

State Monitoring Activities Related to *Pfiesteria*-Like Organisms

Robert E. Magnien

Tidewater Ecosystem Assessment Division, Maryland Department of Natural Resources, Annapolis, Maryland, USA

In response to potential threats to human health and fish populations, six states along the east coast of the United States initiated monitoring programs related to *Pfiesteria*-like organisms in 1998. These actions were taken in the wake of toxic outbreaks of *Pfiesteria piscicida* Steidinger & Burkholder in Maryland during 1997 and previous outbreaks in North Carolina. The monitoring programs have two major purposes. The first, rapid response, is to ensure public safety by responding immediately to conditions that may indicate the presence of *Pfiesteria* or related organisms in a toxic state. The second, comprehensive assessment, is to provide a more complete understanding of where *Pfiesteria*-like organisms may become a threat, to understand what factors may stimulate their growth and toxicity, and to evaluate the impacts of these organisms upon fish and other aquatic life. In states where human health studies are being conducted, the data from both types of monitoring are used to provide information on environmental exposure. The three elements included in each monitoring program are identification of *Pfiesteria*-like organisms, water quality measurements, and assessments of fish health. Identification of *Pfiesteria*-like organisms is a particularly difficult element of the monitoring programs, as these small species cannot be definitively identified using light microscopy; newly applied molecular techniques, however, are starting to provide alternatives to traditional methods. State monitoring programs also offer many opportunities for collaborations with research initiatives targeting both environmental and human health issues related to *Pfiesteria*-like organisms. **Key words:** Delaware, Florida, harmful algal blooms, Maryland, monitoring, North Carolina, *Pfiesteria*, *Pfiesteria*-like organisms, *Pfiesteria piscicida*, South Carolina, Virginia. — *Environ Health Perspect* 109(suppl 5):711–714 (2001). <http://ehpnet1.niehs.nih.gov/docs/2001/suppl-5/711-714/magnien/abstract.html>

Pfiesteria piscicida Steidinger & Burkholder has been suspected as the causative agent in a number of fish health events and fish kills since the early 1990s in several estuaries of North Carolina and Maryland on the east coast of the United States (1–4). Human health effects have also been associated with exposure to *P. piscicida* in the laboratory and with exposure to waterways in which *P. piscicida* has existed in a toxic state (5,6). A newly described species, *Pfiesteria shumwayae*, has also been found to be toxic (7), and additional related species often found in association with *Pfiesteria* are being investigated for their possible role in fish health problems. In this article, the term *Pfiesteria*-like organisms (PLOs) refers to the different species of *Pfiesteria* as well as closely related dinoflagellates suspected of having impacts on fish health.

In the spring of 1997, the State of Maryland established an ad hoc monitoring program to assess environmental conditions in response to serious fish health and human health problems associated with waterways on the mid-Delmarva Peninsula, which lies between the Chesapeake Bay and the Atlantic Ocean. This monitoring program was expanded during the summer as additional evidence emerged that *P. piscicida* was in an actively toxic state in the monitored waterbodies (4) and that individuals who came in contact with these water bodies exhibited neurologic deficits (6). The monitoring effort provided information that helped the

Governor and other state and local government officials to determine whether waterways should be closed because of potential *P. piscicida*-related risks to human health. During the summer and fall of 1997, three waterways in Maryland were closed during and after active toxic outbreaks documented by the new monitoring efforts (4).

The fish and human health concerns in Maryland, and previously documented fish health problems associated with *P. piscicida* in North Carolina, led several east coast states, where the presence of *Pfiesteria* had been confirmed or suspected, to establish additional monitoring capabilities in 1998. These additional programs were supported by new federal and state resources made available in the wake of problems that Maryland experienced in 1997. Some of the new monitoring was built upon existing programs being conducted for different purposes. A number of states also entered into cooperative arrangements with research initiatives to more efficiently and comprehensively monitor *Pfiesteria* populations and the conditions associated with them.

In this article I provide an overview of the *Pfiesteria*-related monitoring approaches undertaken since 1998 by Maryland and five additional east coast (USA) states.

States Involved in PLO-Related Monitoring Programs

The monitoring programs reviewed in this article include those of six east coast states:

Maryland (MD), Delaware (DE), Virginia (VA), North Carolina (NC), South Carolina (SC), and Florida (FL) (Figure 1). Each state undertook PLO-related monitoring programs no later than 1998 and each has continued some level of monitoring since then, although changes have been initiated for various reasons, including advances in scientific methods and changes in funding.

In each of the six states, at least one *Pfiesteria* species has been found and some degree of policy attention and new research have been directed toward PLOs. Each of these states has a “hotline” operated 24 hr per day to receive calls about fish kills, fish health events, or human health problems that could be associated with PLOs. Four of the six states—MD, DE, VA, NC—have written policies that provide guidance for determining when water bodies may constitute a public health risk because of the possibility that *Pfiesteria* species are in a toxic state. When that happens, the water bodies are temporarily closed to boating, fishing, and other recreational or commercial activities. The six states have also been selected by the Centers for Disease Control and Prevention for surveillance of possible estuary-associated syndrome, a set of criteria used to screen for individuals who may have experienced a common set of medical problems following exposure to estuarine waters (8). Three states, MD, VA, NC, are also conducting epidemiological studies to examine possible relationships between *Pfiesteria* and human health.

Monitoring for PLOs has been conducted in a number of other east and gulf coast

This article is based on a presentation at the CDC National Conference on *Pfiesteria*: From Biology to Public Health held 18–20 October 2000 in Stone Mountain, Georgia, USA.

Address correspondence to R.E. Magnien, Tidewater Ecosystem Assessment Division, Maryland Dept. of Natural Resources, 580 Taylor Ave., D-2, Annapolis, MD 21401 USA. Telephone: (410) 260-8630. Fax: (410) 260-8640. E-mail: rmagnien@dnr.state.md.us

The following individuals graciously contributed information for their respective states: Maryland: D. Goshorn, B. Michael; Delaware: E. Humphries, E. Dickey; Virginia: F. Daniel; North Carolina: J. Overton, E. Fensin, J. Burkholder, H. Glasgow; South Carolina: A. Lewitus; Florida: J. Landsberg, K. Steidinger. The National Oceanic & Atmospheric Administration, the U.S. Environmental Protection Agency, and state governments provided funding for the state monitoring activities described herein. The manuscript was improved by comments received from two anonymous reviewers.

Received 8 January 2001; accepted 20 June 2001.

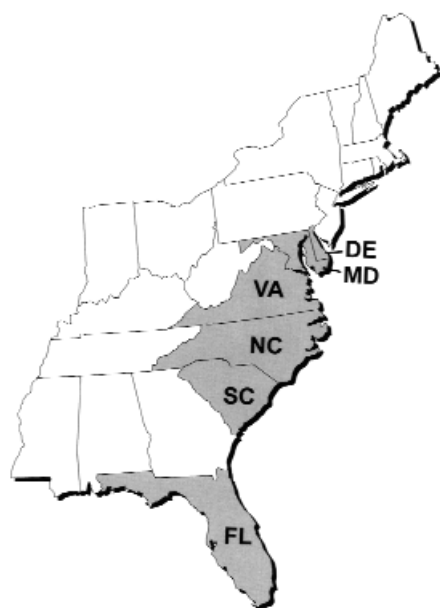


Figure 1. Map of the east coast of the United States, highlighting the 6 states that are conducting monitoring for *Pfiesteria*-like organisms and are the subject of this article.

states, although the level of effort is generally less than in the six states highlighted here and this type of monitoring has generally been initiated only more recently. To date, *Pfiesteria* spp. have been found by molecular detection methods in four states—New York, New Jersey, Georgia, and Texas—besides the six discussed in this article (9).

Objectives of PLO-Related Monitoring Programs

All six states reviewed here have similar objectives for the monitoring of regions that may be susceptible to toxic outbreaks of *Pfiesteria*. The commonality of objectives has evolved from common information needs and active participation by these six states in a number of regional forums on monitoring and policy issues since the toxic outbreaks of *P. piscicida* in Chesapeake Bay in 1997. These objectives are linked to two major purposes for conducting this type of monitoring. The first major purpose is to ensure public safety by responding immediately to conditions that may indicate the presence of *Pfiesteria* in a toxic state. This type of monitoring is referred to here as “rapid response” monitoring. It is conducted in response to information received by a state’s hotline, routine monitoring programs, or other sources. The objectives of this type of monitoring are 3-fold:

- to protect human health by identifying potentially toxic PLO outbreaks
- to diagnose fish health or fish kill events
- to provide data for human health studies

The second major purpose of PLO-related monitoring is to provide a more

complete understanding of where *Pfiesteria* and similar organisms may become a threat, to understand what factors may stimulate their growth and toxicity, and to evaluate the impacts of these organisms upon fish and other aquatic organisms. This type of monitoring is referred to here as “comprehensive assessment” monitoring. The objectives of this type of monitoring are 5-fold:

- to evaluate associations between PLOs and environmental variables
- to evaluate associations between PLOs
- to determine distribution of *Pfiesteria* and other PLOs
- to learn if management measurements to improve environmental conditions are having the desired effects
- to provide data for human studies

Elements of PLO-Related Monitoring Programs

The PLO-related monitoring programs for each state contain a number of common elements even though the specific measurement techniques, numbers of stations, level of effort, etc., may vary between states. Furthermore, many of the new monitoring elements for PLO-related issues have been added to and integrated with existing estuarine monitoring programs that differ among states. Some states have also enhanced watershed monitoring efforts in response to PLO issues to examine pollutant loading sources; those efforts will not be discussed here.

In this section I summarize the common monitoring design elements, discuss reasons for including them, and, where appropriate, point out some of the approaches being pursued.

PLO Species Identification

Fundamental to each of the monitoring programs is the identification of PLOs. This aspect of the programs has been very dynamic, as *Pfiesteria* spp. are newly described and a number of the similar species sometimes found in association with *Pfiesteria* or fish health events have not yet been named or fully described. Because *Pfiesteria* and PLOs are relatively small, the features needed for species identification are not visible using light microscopy. Therefore, species identification must often be made using laborious scanning electron microscopy (SEM) procedures following culturing to increase cell densities beyond that which existed at the time of collection. Newly applied molecular identification techniques, however, are rapidly changing the options available to identify PLOs (9–11).

Currently, a combination of the available techniques is required to meet monitoring objectives. Light microscopy provides a rapid tool (within 24 hr in most cases) to determine whether densities of PLOs are sufficient to

potentially be harmful. It is assumed that any *Pfiesteria* cells would be enumerated by this technique and, therefore, if densities are lower than about 100 cells/mL, investigators generally conclude that there is no serious threat to fish or human health at the time of sample collection. Higher densities of cells may pose a threat, but because definitive identification cannot be performed with light microscopy, additional techniques are needed to clarify species identification and toxicity. Epifluorescence light microscopy can be used to distinguish between heterotrophs and autotrophs, thereby helping to eliminate from the enumerations autotrophic species morphologically similar to *Pfiesteria*. *Pfiesteria* is a heterotroph but can function as an autotroph if it sequesters chloroplasts from its prey (12); therefore, care must be used in applying this technique and interpreting the resulting data. Cell densities must also be cautiously interpreted if they are from samples not collected while a fish kill is in progress or after fish lesions have been initiated. Field samples are often taken after a kill has occurred or when lesions show signs of being days to weeks old. Given the “ambush predator” nature of *Pfiesteria*, it is very likely that cell densities in the water column can drop significantly in the hours and days following a toxic event.

Light microscopy is the only technique currently used by state agencies to provide densities of PLOs. Because these are not the only data needed to make decisions about the potential toxicity of a water body, the counts are used simply as a first step in a rapid response evaluation. If light microscopy reveals significant densities of PLOs, this finding can lead to further processing of samples. Methods for doing so may include the use of molecular techniques, bioassays to develop sufficient densities for SEM identification, and bioassays to determine toxicity to fish. In some rapid response protocols, such as those used in Maryland, samples are sent immediately for testing using molecular techniques so that both light microscopy counts and molecular identification results are available within 24 hr. Decisions about whether to start more lengthy and laborious bioassays typically await the results of PLO counts and, more recently, molecular identification results. In some state comprehensive assessment programs, such as those in Virginia, where *Pfiesteria* distributions are being studied, PLO counts have been used to screen for samples that will undergo more detailed testing for species identification.

In areas of potential *Pfiesteria* toxicity, as indicated by fish or human health problems and positive results from light microscopy and/or species identification using molecular techniques, bioassays can be used to further evaluate samples for species identification and toxicity. Incubation of samples with algal

prey suitable for PLOs in the presence of fish is the only method currently available to determine if the sample contains toxic strains of *Pfiesteria* (13,14). After incubation, *Pfiesteria* and other PLOs can be identified by either molecular techniques or SEM. Bioassays using algal prey in the absence of fish are believed to be unreliable for detecting toxic *Pfiesteria* strains (13,14). It is important to start bioassays as soon as possible following field collection because physiological changes (e.g., encystment) occur in a matter of hours to days, thereby delaying the expression of toxicity in bioassays even if the population was actively toxic when collected (13).

Molecular identification techniques for PLOs are advancing rapidly and offer the benefits of species-specific identification in less than 24 hr if needed (10,11). These techniques have been integrated into most state monitoring programs over the past 2 years. In Maryland, molecular techniques have been used routinely since 1999 to quickly identify the two species of *Pfiesteria* in rapid response events and to identify *Pfiesteria* in samples of water and sediment that are taken to meet comprehensive assessment objectives. These techniques also offer the promise of providing semiquantitative information (e.g., cells/mL) in the near future (11); this would be a great benefit to state monitoring programs, as it would eliminate some of the uncertainty that exists in PLO density determinations using light microscopy. If the gene expression underlying *Pfiesteria* toxin production were determined, molecular assays targeting toxicity-associated mRNA transcripts could also, theoretically, lead to methods that distinguish between toxic and nontoxic states (15).

Water quality. All six states routinely sample water quality as part of their PLO-related monitoring programs. An understanding of water quality is critical to a number of objectives for both the rapid response and comprehensive assessment monitoring. For rapid response monitoring, water quality measurements for variables that can be determined immediately by *in situ* probes (dissolved oxygen, pH, temperature, salinity) can aid in the identification of causes of fish health or fish kill incidents. Nutrient and chlorophyll measurements taken during either rapid response or comprehensive assessment monitoring can be used to evaluate associations between these variables and PLOs. Maryland has also included in comprehensive assessments the determination of phytoplankton biomass by horizontal *in situ* fluorometry to assess longitudinal patterns of phytoplankton biomass within river systems. These longitudinal assessments of phytoplankton biomass maxima are important because both PLOs and Atlantic menhaden (*Brevoortia tyrannus*), a fish associated with

Pfiesteria during fish kills and lesion events (1–4), have been associated with elevated phytoplankton biomass (4,16). Sampling of water quality for comprehensive assessment has generally been conducted at weekly to monthly intervals except during winter months in temperate climates. More recently, a number of states have added continuous *in situ* monitors to capture short-term events such as low dissolved oxygen excursions and flow-related changes that may be important influences on PLOs and fish health.

Fish health. All six states have the capability to respond to fish kills and fish health events. These capabilities were in place long before PLOs were an issue. Yet certain fish health events, such as lesioned Atlantic menhaden, have led a number of states to give increasing attention to these incidents; they have increased their staffs and added boats and other resources to help them conduct a much larger number of investigations with a more comprehensive suite of measurements at the event site. For example, additional protocols have been developed to record and manage data related to externally visible anomalies.

Because Atlantic menhaden have been the fish most often associated with toxic *Pfiesteria* events, much of the new fish monitoring effort has been directed to this species. Sampling of menhaden has required the use of cast nets, often the most effective way to capture the small young-of-the-year during summer; they are not captured efficiently in the larger mesh typical of other gear such as trawls or fixed nets. Typical data recorded from fish sampling programs include species, length, and category of externally visible anomaly if present. Tissue samples may be taken for pathological investigations, depending upon the circumstances.

Conclusions

Each of the six states included in this article has responded to the potential threats posed by PLOs by increasing monitoring efforts. Because these states have common information needs and have been participating in regional conferences and workshops, the monitoring programs have been developed with similar objectives and monitoring designs. Despite the commonalities, however, unique circumstances and opportunities in each state have led to differences in the programs. For example, sampling locations in each state have been chosen on the basis of local concerns such as areas that have experienced fish or human health problems or areas that have conditions such as poor flushing and nutrient enrichment that are thought to favor PLOs. In most cases the new monitoring programs have also been linked to existing state monitoring programs. This link

provides opportunities for efficiencies, but it also creates limitations due to differing objectives. Most of the new state monitoring programs also rely on the expertise of local researchers, especially for the identification of PLOs. The result has been productive collaborations, providing the states with leading-edge measurement techniques and giving the researchers access to environmental samples for testing new identification procedures and other experimental studies. Several states send samples to multiple laboratories so that results can be compared and used to improve procedures. There are also a number of strong collaborations between state monitoring programs and harmful algal bloom research, such as that supported by the federal Ecology and Oceanography of Harmful Algal Blooms (ECOHAB) program. These collaborations provide an opportunity to utilize state monitoring data to help answer a number of basic research questions concerning PLOs by complementing the more site-specific or experimental research approaches. This combination of comprehensive monitoring information and targeted experimental approaches will be needed to answer the many complex questions about factors that stimulate PLOs and to provide information about their impacts upon fish and other aquatic resources.

REFERENCES AND NOTES

- Burkholder JM, Noga EJ, Hobbs CH, Glasgow HB. New "phantom" dinoflagellate is the causative agent of major estuarine fish kills. *Nature* 358:407–410 (1992).
- Burkholder JM, Glasgow HB, Hobbs CW. Fish kills linked to a toxic ambush-predator dinoflagellate: distribution and environmental conditions. *Mar Ecol Prog Ser* 124:43–61 (1995).
- Burkholder JM, Glasgow HB. *Pfiesteria piscicida* and *Pfiesteria*-like dinoflagellates: behavior, impacts, and environmental controls. *Limnol Oceanogr* 42:1052–1075 (1997).
- Maryland Department of Natural Resources. Water Quality, Habitat and Biological Conditions of River Systems Affected by *Pfiesteria* or *Pfiesteria*-like Organisms on the Lower Eastern Shore of Maryland: 1997 Summary. Annapolis, MD: Maryland Department of Natural Resources, 1998.
- Glasgow HB, Burkholder JM, Schmechel DE, Tester PA, Rublee PA. Insidious effects of a toxic dinoflagellate on fish survival and human health. *J Toxicol Environ Health* 46:501–522 (1995).
- Grattan LM, Oldach D, Perl TM, Lowitt MH, Matuszak DL, Dickson C, Parrott D, Shoemaker RC, Kauffman CL, Wasserman MP, et al. Learning and memory difficulties after environmental exposure to waterways containing toxin-producing *Pfiesteria* or *Pfiesteria*-like dinoflagellates. *Lancet* 352(9127):532–539 (1998).
- Glasgow HB, Burkholder JM, Morton SL, Springer J. A new species of ichthyotoxic *Pfiesteria* (Dinamoebales, Dinophyceae). *Phycologia* 40:60–71 (2001).
- CDC. Surveillance for possible estuary-associated syndrome—six states, 1998–1999. *Mor Mortal Wkly Rep* 49(17):372–374 (2000).
- Rublee PA, Kempton JW, Schaefer EF, Allen C, Harris J, Oldach DW, Bowers H, Tengs T, Burkholder JM, Glasgow HB. Use of molecular probes to assess geographic distribution of *Pfiesteria* species. *Environ Health Perspect* 109(suppl 5):771–773 (2001).
- Oldach DW, Delwiche CF, Jakobsen KS, Tengs T, Brown EG, Kempton JW, Schaefer EF, Steidinger K, Glasgow HB, Burkholder JM, et al. Heteroduplex mobility assay guided sequence discovery: elucidation of the small subunit (18S) rDNA sequences of *Pfiesteria piscicida* and other related dinoflagellates from complex algal culture and environmental sample DNA pools. *Proc Natl Acad Sci U S A* 97:4303–4308 (2000).
- Bowers HA, Tengs T, Glasgow HB, Burkholder JM, Rublee PA,

- Oldach DW. Development of real-time PCR assays for rapid detection of *Pfiesteria piscicida* and related dinoflagellates. *Appl Environ Microbiol* 66(11):4641–4648 (2000).
12. Lewitus AJ, Glasgow HB, Burkholder JM. Kleptoplastidy in the toxic dinoflagellate, *Pfiesteria piscicida*. *J Phycol* 35:303–312 (1999).
13. Burkholder JM, Marshall HG, Glasgow HB, Seaborn DW, Deamer-Melia NJ. A standardized fish bioassay process for detecting and culturing actively toxic *Pfiesteria*, used by two reference laboratories for Atlantic and Gulf coast states. *Environ Health Perspect* 109(suppl 5):000–000 (2001).
14. Burkholder JM, Glasgow HB, Deamer-Melia NJ. Overview and present status of the toxic *Pfiesteria* complex. *Phycologia* 40:106–134 (2001).
15. Oldach DW, Brown E, Rublee P. Strategies for environmental monitoring of toxin producing “phantom dinoflagellates” in the Chesapeake. *Md Med J* 43(3):113–119 (1998).
16. Friedland KD, Ahrenholz DW, Guthrie JF. Formation and seasonal evolution of Atlantic menhaden juvenile nurseries in coastal estuaries. *Estuaries* 19(1):105–114 (1996).