

And the Waters Turned to Blood

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Principles of Environmental Toxicology
December, 2000

Abstract:

Published in 1997, Rodney Barker's book, And the Waters Turned to Blood, outlines the events that took place in the discovery of a toxic dinoflagellate, *Pfiesteria piscicida*. Since this dinoflagellate alga was discovered in fish cultures at North Carolina State University, it has impacted many East Coast estuaries. *Pfiesteria* has 24 life stages that can be categorized as amoeboid, flagellated, or encysted. The amoeboid and flagellated stages can release toxins in response to fish secreta/excreta. The toxins are known to negatively affect both fish and humans that come in direct contact with it. *Pfiesteria* seems to have its own distinct water quality and limnological optima, which includes a temperature of 25 °C, and a salinity of 15 psu. Nutrient loading in the form of phosphorus and nitrogen enhances its growth, and are being added to the estuaries via mining, wastewater treatment, and hog and chicken farming. The organism tends to bloom following certain hydrobiological changes, including turnover, upwelling, and increased thermal stratification. Typically blooms arise in areas of low dissolved oxygen, which can be a common occurrence in estuaries in summer months. Managers are currently seeking tools that can be used to help reduce the probability that the organism will enter its toxic form; artificial mixing and controlling nutrient loading will definitely reduce toxic bloom occurrences. Further research opportunities are available to collect more information that may help managers effectively control toxic *Pfiesteria piscicida*.

Introduction

Rodney Barker's book, And the Waters Turned to Blood, was published in 1997. This book provides a very descriptive account of what has happened in the lives of those closely associated with a very controversial toxic dinoflagellate called *Pfiesteria piscicida*. This microscopic alga was accidentally introduced into cultured tilapia tanks at the North Carolina State University Veterinary School in 1988 by using freshly collected water from a nearby estuary (Burkholder et al. 1992). It killed all of the tilapia in those culture tanks and since then has been found in many estuaries and coastal waters of the mid-Atlantic and southeastern United States. It has been documented as causing fish kills in North Carolina estuaries (Burkholder et al. 1992) and in the largest and most productive estuary (Cerco et al. 1993) in the U.S., the Chesapeake Bay system (Lewitus et al. 1995). Both of these systems are characterized by large inputs of freshwater into the seawater, forming brackish zones. It is in these zones that the organism concentrates its efforts and causes the most damage. Although not necessarily causing fish kills, *Pfiesteria* and *Pfiesteria*-like organisms have been identified as far south as the Atlantic and Gulf coasts of Florida (Figure 1). The possibility exists that the organism has prevailed on the East Coast of the U.S. because of the high amount of pollution and subsequent nutrient loading in the water.

Pfiesteria is a very unusual dinoflagellate because it produces high quantities of exotoxins, shows a direct chemoreceptory response to fish secretions/excretions, and has a very complex life cycle (Glasgow et al. 1995). This dinoflagellate has caused concern for both fish survival and human health. The recent rise in the reports of *Pfiesteria* blooms appears to coincide with an increased nutrient loading of affected

waters. Many management concerns have arisen to try and limit the further eutrophication of coastal waters to decrease the chance of *Pfiesteria* caused fish kills (Burkholder 1998).

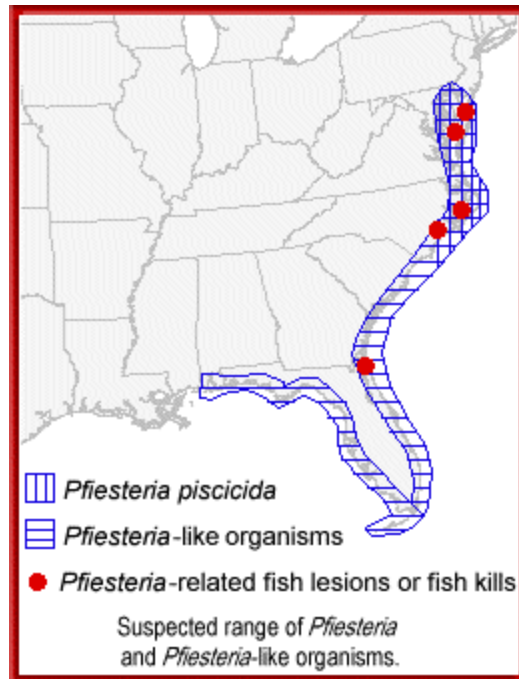


Figure 1: Range of *Pfiesteria* and *Pfiesteria*-like organisms.

Objectives

The objectives of this document are to provide an unbiased review of And the Waters Turned to Blood, and the more recent published literature on *Pfiesteria*. The results of the review will be summarized into four general topics:

- ? The life cycle of *Pfiesteria*
- ? The effects of *Pfiesteria* on fish survival
- ? The effects of *Pfiesteria* on human health
- ? The preferred water quality and limnological considerations of *Pfiesteria*

Results/Discussion

Life Cycle

Pfiesteria is a very complex organism. It has at least 19 identified stages in its life cycle, many of which are quite deadly (Glasgow et al. 1995). These stages include flagellated, amoeboid, and encysted forms. The flagellated and amoeboid forms vary in size from 5-450 μ m and are known to be toxic to fish. The encysted form includes all of the dormant stages and ranges in size from 7-60 μ m in diameter. Each form of the dinoflagellate has a different role in the survival of the cell. The complex life cycle of the organism is shown in figure 2.

Flagellated Stage

The flagellated cells are highly motile (Barker 1997). This is very advantageous for *Pfiesteria* because it allows rapid vertical movement along gradients related to light, temperature, and nutrients. This movement is critical for the survival of the cell. When resources are limited in one area, the flagellated cell can move to where the conditions are more favorable for its success (Glasgow et al. 1995). For example, dinoflagellates will often orient themselves near the surface during photosynthetically favorable light conditions but can then move deeper in the water column during aphotic times, where nutrients are more abundant (Paerl 1988).

The flagellated form of *Pfiesteria* is unique because it can detect an unknown fish substance that is secreted or excreted in the water (Barker 1997). After the substance is detected, the toxic flagellated vegetative cells release their deadly toxins that immobilize and kill the fish (Lewitus et al. 1995). The cells then use their flagella to

propel themselves toward the sloughing fish tissue. Like the amoeboid stages, these flagellated stages can engulf their prey by attaching to prey cells using a peduncle and suctioning the prey contents. Within hours of the fish death, the flagellated cells either transform to amoeboid stages or encyst and sink to the bottom sediments. This rapid transformation has often made it very difficult to detect the presence of the toxic flagellated form in fish kill sites.

Amoeboid Stage

The amoeboid stages of *Pfiesteria* are toxic as well. They can be found in both the water column and bottom sediments. They are known to engulf other organisms like bacteria and algae or feed on fish tissues (Barker 1997). They are the true engulfing form of *Pfiesteria* and though they don't enter the flesh of their prey, they reside in the water column and feed on the sloughed epidermal tissue and blood from the ulcerations on the body of their prey. Much like the flagellated form, the amoeboid form is able to detect the presence of fish tissues in the water.

Encysted Stage

The non-toxic encysted stage of *Pfiesteria* is the resting form of the organism (Paerl 1988). These cells are thick-walled and are usually found in the benthic sediments of estuaries but can often take on a pelagic lifestyle as well. *Pfiesteria* can survive many years in the encysted stage. The organism will stay in this stage until it is ready to emerge as a vegetative cell when the conditions are favorable. It can rapidly transform into a flagellated vegetative cell, migrate rapidly to stable surface waters, and release its deadly toxins when it detects a fish substance. It has been shown that several unfavorable conditions, especially temperature, lead to encystment. Usually the

colder months of the year are the time when conditions are unfavorable and the cells enter the encystment, or dormant form.

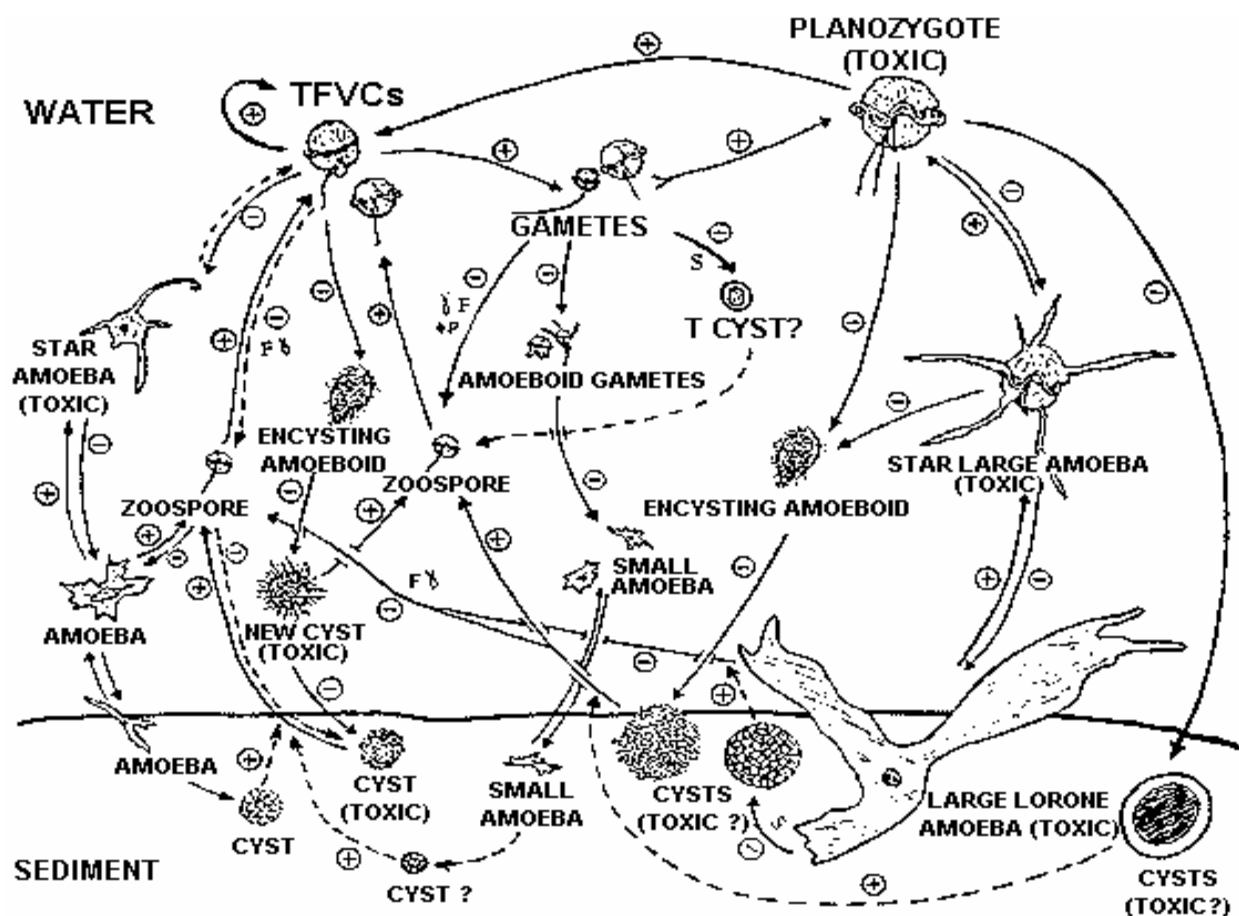


Figure 2: Life cycle of *Pfiesteria piscicida*

Life Stage Decision

Determining when *Pfiesteria* enters a certain life stage and why it enters that life stage at that particular time can help us better understand how the organism “thinks”.

Laboratory experiments have shown that the particular life stage *Pfiesteria* enters is

very dependent on what is available in its environment (Burkholder 1992). The experiments show that the organism needs live fish cells or excretions in order to remain in the toxic and feeding flagellated or amoeboid forms. *Pfiesteria* will encyst or enter its dormant form within hours of fish death unless live fish are added to the water in which it is occupying. This shows that the organism can sense the fish excretions and modify itself in order to take advantage of the conditions it is under. Electron microscope images of each form can be seen in figure 3.

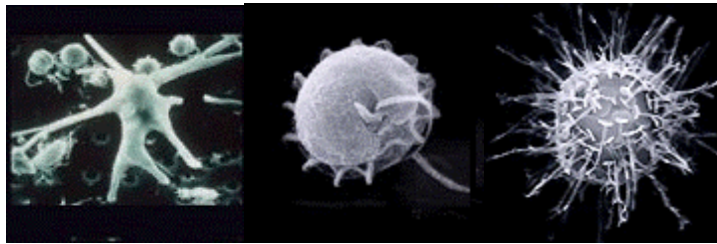


Figure 3: Amoeboid form, flagellated form, cyst form.

Fish Survival

Pfiesteria is probably most known for its damage to fish populations (Barker 1997). A wide variety of finfish and shellfish have suffered because of the toxic organism. It has killed every individual of every type of finfish and shellfish that it has been tested with in culture. Among these are blue crabs, oysters, clams, scallops, striped bass, mullet, croakers, spot, eel, menhaden, pinfish, flounder, and largemouth bass. It tends to be most effective on schooling fish and most of the fish kills in the estuaries have been on juvenile menhaden, a very common East Coast schooling fish. The menhaden often activate the *Pfiesteria* by their secretions but once the toxins are released, they can hurt any finfish or shellfish in the area.

Fish Symptoms from Toxins

When the potent toxins are secreted into the water, the fish become lethargic and are immobilized. They have a severely reduced fright response and often gulp air at the surface in respiratory distress (Glasgow et al. 1995). They apparently lose their equilibrium and become disoriented. This allows the toxins to do a vast amount of physiological damage to the fish, which may result in open bleeding sores, or ulcerations. Figure 4 shows these typical ulcerations on juvenile menhaden. The sloughed epidermal tissue from the ulcerations on the fish is what *Pfiesteria* feed on. *Pfiesteria* is also known to injure the fish skin so that they lose their ability to maintain an internal salt balance. The fish will become very weak and without its outer mucus layer, is very susceptible to secondary diseases. Toxin-induced muscle paralysis is the apparent cause of death resulting from a decrease in the respiratory processes in the body of the fish (Glasgow et al. 1995).



Figure 4: Typical ulcerations caused by *Pfiesteria*

Human Health

Just as *Pfiesteria* has a damaging and often lethal effect on fish, it can cause severe problems in humans as well (Glasgow et al. 1995). When the organism was first discovered and tests were being performed in the laboratory, *Pfiesteria* was not thought

to have an affect on humans (Barker 1997). It was not until technicians and scientists working with the organism in the lab (like Burkholder and Glasgow) started showing various symptoms of the toxin that *Pfiesteria* was suggested to be potentially damaging to humans. Working in close proximity with high concentrations of *Pfiesteria* has proven very dangerous in laboratory conditions. Direct skin contact with the toxic waters can cause damage to humans. Not only have these symptoms been reported in laboratory settings but various fishermen and recreational water-users in direct contact with fish kill waters have also been affected. Barker (1997) wrote of *Pfiesteria*'s potential be damaging to humans who inhale toxic aerosols from laboratory cultures (Glasgow et al. 1995). Both Dr. Howard Glasgow and Dr. JoAnn Burkholder showed symptoms from airborne exposure.

Human Symptoms from Toxins

The effect of the organism on humans is usually quite slow in progression. Most of the problems occur after people have worked with toxic *Pfiesteria* cultures for a couple of hours each day over several weeks (Barker 1997). The comparisons of the human exposures have shown a wide variety of symptoms. These include headaches, abdominal cramps, nausea, and vomiting. More serious effects can occur as well, including the loss of short-term memory and an impaired ability to think or reason. These serious effects occurred in Dr. Howard Glasgow and Dr. JoAnn Burkholder. Although it may take time, if the symptoms are detected immediately, removal from the presence of the toxins will induce improvements in overall human condition. For example, it took Dr. Howard Glasgow a couple of months to recover from his impaired

mental state, apparently caused by *Pfiesteria* (Barker 1997). Even then, Barker reports that Glasgow never regained his full mental capabilities.

Water Quality and Limnological Considerations

Through much study and research, an abundance of information about *Pfiesteria* and its water quality optima have been discovered. There are numerous laboratory studies that have outlined where the organism exists and in what conditions it tends to be the most threatening to fish. Taking care to study the ecological and environmental setting of the organism will allow for a deeper understanding of its processes and increase the probability of its detection.

Urban Development

The estuaries that house the *Pfiesteria* fish kill sites are plagued with many problems associated with agricultural and industrial development along their shores. The presence of large, growing cities like Baltimore and Washington D.C. in Chesapeake Bay have aided in the loss of submerged aquatic vegetation, wetland vegetation, and riparian areas leading to an increasing eutrophication of the estuaries (Cerco et al. 1993). These vegetation areas are vital for the removal of nutrients from the water.

Some studies have concluded that nutrient gradients follow the gradient in percent residential areas (McDonnell et al. 1994). These studies have shown that areas with more development per area of land show an increased level of NO_3^- . It has been suggested that the majority of nutrient inputs in these residential areas may be from septic tank plumes. Regardless of the input, levels of NO_3^- in estuaries are

considerably higher in developed areas. Although nitrate levels increased in developed areas, phosphate levels remained the same. This can be expected because phosphate is readily adsorbed by soils.

Temperature and Salinity

Pfiesteria can exist in a wide range of temperatures (Burkholder et al. 1992). This large tolerance range allows the dinoflagellate to survive the 4 °C temperatures on the estuary substrate in the winter. It seems to be most active in toxic outbreaks during warmer months when the temperature is about 25 °C. It is also known to have a wide salinity range of 2-35 ppt (Burkholder et al, 1992). The optimum salinity for *Pfiesteria* growth is in brackish waters, at about 15 ppt, but it has killed fish from nearly freshwater up to fully salinified seawater. These brackish waters are found in many of the East Coast estuaries where freshwater tributaries enter saline bays.

Nutrient Loading

High nutrient loading of phosphate and nitrate is known to stimulate the outbreak of *Pfiesteria*. Although low nitrate enrichment mildly stimulates growth of the organism, it has not shown to be a dominant factor in its total toxicity (Glasgow et al. 1995). Phosphate enrichment, on the other hand, has been shown to greatly increase *Pfiesteria* growth. Recent experiments have concluded that there is a positive relationship between the organism abundance and phosphate concentration in the water-column (Mallin 1994). In areas suffering the most fish kills, there has been an indication of high phosphate levels. This is being blamed on numerous inputs like hog and chicken farming, wastewater treatment facilities, and phosphate mining. One fish kill site in the Pamlico River Estuary was located directly adjacent to a phosphate

mining industry that has been discharging high levels of phosphorus for several years. The increased numbers of people in these areas have caused increased amounts of sewage and fertilizer being dumped into the river (Pain 1996). Farmers in the fish kill areas are taking much of the blame for nutrient loading. The manure piles from their farms often drain directly into the rivers or estuaries, carrying with them high levels of nitrate and phosphate.

Hydrobiological Changes

Many hydrobiological changes in bodies of water where *Pfiesteria* abound appear to aid in their abundance. It seems that increased eutrophication of coastal and estuarine waters has offered a more preferable habitat for *Pfiesteria* (Lewitus et al. 1995). These waters are much more productive and are typically very warm, often with low amounts of dissolved oxygen. The low dissolved oxygen can be attributed to algal and heterotrophic respiration, nitrification, and the chemical oxygen demand of the sediments (Cerco et al. 1993). It has also been observed that a stable body of water, or one that has strong vertical stratification, favors the accumulation of the organism (Paerl 1988). This may be because the increased temperatures in the epilimnion as well as the low levels of oxygen are more suitable for the growth of dinoflagellates.

The dispersal of dinoflagellates in a water column can be aided by regions of upwelling and downwelling, as well as wind driven currents (Paerl 1988). These areas in the water are often characterized as having an increased build up of foam on the surface. *Pfiesteria* may find this type of environment favorable to occupy and are therefore transported by the wind to other areas. Periods of turnover appear to aid in the dispersal of *Pfiesteria* as well. This may be due to the fact that it brings the

dormant, encysted form of the organism up where the conditions are more favorable for it to exist as the toxic amoeboid or flagellated forms. Another common occurrence is to find schooling fish, like Menhaden, in the upper layer of the water column where they may be detected by the dinoflagellate.

Ecological Setting

The problems associated with *Pfiesteria* are generally very localized to particular areas (Barker 1997). Although the organism is found all over the eastern seaboard, fish kill outbreaks occur in freshwater rivers that dump into the estuaries. These sites are usually located where the rivers flow into the more saline waters. This means that the organism may optimize its efforts in waters that are brackish, or that have inputs of both freshwater and seawater. The warmer summer months also show a higher frequency of fish kills and *Pfiesteria* outbreaks. This is most likely related to the fact that nutrient levels, like phosphorus and nitrogen, are highest during these hot summer months. Low dissolved oxygen levels can also be associated with the hottest time of the year and can cause fish to become stressed. These higher stress levels will leave the fish more susceptible to attack by the *Pfiesteria* organism.

Conclusions

As more information is being gathered about *Pfiesteria*, water and fish managers are becoming more aware of what type of strategies may work to help alleviate the problem. First and probably most important is the ability to predict and control harmful blooms. An extensive monitoring plan must be developed where more data can be collected for further research and study. In order to put this research into motion,

sufficient funds must be available. Many of the local Universities are currently funding much of the research about *Pfiesteria*. It is a large area of concern because of the impact on the East Coast and funding for further study is typically not limited. So far, successful models have not been developed to predict the behavior and growth of the organism. Because of this, management has been focusing on mitigating the impacts that *Pfiesteria* has on fish and humans. One way in which this can be done is by decreasing the nutrients being loaded into the waters. Since hog and chicken farmers are getting most of the blame for this, the North Carolina legislature has required permits for the creation of new farms or the expansion of existing ones. This way proper disposal of the nutrients can be achieved without runoff into estuaries. Strong vertical mixing will often discourage, and even eliminate the chances for the dinoflagellate to develop. This knowledge can be implemented as a management tool in controllable environments, like aquaculture facilities. Artificial destratification can often control many alga species and could possibly do the same for *Pfiesteria*.

Although many studies have been completed on the problems that *Pfiesteria* can cause, there are many more areas where very little is known about the organism. Advanced technology, like remote sensors and molecular probes, are being developed that aid in rapid detection of the organism. However, the toxic substance released by *Pfiesteria* in the presence of fish is still chemically unidentified. Also, the fish secreta/excreta that causes the dinoflagellate to release its toxin remains chemically unidentified. The characterization of such chemical constituents could aid in controlling or reducing the harmful effects created by the toxin.

In order for progress to occur regarding *Pfiesteria* “clean up”, cooperation between all environmental biologists, ecologists, and policy makers is vital. The evidence provided by field biologists and ecologists must translate into regulated policy in order to see improvements in estuary health. Equally important to policy regulation is policy enforcement. This enforcement will ultimately lead to results in our nation's estuaries. Unless nutrient inputs can be reduced and the process of eutrophication brought to a halt, estuaries will remain at the mercy of the many organisms, like *Pfiesteria*, that thrive on its polluted waters.

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