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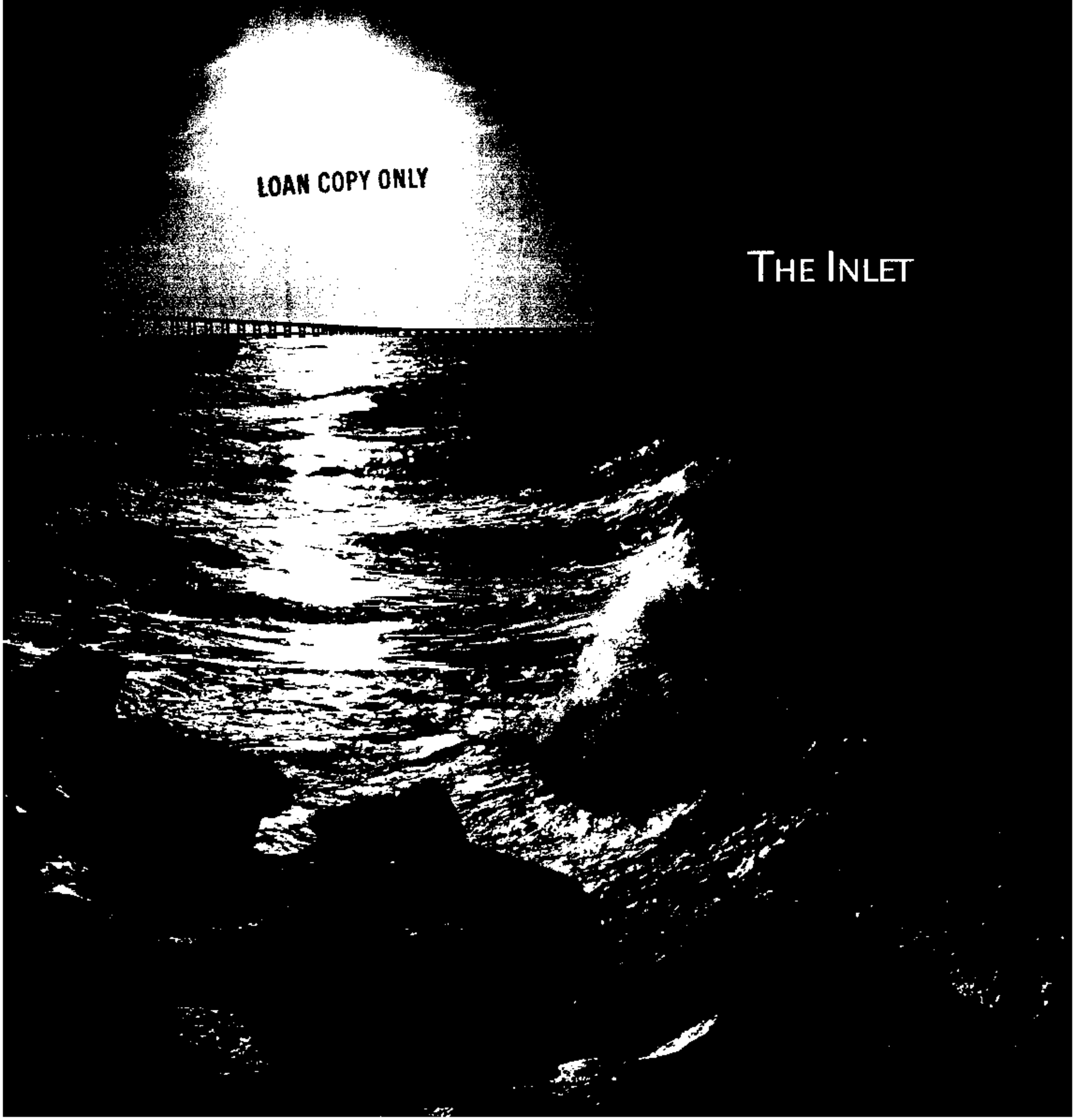

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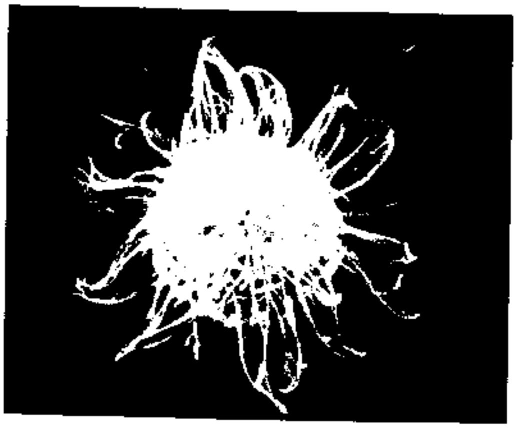
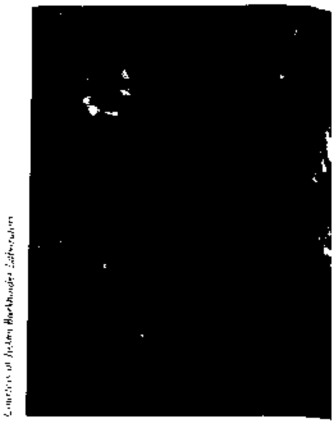


Courtesy of JoAnn Burkholder Laboratory



Pfiesteria *has 24 life stages ... including star ameoba* *... cyst from toxic zoospore* *... planozygote*

Beyond *Pfiesteria*: Harmful Algae in Coastal Waters

By Jeannie Faris Norris

It's been called the "cell from hell" and compared — absurdly — to the deadly *Ebola* virus. *Pfiesteria piscicida* has been given horror movie proportions by some accounts.

But *Pfiesteria* is not a freak of nature, nor is it a monster. It has not killed people, and its threat to human health has yet to be proven. Rather, *Pfiesteria* is a harmful algal bloom (HAB), one of many that plague coastal waters around the country.

"HABs in general are a problem that I think we should be paying a lot more attention to," says JoAnn Burkholder, a North Carolina Sea Grant researcher and codiscoverer of *Pfiesteria piscicida*. "*Pfiesteria* is a recent poster child, and it's only one of many that are really important in coastal areas and are causing a lot of havoc."

Virtually invisible, small numbers of HABs such as *Pfiesteria* may live in coastal waters at any time. But in the right environment, they can form large colonies or blooms that discolor water and deplete oxygen that other marine organisms need to survive. HABs also can cause human illness and death, alter marine habitats through shading and overgrowth, and close coastal businesses.

The recent spate of headlines might suggest otherwise, but harmful algae are nothing new. They have been around for thousands of years, says Barbara Doll, North Carolina Sea Grant's water quality specialist. The Old Testament of the Bible contains the first known description of a red tide, and Native Americans in the Pacific Northwest were aware of shellfish poisonings centuries ago.

Courtesy of JoAnn Burkholder Laboratory

The toxic stages of Pfiesteria *have been linked to sores and fish kills, particularly among menhaden.*

But in recent decades, the United States has experienced an escalating trend in harmful algal blooms such as *Pfiesteria*, says Don Anderson, a scientist for Woods Hole Oceanographic Institution Sea Grant and one of the world's leading experts on HABs.

Before the early 1970s, only a few regions of the United States suffered outbreaks of toxic algae. Now, virtually every coastal state is threatened by at least one toxic species that can be found over large areas. Among many others, they include red tides in the Gulf of Mexico and along the southeastern coast; brown tides off New York and Texas; and dangerous, even deadly, shellfish poisoning off Maine, the Pacific Northwest and Alaska.

"This is a much, much bigger problem than *Pfiesteria*," says Anderson, who leads one of the nation's premier research labs addressing HABs. "There are similar organisms elsewhere in the country."

Nationally, the losses to coastal resources and communities may exceed

JoAnn Burkholder and Howard Glasgow

... toxic zoospore

JoAnn Burkholder and Howard Glasgow

... and lobose ameoba.

$1 billion, according to the National Oceanic and Atmospheric Administration's Coastal Ocean Program.

In North Carolina, the 1987 red tide that closed shellfishing along more than 250 miles of shoreline during peak harvest season caused $25 million in losses to the seafood and tourism industries, Doll says. And nationally, the fishing industry alone loses $35 million to $65 million per year to outbreaks of red tide, brown tide, oxygen-depriving algae blooms and toxic algae such as *Pfiesteria*, says Sen. Olympia Snowe, R-Maine, chair of the Senate Commerce subcommittee on science and fisheries.

"Even more troubling is that science cannot fully explain why this is happening or how to prevent it in the future," Snowe says.

With losses of this size threatening the country's coastlines, scientists are searching for the causes of the increase and spread of HABs.

Some evidence points to widespread eutrophication, which is caused by excess nutrients such as nitrogen and phosphorus that filter from wastewater treatment plants, cropland, livestock facilities and urban runoff. The nutrients stimulate the growth of algae and aquatic plants and may contribute to the proliferation of HABs.

Climate change may be another culprit, causing shifts in sea surface temperature and elevation that may create more suitable environments for harmful algae.

Finally, some researchers suspect that heightened awareness and better reporting may be partly responsible for the observed increases in HABs. ◘

Pfiesteria — Perplexing Fish Killer

If ever a harmful algal bloom had a Jekyll-and-Hyde personality, it is *Pfiesteria piscicida*.

From a harmless cyst that lies in the sediment, this organism can transform itself into a toxic dinoflagellate that kills fish. JoAnn Burkholder, a North Carolina Sea Grant researcher who codiscovered *Pfiesteria*, says she believes it becomes toxic when it detects something that the fish excrete or secrete, but she has yet to identify the substance.

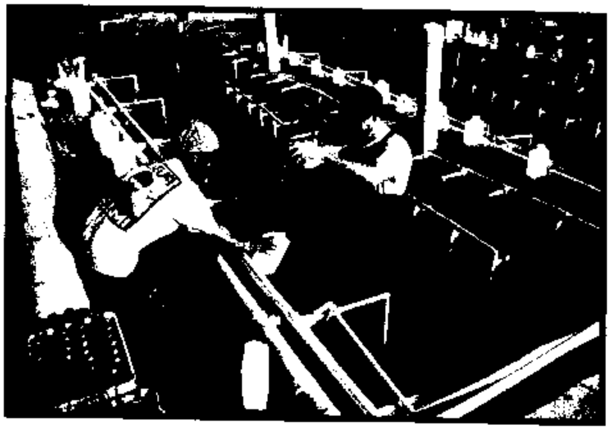
"We're working very, very hard to determine what it is in fish excreta or secreta that stimulates *Pfiesteria* to this Jekyll-and-Hyde transformation," Burkholder says. "We don't know the substance."

Benign most of the time, the organism is toxic in four of its 24 life stages. Exposure to active *Pfiesteria* cultures in the laboratory kills healthy scallops and striped bass in minutes. At chronic, sublethal levels, its toxins create bleeding lesions and destroy the epidermis of finfish and the carapace of blue crabs. It can also depress fishes' white blood cell counts to 20 percent of normal.

Human health effects — including cognitive impairment, memory loss, nausea and asthmalike distress — have been linked to *Pfiesteria* exposure in the laboratory. The effects on people who work on waters affected by fish kills are being studied.

The cellular features of *Pfiesteria*-like organisms suggest they are among the oldest living dinoflagellate species, Burkholder says. *Pfiesteria* was first reported in *Nature* in 1992, and it has since been linked to major fish kills affecting millions of fish in North Carolina's estuarine waters, according to N.C. Division of Water Quality figures.

These organisms thrive in poorly flushed estuaries with water degraded by human sewage, animal wastes and other forms of nutrient overenrichment, Burkholder says. But large fish kills can result from a variety of causes — *Pfiesteria* is one, low dissolved oxygen is another — and they often cannot be attributed to a single cause. ◘ —J.F.N.

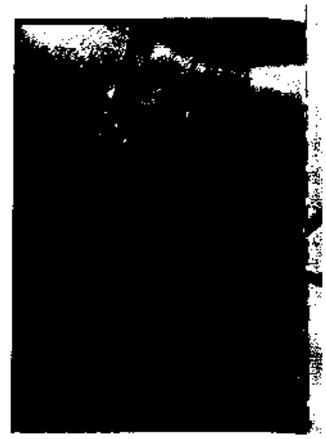


Courtesy of Hans Paerl

Mike Piehler (left) and Jay Pinckney, postdoctoral research scientists, test water quality at the Institute of Marine Sciences (IMS) experimental pond.

Courtesy of JoAnn Burkholder Laboratory

Pfiesteria *research is conducted at JoAnn Burkholder's toxic containment culture unit.*



David Griffith surveyed crabbers who work

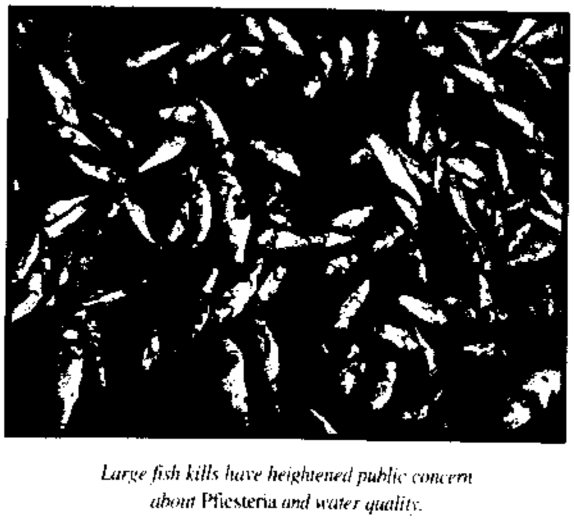
Pfiesteria Research: Scientists Study Causes & Effects

By Jeannie Faris Norris

The hot, still days of summer have arrived. Vacationers may flock to our beaches for sun and surf, but just a few miles inland, conditions in our brackish waters are ripening for fish kills and *Pfiesteria piscicida*.

Pfiesteria made national news last year when it was discovered in tributaries of the Chesapeake Bay, killing thousands of fish and forcing closure of two rivers. Large kills in North Carolina in 1991 and 1995 also corresponded to the organism's presence.

Considerable hype and attention have since been focused on *Pfiesteria*, but we still don't know precisely what causes these kills and what impact they have on human health and seafood. The process of scientific discovery can be slow. But we're a few steps closer to the answers thanks to recent reports from four teams of scientists assembled by North Carolina Sea Grant. In 18-month studies, these teams examined how *Pfiesteria* affects the health of crabbers in fish-kill areas, the learning ability in laboratory rats and the quality of seafood taken from kill waters. A fourth team examined how the nutrients that seep and spill into state waterways affect one of *Pfiesteria*'s nontoxic life stages.



Courtesy of JoAnn Burkholder Laboratory

Large fish kills have heightened public concern about Pfiesteria *and water quality.*

Some facts are already known. *Pfiesteria*-like organisms have been found in coastal waters from Delaware Bay to the Gulf of Mexico. Recent research suggests there are as many as four species. The complex life cycle of the organism has at least 24 distinct stages that range from a sediment-dwelling cyst to a free-swimming zoospore. Certain stages may release toxins that affect the neural and immune systems of fish. They also directly or indirectly cause fish skin to blister and slough off, creating sores that are then invaded by opportunistic pathogens such as bacteria and fungi.

Because this unusual organism has raised questions about human health, consumer safety and water quality, the N.C. Department of Environment, Health and Natural Resources (now the Department of Environment and Natural Resources) set aside funds for studies of *Pfiesteria*-like organisms. North Carolina Sea Grant administered the funds and awarded them to scientists at Duke University, East Carolina University, North Carolina State University and the University of North Carolina at Chapel Hill.

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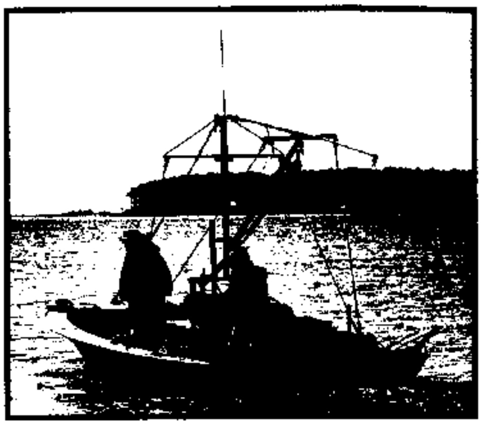
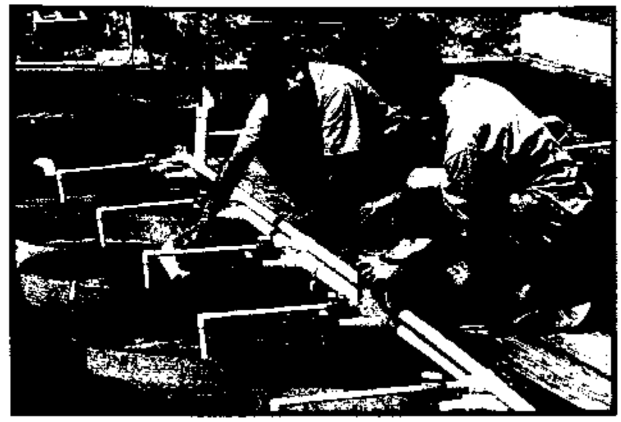


Crabbing requires fishers to spend hours on the water placing and pulling pots.



Hans Paerl (left) samples an IMS mesocosm with Matt Fitzpatrick, a former technician. They are studying how algae respond to the addition of nutrients.

Human Health Effects

In one study, a team of ECU researchers compared the health and habits of crabbers to those of a group of nonfishers. The team also examined where crabbers set their pots and where fish kills, algal blooms and other water quality problems have taken place.

The researchers concluded that casual contact with eastern North Carolina waters under normal conditions — in the absence of fish kills, algal blooms, red tides or other indicators of poor water quality — poses extremely low health risks. Reports of ill-health effects caused by high concentrations of *Pfiesteria*-like organisms during fish kills appear to have been "greatly exaggerated," says David Griffith, the ECU anthropologist who headed the research team.

Griffith notes that North Carolina has water quality problems, but his research did not find a link between poor water quality and bad health.

"The threat is exaggerated for the human population. But I can't speak for fish," Griffith says. "This is a very narrow kind of finding that people who work all day on the water for their entire lives are not sick just because of that."

In 1996, the researchers interviewed 253 crabbers who worked in the Neuse and Pamlico rivers and in the southern sections of the Pamlico Sound (Pamlico crabbers). They compared the crabbers' replies to questions about their recent health to responses from two control groups: 115 crabbers working in Albemarle and Currituck sounds, the Alligator River and northern areas of the Pamlico Sound (Albemarle crabbers) and 125 nonfishers living in the crabbers' communities.

Continued

Heterosigma – Devastating to Aquaculture

Chocolate-brown waters are a sign that *Heterosigma carterae* is blooming. To aquaculturists on the West Coast, these brown waters forewarn a loss of green in their pockets.

The *Heterosigma* toxic algae has been reported on every continent except Antarctica, and it has caused multimillion-dollar fish kills worldwide, says Rose Ann Cattolico, a Washington Sea Grant research scientist.

Sea bream, salmon, yellowtail, sea bass, trout and flounder have been killed by *Heterosigma* blooms. In the Pacific Northwest salmon industry, losses have reached more than $20 million over the past eight years, and Japan estimates that *Heterosigma* has cost its aquaculture industry more than $2 billion over a 16-year period.

Beyond this economic havoc, the ecosystem suffers as well. "You don't have to have lots of dead fish in the water in order to have an effect on your ecosystem," Cattolico says. "There are many impacts on many organisms and at different levels."

Specifically, *Heterosigma* blooms can interfere with the survival and reproduction of invertebrates. When juvenile oysters or clams are fed *Heterosigma*, their growth rates are 20 percent lower than those of shellfish fed *Skeletonema costatum* (healthy food for oysters). Adult shellfish are not directly affected by *Heterosigma*, but oysters refuse to feed when this toxic algae is in the water.

"And if they aren't feeding, they're not surviving with the same success rate," Cattolico says.

Likewise, sea urchin eggs will not grow at all in water where *Heterosigma* is present. For aquaculturists who cater to Asian markets where people covet sea urchins as a meal, *Heterosigma* can be a devastating setback to business. ◘

—J.F.N.

The researchers found that in most cases, the two groups of crabbers and the community controls reported similar levels of illness and injury. However, both groups of crabbers reported higher — and nearly identical — incidences of skin disorders than the community controls. The researchers concluded that skin disorders are a common occupational hazard among crabbers and cannot be attributed to *Pfiesteria* or similar dinoflagellates.

Increased exposure to water (as measured by the number of traps the crabbers pull per day) did not result in increased incidence of illness. And there was no statistical difference in levels of illness between those who worked in areas with fish kills and those who worked in areas with few or no kills. The two areas known to have had fish kills — the Neuse River and the Pamlico and Pungo rivers — did have lower well-to-ill ratios than areas less prone to kills. This finding may be some cause for concern about the human-health impacts in these areas as opposed to others, the researchers reported.

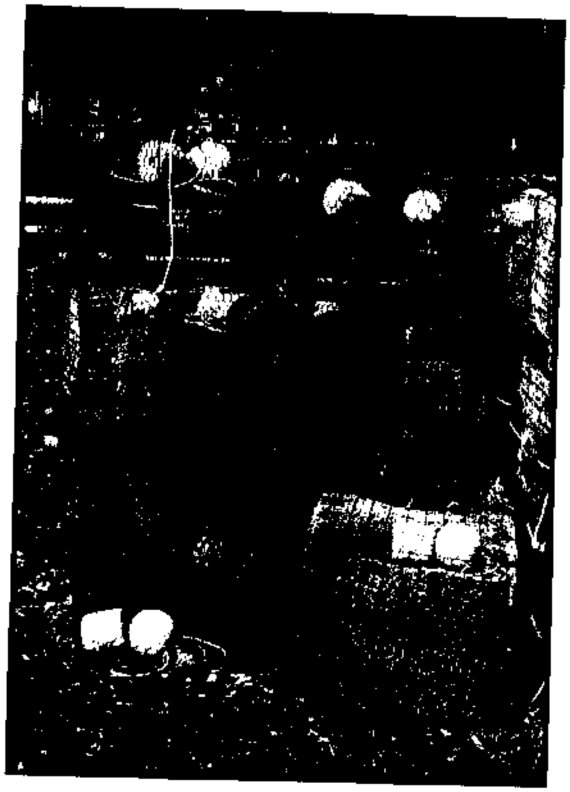
Learning Impairment in Rats

In another study, Duke University scientist Ed Levin found that rats injected with *Pfiesteria* display a significant learning deficit when called on to attempt new tasks as long as 10 weeks later. Levin's work was published in the December issue of *Environmental Health Perspectives*, the journal of the National Institute of Environmental Health Sciences.

"It seemed to be relatively specific in that the rats seemed to be otherwise healthy and their appearance seemed normal," Levin says.

In addition to appearing physically well, the rats showed no *Pfiesteria*-induced effects in blood count or in a standard pathological screening of brain, liver, lungs, kidneys and spleen.

Water samples were taken from aquariums in which *Pfiesteria* were killing fish. The samples were frozen, thawed and injected into rats. The significant learning impairments were documented in rats required to learn a new task after they received recently frozen injections of *Pfiesteria*. However, no effect was seen in the recall of previously learned tasks. These tests were not carried out beyond 10 weeks.



Griffith estimated each crabber's exposure to the water by the number of pots he or she worked.

The persistent learning impairment in rats might be comparable to the cognitive deficits humans have shown after exposure to *Pfiesteria* in the lab, the researchers concluded. The findings were replicated in several studies, but more are needed to determine which chemical causes the effects, Levin says.

"We're going through different extracts to see which (chemical) is the bad actor," Levin says. "We're using procedures to help identify the critical toxin, the critical chemical in *Pfiesteria*."

From that information, researchers can help determine what exposure levels warrant concern and develop therapeutic treatments for the learning deficits.

Environmental Causes

At the UNC Institute of Marine Sciences, researchers Hans Paerl and James Pinckney examined whether nutrients spur growth of the dominant nontoxic zoospore stage of *Pfiesteria* and whether nutrient-induced increases in phytoplankton — a primary food source for *Pfiesteria* — boosted the dinoflagellate's numbers.

They concluded that *Pfiesteria* does not respond strongly to the addition of nitrogen and phosphorus — nutrients that commonly reach state waterways through runoff and discharge pipes. But phytoplankton do respond to nitrogen, and increases in phytoplankton appear to direct changes in *Pfiesteria*-like zoospores. Thus, it appears that phytoplankton are the nutrition source that supports the growth of *Pfiesteria*-like zoospores.

"We saw the prey organisms increase, but we didn't see *Pfiesteria* increase at the same time," Paerl says. "That says that a slug of nutrients that enters the river probably doesn't have an immediate short-term stimulator effect on *Pfiesteria*, but in the long term you can see a pattern."

Their experiments used water from a location on the Neuse River where both *Pfiesteria* and fish kills have been reported. The water was transferred to mesocosms — translucent fiberglass tanks. The scientists then simulated natural conditions and added inorganic nitrogen and phosphorus. These forms are the most likely types of growth-stimulating nutrients encountered by phytoplankton and *Pfiesteria*-like dinoflagellates in the Neuse estuary.

Paerl and Pinckney concluded that reducing phytoplankton growth would translate into broad water quality improvements, including a decline in the frequency and magnitude of algal blooms, oxygen depletion, and fish and shellfish deaths.

The Neuse River, in particular, would benefit from proposed nutrient-reduction strategies to improve its water quality and reduce fish mortality, Paerl says.

"By reducing nitrogen loading and reducing the major phytoplankton responses that we saw in bioassays, it's likely to help minimize outbreaks of *Pfiesteria* as well," he says.

The results confirmed 10 years of nutrient-addition experiments on the Neuse River, showing the importance of nitrogen as a growth-limiting nutrient, Paerl says.

Exposure to Seafood

Meanwhile, Duke University scientist Patricia McClellan-Green and NC State University researchers Lee Ann Jaykus and David Green studied the possibility of consumer health risks from eating fish exposed to *Pfiesteria*. They wanted to know if fish exposed to the dinoflagellate absorb its toxin.

The fish used in the study were collected live on Sept. 3, 1996, during an active kill on Northeast Creek, a tributary on the upper portions of the New River. The scientists used fillets — edible portions of the fish — for their studies.

The team reported that only one species of fish sampled from the kill site — Atlantic menhaden — had toxin in its tissues. But because other dinoflagellates were present in the water, the team had no way of knowing whether the toxin was from *Pfiesteria*. Other fish collected at the same time — spot, striped mullet, croaker, Spanish mackerel, silver perch and pinfish — did not appear to contain toxins.

"The fact that none of the tissues from other fish was positive is very encouraging," McClellan-Green says.

The researchers are confident that the test they adapted for this research can be used as an early screening method for fishery products and water samples collected at the site of fish kills. They are continuing to refine these techniques and use them with other methods for detecting *Pfiesteria* toxins in the environment.

Rather than ending inquiries into the effects of *Pfiesteria*, these four peer-reviewed research projects help to identify areas that need further exploration, says Sea Grant Director Ron Hodson. He emphasizes that the work is just beginning.

"With the interest of federal agencies and state agencies, I'm very optimistic that funds will be made available to continue that research," Hodson says. ◘

Brown Tide – Deadly to Shellfish

Brown tide is a destructive algal bloom that can starve shellfish, damage critical nursery habitat and interrupt the marine food web.

Its blooms are caused by a bacteria-sized algae, ***Aureococcus anophagefferens***, during the late spring and summer in coastal embayments from Narragansett Bay, R.I., to Barnegat Bay, N.J., says Darcy Lonsdale, a New York Sea Grant researcher. Different brown tide species are found in the Gulf of Mexico.

"There is no reason to suspect that this alga is directly harmful to humans," Lonsdale says. "But we are concerned because it is apparently quite harmful to organisms that consume phytoplankton."

Brown tide can starve shellfish – especially mussels, scallops and hard clams – by reducing their feeding response. Juvenile hard clams tend to grow slower during brown tides, and scallops seem to stop feeding when concentrations reach 250,000 cells per milliliter of seawater (a milliliter equals about 20 drops) – the point at which researchers begin to see negative effects. At times, brown tide blooms reach concentrations of more than 1 million cells per milliliter.

"When shellfish are out in the bay and they start to experience higher and higher concentrations of brown tide, they stop feeding, close up and starve," Lonsdale says.

The price for these outbreaks in terms of lost seafood sales can be steep. In 1988, New York state suffered a $2 million loss from reduced scallop landings caused by brown tide.

Besides starving shellfish, brown tide damages habitat by darkening the water and diminishing the light needed by bottom-dwelling eelgrass, a nursery and refuge for many fish species. The algae also interrupts the food web at about 500,000 cells per milliliter by decreasing protozoa, a key nutritional link between phytoplankton and their predators, such as some juvenile fish. With less food available, juvenile fish may grow more slowly or die. ◘ – J.F.N.