susan directed her questions to him: "What caused this fish kill? Was it the heat? It seems to be affecting a lot of animals these days."

"Well, the high temperatures are often related to low oxygen levels in the water," Mark replied. "Come on over here. I'll show you some data maps we have been collecting from the Neuse River."

Mark showed Susan a series of dissolved oxygen maps (http://www.esb.enr.state.nc.us/NeuFolder/images/060708do.gif), pointing out to her that the colors on the maps indicated different concentrations of oxygen.

"The blue colors indicate high amounts of oxygen (in mg/L) and so these areas are good for the fish," Mark explained. "The red colors indicate areas in the river where dissolved oxygen is low, around 0 to 3 mg/L. Here is where fish would have a problem. Typically, dissolved oxygen, or DO, readings of 2 mg/L or less are harmful to fish."

Studying the map for August 2002, Susan saw changes occurring over the month. She noticed that at times the river seemed to house enough dissolved oxygen for the fish, but at other times, for example, on August 21st and August 27th, red areas were found, indicating that dissolved oxygen was very low in these sections of the river.

"Wow, I didn't realize the dissolved oxygen could fluctuate so much over a short period of time," said Susan.

"What is the DO here today?" Kathy was working with another small group of people nearby, but became more interested in the conversation between Mark and Susan.

Mark responded, "The reading here right now is 6 mg/L. It may have been down to 2 at some point, but I don't think so."

"How did the fish die then?" Susan was really interested now. But Kathy interrupted her. "Hey, wait," she said. "Before you answer that, Mark, you never explained how temperature and dissolved oxygen are connected."

"Oh, you're right. I'm sorry," Mark replied. "The amount of oxygen that water can hold depends a lot on its temperature. The cooler the water, the more oxygen it can hold and the warmer the water, the less oxygen it can hold. Look at the map for December of 2001. Notice how much more dissolved oxygen the water holds. Almost everyday there is blue indicated or dissolved oxygen readings in the 8 to 10 mg/L range. Think about how this compares to the maps of August you just looked at, where it was unusual to see blue colors."

"That's pretty cool. I've never thought about that before," replied Kathy.

"Okay, okay. Then how did the fish die? They had enough oxygen to live. Did they run out of food?" Susan asked.

Remembering what it was like to first learn about a topic about which he felt passionate, Mark smiled. "Actually, there is plenty of food here. Notice how turbid the water is. There are lots of nutrients that come down from the rivers. That's what makes estuaries so valuable. Lots of nutrients for phytoplankton equal lots of potential photosynthesis. In fact, this Albemarle-Pamlico Estuarine System is the most important fish nursery ground on the U.S. Atlantic Coast.[1, 2] The plankton found in such estuaries can serve as food for other organisms in the food web. With such abundant food available, estuaries serve as valuable nursery ground for many different kinds of fish. What's so ironic about nutrient influx is that too much can be bad. I guess too much of anything is bad in most cases." He chuckled a bit before continuing, "Seriously, too many nutrients can cause certain species of phytoplankton to photosynthesize more actively and ultimately reproduce more successfully, producing more phytoplankton as a consequence."

"Is that why some ponds around my house have a mat of algae on the surface?" Susan asked.

"Exactly! What a great observation! The problem with this is that the algae or other phytoplankton we're discussing can block light from reaching further down in the water column. This prevents phytoplankton from living deep in the water column, because, of course, they need light for photosynthesis. When the algae or phytoplankton on the surface die and sink to the bottom, they are decomposed by bacteria. The decomposers consume a lot of oxygen. This limits the oxygen in the deeper waters. The oxygen at these depths is not replenished by photosynthesis, because the light cannot penetrate the dense growth of algae on the surface. As a result of the oxygen depletion, fish and other organisms begin to die."

"Wait a minute. You said earlier that there was enough oxygen for the fish and now you're saying there's not enough?" Kathy emphasized this point again. "I'm confused."

"You're right, Kathy. There is enough oxygen here. Oxygen depletion can kill fish, but it's not the answer to this fish kill."

"Nutrients are related to the problem, but not in the sense that I just described," Mark continued to explain. "Nutrient influx doesn't only have the potential to stimulate the overpopulation of photosynthesizers, it can also stimulate some plankton species to produce toxins and may indirectly stimulate some to change body forms."[3, 4]

"That's so strange—like a science fiction movie or something." Susan was baffled. Kathy too looked a bit confused.

"This dinoflagellate organism, *Pfiesteria*, is very impressive," Mark continued. "It has at least 20 body forms and …."[5, 6]

Susan and Kathy both cut in almost at the same time "What?! Why does it have so many different body types?" Susan continued, "And what do they all look like?"

Mark explained, "Well, there are a lot of microscopic ameobae forms; some are more rigid than others, but they all move slowly through the water using pseudopods or false feet. There are also microscopic flagellated stages as well as zoospore and cystic stages. These forms can either be found in benthic sediments or suspended in the water column.[7] Take a look at these images taken from a light microscope (http://www.ncsu.edu/wq/harmfulalgae-estuarine/dinoflagellates/pfiesteria/). Most of the time, however, you would never know *Pfiesteria* was there. They are not toxic and usually do not bother fish."

Dr. Trout, who overheard his graduate student, came over. "Mark, don't lead these ladies into thinking that scientists know for sure what is happening with this interesting species. There is a group of scientists that are not sure there are such complex body forms. Wayne Litaker and his colleagues have evidence supporting a much more simplistic life cycle for *Pfiesteria*, one without all the amoeboid forms."[8]

"You're right, of course, Dr. Trout," Mark commented. "Recently the 20-plus stages have been called into question. It's still not resolved though. Burkholder and Glasgow[9] contend that the amoeba stage wasn't found by Litaker and his colleagues[10] because they did not use a toxic strain of *Pfiesteria*. Rather, they say a non-inducible, benign strain was used and thus it wasn't predicted to produce amoeba forms. These researchers further argue that the 10-year-old sample that was used by the Litaker team[11] was too old, for the longest a toxic *Pfiesteria* strain maintained toxic properties was four years.[12] In this sense, the argument is that the absence of an amoeba form does not prove such forms never occur.[13] In addition, Dr. Trout, isn't there some new evidence that perhaps *Pfiesteria* kills not by toxin, but rather by direct contact?"[14,15]

"Yes, Mark, that's correct." Susan sensed that Dr. Trout was very proud of his graduate student.

"Again, though, the answer is still unknown." Dr. Trout went on to explain. "Burkholder and some of her colleagues[16] found that non-inducible strains can directly kill fish, but that it is a slower process than if toxin were released by toxic strains of *Pfiesteria*. Other researchers, as you correctly point out, Mark, find dinoflagellates can kill fish directly[17] and these researchers maintain that there is no evidence for amoeboid toxic forms.[18] Another interesting thing about *Pfiesteria* is that they become photosynthetic by first ingesting algal prey and then keeping the photosynthetic organelles called chloroplasts from these prey."[19]

Dr. Trout continued, "You see, ladies, this is one of the great thrills of science. As more people investigate, as more technology becomes available, and as more questions are asked, we continue to learn and debate more things."

Kathy jumped in, "I think this is what my dad means when he says 'the more you learn about something, the more you realize you don't know anything.'"

"Well," Dr. Trout said, "I guess that's true somewhat. Scientists can study something for years and continue to learn more about the topic. The point is that science doesn't always have a clear-cut answer to questions immediately. This *Pfiesteria* controversy is far from over and that is part of the reason why Mark and I are here collecting data today." Dr. Trout paused and then continued, "Well, speaking of collecting data, I better get back to my work."

After Dr. Trout left, Mark picked up where he had left off. "Biologists have investigated the role of nutrients in the *Pfiesteria* story. Nutrient influx may come from land runoff or may come from the decomposition of dead plant and animal remains that have settled on the bottom. With a nutrient influx and with lots of sunshine, photosynthesis can increase. This in turn can help support large populations of *Pfiesteria* and may also help to attract fish. Most *Pfiesteria* outbreaks in the past have occurred when water temperatures were at 26°C or above,[20] and conditions like that provide a lot of opportunity for photosynthesis."

Mark continued: "Look at this map. When nutrients, such as nitrates, come in huge quantities into the estuary, photosynthesis increases and more phytoplankton are produced. More phytoplankton provide more food for zooplankton. More phytoplankton and zooplankton provide more food for fish. When large schools of fish are present, they can excrete or secrete substances into the water that act as a cue for *Pfiesteria* to produce a deadly toxin, which results in fish kills.[21,22] Many forms of *Pfiesteria* can produce the toxin. Active amoeboid and flagellated cells can produce the toxin immediately. Encysted cells can change forms and then begin to produce the toxin.[23] The toxin makes the fish lethargic, which prevents them from swimming quickly away from the toxin. The toxin also destroys the skin of the fish.[24,25] Some people have described some of these fish as the "living dead" because they have pieces of flesh missing. *Pfiesteria* will feed on this tissue and other substances that are leaking from the sores."

"That sounds like a horrible way to die! Could the toxin kill people if they were swimming in the water?" Kathy asked, sounding a bit anxious.

Mark picked up a dead fish and pointed to open sores all over its body. "These types of sores have been found on 19 species of finfish and shellfish tested in the lab. In the field, 13 additional fish species have been affected.[26] Such sores have been seen on some people as a result of the toxin. No deaths, however, are known in humans as a result of *Pfiesteria*. There is a toxin that can be aerosolized and become airborne. That is a little scary, because it has been linked with memory impairment in humans. Fortunately, the memory loss is usually only temporary."[27]

"But the *Pfiesteria* could produce toxins for weeks or years, couldn't they?" Susan asked.

"Well, hopefully, this is not an issue because once the fish are dead, the active stages of *Pfiesteria* change back into dormant cysts," Mark explained. "They seem to disappear from the estuary—hence the name "ghost" or "phantom dinoflagellate." Still there is evidence that the toxin can compromise the fish immune system when given an acute exposure to the toxin over days to weeks. The hatching success of the eggs of some fish, such as striped bass and killifish, may be influenced as well. This suggests that the reproductive health of the fish might be harmed as a result of exposure to the toxin."[28]

Susan decided to push the subject, "How do you know the toxin won't kill people or harm their reproduction?"

"Well, you are right that the toxin is potentially nasty stuff." Mark continued, "When I work in the lab, I have to wear a protective suit over my entire body. This prevents the airborne toxin from affecting me. There are all kinds of state and federal requirements to conduct research on *Pfiesteria*. For example, there are special buildings with limited access, and the lab rooms all have special containment systems to prevent environmental contamination. Still, people who ate seafood collected from areas where *Pfiesteria* outbreaks were in progress did not seem to be harmed. But, I would definitely never advise anyone to eat such seafood. In addition, the toxin seems to kill fish for only three to eight hours. It appears to break down once in the water. After this time, it should be safe for humans to enter the water again."[29]

Mark continued, "There are some health issues. Besides the sores and memory disfunction that I mentioned, there have been symptoms of blurred vision, respiratory difficulty, muscle cramping, and vomiting along with headaches for those researchers who inhaled the airborne toxin.[30,31] What we have to concentrate on are the positives of this situation. We know that temperature and nutrient influx can make a difference. A study done by Glasgow and Burkholder in 2000 found that some effluent came to the Neuse system from municipal wastewater treatment plants, as well as from swine and poultry production areas.[32] Manure produced by animals can contribute to the nutrient load. The good news is that over a five-year study period, the amount of phosphate decreased. The bad news is that the amount of nitrogen entering the system increased. We can try to make a difference here. We can also think about controlling nutrient runoff from other locations like manicured lawns."

Linda came over to her friends at that point. "So do you guys know what killed all these fish? My group doesn't have a clue."

"Oh!" both Susan and Kathy exclaimed, "Let us tell you about the ghost dinoflagellate *Pfiesteria*…."

Questions

1. Why are estuaries, such as the Pamlico Estuary, so important?
2. How would you be able to determine from where nutrient influx comes? Could you prove this?
3. High levels of nutrients moving into the estuary have been linked to *Pfiesteria* outbreaks. What nutrient sources might contribute to this problem? You should be able to list several potential nutrient sources.
4. The Environmental Protection Agency (EPA) has regulations to manage water pollution. Examine those found at http://www.epa.gov/lawsregs/laws/cwa.html. Given that nutrient influx can be considered a form of pollution, do you think additional regulation is needed? How could this agency regulate all nutrient influx (e.g. suburban runoff)?
5. Summarize the two different camps of thought on the life cycle of *Pfiesteria*. Which set of arguments do you find more appealing? Defend your answer. How would you go about resolving this controversy? What studies and/or findings are needed to help in this process? Given there is an active controversy in the scientific community on the *Pfiesteria* life stages, should public policy decisions be made when the scientific community is not in agreement? What would happen if action on this problem were delayed until the controversy was resolved? What if action were taken without all the "facts" known?
6. Under which circumstances should we try to control the population numbers of *Pfiesteria*? If human health was not threatened, but commercially valuable fish species were harmed, should we control *Pfiesteria*? If the fish species harmed were not commercially significant, should we control *Pfiesteria*? Justify your answers in each case.
7. What will scientists need to do before they can develop a test for sampling water for the toxin?
8. What concerns may non-scientists have regarding a test for the toxin?
9. Why might other biologists be interested in studying this organism or its toxin? What other applications might there be?

References

Maps and *Pfiesteria* images are used with permission from the Center for Applied Aquatic Ecology (CAAE), North Carolina State University, http://www.ncsu.edu/wq/.

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