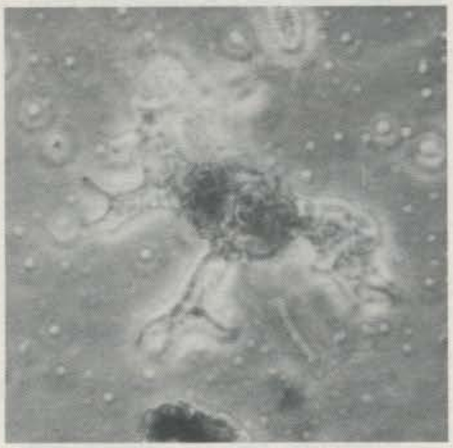
M A R Y L A N D

Research, Education, Outreach *March 1993*

MARINE NOTES

SPOTLIGHT ON RESEARCH

Alien in Our Midst?



Pamlico Sound: The toxic phantom algae

Chesapeake Bay: The phantom algae again?

Phantom Algae Suspected in Bay

In May, 1991, in North Carolina's Pamlico Sound, one million menhaden died suddenly. This massive fish kill would still remain a mystery, if not for the persistent and inspired work of one scientist at North Carolina State University. Her work, widely publicized in the national media, has led to the dramatic discovery of a new species of dinoflagellate — variously called the "phantom dinoflagellate" or the "killer algae." Strong evidence suggests that this mysterious species has now been discovered in the Chesapeake Bay.

The story of how researchers tracked this inscrutable killer reads a little like a detective story, and a lot like a script for a horror movie. One might call it Alien IV.

Silent Stalker

The story begins not in the Pamlico estuary but in a series of fish tanks in the veterinary school at North Carolina State, where Drs. Ed Noga and Steve Smith kept brood stock of striped bass and other species for use in aquaculture studies and research. One day the fish died. They began to develop skin lesions. They circled and gasped for air at the surface. They lost all sense of balance and swam upside down and sideways. Their skin began to slough off in large patches. And then they died, quickly. According to one estimate, $4,000 worth of striped bass died in a single hour.

"Samples of the water were sent to several laboratories," says researcher JoAnn Burkholder, "but no one could find any of the usual suspects." The normal pathogens, she says, did not appear, nor could anyone find any toxic chemicals or other possible causes of the fish's rapid death. The only unusual thing found by Smith and Noga were the small dinoflagellates swimming through the water while fish were dying. Shortly after the death of the fish, those algae seemed to disappear. Burkholder noted that all that was left were tiny round cysts, invisible to the unaided eye, and totally inert, as still and inanimate as the grains of sand that surrounded them.

"The cysts were sent to several prominent researchers," Burkholder says, "but no one knew exactly what to make of them." Most of the researchers did not have time, she said, to delve into the enigma, and the cysts did not look particularly promising: they just lay there.

Burkholder, a scientist at North Carolina State University, did have the time to investigate the cysts, and what she found has amazed the scientific community, which at first maintained a healthy skepticism about her results.

(Continued on page 2)

Phantom Algae, *cont.*

Now, with evidence mounting — in the form of reproducible experiments, microscopic photography and even videotape — it is hard to argue with these findings: that JoAnn Burkholder has discovered a new species of dinoflagellate which she calls *Pfiesteria piscimorte*, a species which can change shape and size, which is difficult to kill, and which itself kills with an efficiency — almost a fervor — that rivals anything found in nature, or in Hollywood.

The Smoking Gun

All that remained after these North Carolina fish kills were the tiny cysts. But Burkholder, Noga and Smith found that if a live fish was reintroduced to the tank a remarkable thing happened. The cysts broke open — not unlike the eggs of the Alien films — and what emerged was a dinoflagellate, a tiny marine plant, some species of which are known to cause "red tides." But these dinoflagellates seemed particularly purposeful. They whipped their long flagellum (a thread-like tail) and headed straight for the swimming fish — and attacked it, like a school of microscopic piranha. The algae excreted a powerful toxin that killed fish, apparently by attacking the nervous system, and then the dinoflagellates fed on the dying tissue, using a tongue-like projection of cytoplasm extruded through their cell wall.

"The trigger seems to be fresh fish excreta," Burkholder says. Once the attack begins in earnest, Burkholder says, the dinoflagellates, now in what she calls their "toxic vegetative state," double their swimming speed and score "direct hits" on the fish, consuming flecks of sloughed fish skin almost immediately.

"This is clearly ambush behavior," she says. The dinoflagellate lies undetected at the bottom of the tank or river or bay, until the presence of live fish triggers their metamorphosis and they swarm for the attack.

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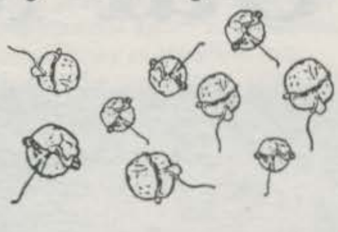


Oddly, according to Burkholder, this killer dinoflagellate appears to reproduce only when killing fish. Apparently, reproductive gametes will form in phosphate-rich water, but they will fuse only in the presence of dead fish. For the phantom dinoflagellate, fish death seems to be a requisite for sexual reproduction.

After about four hours, after the ambushed fish have succumbed to the attack, the dinoflagellates begin to "encyst" again and return to their round, inactive state. According to Burkholder, they can, as cysts, remain dormant for at least two years and still produce toxic cells again when live fish appear.

The Puzzle Takes Shape

Once she had observed this bizarre and lethal behavior in the laboratory, Burkholder needed proof that this same dinoflagellate was responsible for fish kills in the wild. This would be a challenge, she knew, since within a matter of hours after a kill the dinoflagellate would hide, covering itself in its cyst and dropping to the bottom and out of sight.

She worked with North Carolina state biologists and volunteer citizens, depending on them to respond quickly to a reported fish kill — and they did. When those one million menhaden died in 1991, the response team arrived rapidly on the scene and gathered samples of the water and of the dying fish. From those samples Burkholder isolated the phantom dinoflagellate. She had her proof.

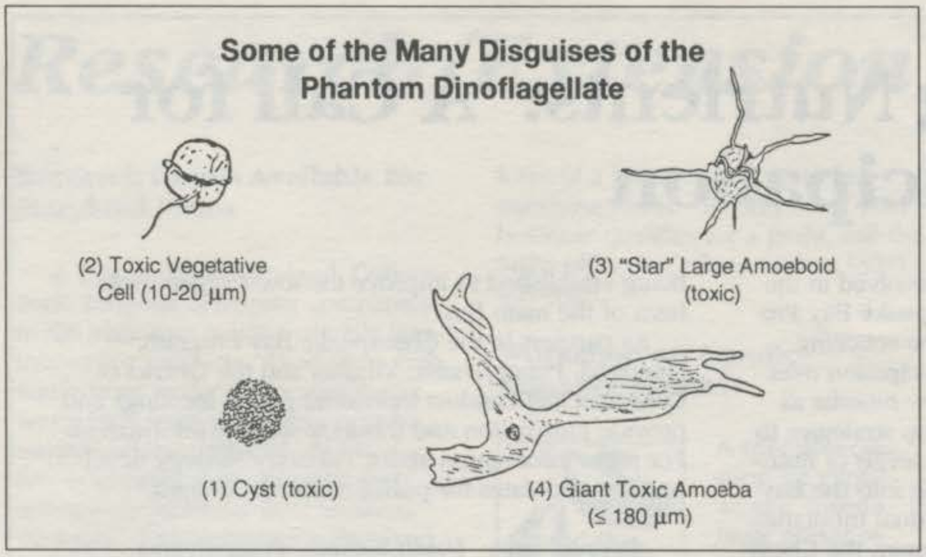
The story of the killer dinoflagellate does not end there. Burkholder reports several other striking findings.

The dinoflagellate is tough. When she used a conventional detergent solution to strip off its outer membranes — part of a common laboratory treatment to assist identification — she found the membranes very resistant, and the dinoflagellate kept swimming. She used 50 proof alcohol . . . and the dinoflagellates kept swimming. It took 100-proof alcohol to do them in. The cysts are even more resistant; after exposure to full strength sulfuric acid, the cysts were still able to "hatch" to release swimming cells.

If these "plants" do not yet begin to resemble sinister aliens, consider that Burkholder has discovered some fifteen different life stages. The most amazing of these she describes in the following way.

While watching the dinoflagellate under the microscope for long periods, Burkholder noticed that some other forms of microscopic marine life would consume them, including rotifers and a certain ciliated protozoan. This suggested to her that there could be a "biological control" for the killer dinoflagellate. But as she watched, she noticed that after a while the dinoflagellates would begin to swarm around the larger protozoan, as if ganging up on it, as if driving it away. And if she continued to watch, she observed that the dinoflagellates would actually begin to change shape. They would, remarkably, form a large amoeba, some twenty times the size of the original dinoflagellate — and then, in an act which turned prey to predator, the amoeba would attack the protozoan and engulf it.

Burkholder now keeps these dinoflagellates in culture, sustaining a population of about 90,000 cells per milliliter. Maintaining the culture requires care: evaporates from the water can contain sufficient toxin to



A microscopic chameleon — or, more accurately, shape changer — the phantom dinoflagellate has, according to JoAnn Burkholder, some fifteen life stages. It can appear as an inert cyst (1), a swimming vegetative cell (2), a large, many-armed amoeboid form (3), or a giant toxic amoeba (4). The giant amoeba, some twenty times the size of the vegetative stage, can attack the dinoflagellate's larger microscopic enemies. Plants that can behave like animals, most dinoflagellates resemble the vegetative stage (2), though Lois Pfiester and other researchers have shown that some freshwater dinoflagellates can also experience radical changes of shape. (After JoAnn Burkholder.)

produce eye irritation, dizziness, or significant respiratory symptoms. To sate the hunger of the dinoflagellates, she must feed them some fifteen fish a day . . .

The species isolated from the Chesapeake Bay is "indistinguishable" under the microscope from the one found by Burkholder

A Worldwide Watch

Even with the remarkable discoveries made by Burkholder and her colleagues, tracking the dinoflagellate and detailing its life cycle will prove difficult. In some phytoplankton communities, the phantom dinoflagellate may constitute only ten percent or less of the population and is therefore difficult to spot. In addition to their amoeboid form, the dinoflagellates occur in many varied shapes and may grow long "arms" that look very unlike normal dinoflagellates.

Following on the heels of these discoveries, researchers are now hunting for the dinoflagellate in some fourteen different countries around the world. The dinoflagellates, Burkholder says, seem to prefer shallow, phosphate-rich, turbid estuarine waters. She hypothesizes that changing environmental conditions may have made the world's rivers and bays more conducive to the success of these dinoflagellates than in the past.

Burkholder reports that, with help from Delaware biologists Ben Anderson and Roy Miller, the dinoflagellate has been isolated from the Indian River in Delaware Bay, and, now that we know what to look for, she expects it to turn up in other coastal waters as well.

Ending a recent lecture at the University of Maryland's Chesapeake Biological Laboratory, Burkholder showed a final slide of the phantom dinoflagellate. It looked very much like the others she had shown, but there was an important difference. This one had been isolated from Jenkins Creek on the Choptank River. According to Burkholder, the dinoflagellate has already killed fish at the University of Maryland's Horn Point Laboratory.

"I would be very surprised," says Alan Lewitus, a research scientist at the Horn Point Laboratory who is studying the dinoflagellate, "if this were not the same species. The evidence is very strong." According to Lewitus, the behavior of the species they have isolated is "identical" to that described by Burkholder, and the amoeboid forms have appeared during fish die-offs in their laboratory. He reports that the species isolated from the Chesapeake Bay show the same unique staining pattern in the laboratory as that found by Burkholder, and the two species are "indistinguishable" under the microscope. He is currently sending samples to JoAnn Burkholder, who will use electron microscopy to further verify similarities.

Lewitus plans to continue his investigation into this new dinoflagellate, studying its possible involvement in fish kills in the Chesapeake Bay. The Maryland Department of Natural Resources and Department of the Environment have four response teams that will be on-call to investigate fish kills, and they will help supply Lewitus with samples to aid in his research.

According to both Burkholder and Lewitus, it seems likely that the phantom dinoflagellate has already made its appearance in the Chesapeake Bay.

— Jack Greer
Maryland Sea Grant College