Report from the NOAA Workshops to Standardize Protocols for Monitoring Toxic *Pfiesteria* Species and Associated Environmental Conditions

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Long-term monitoring of water quality, fish health, and plankton communities in susceptible bodies of water is crucial to identify the environmental factors that contribute to outbreaks of toxic *Pfiesteria* complex (TPC) species. In the aftermath of the 1997 toxic *Pfiesteria* outbreaks in North Carolina and Maryland, federal and several state agencies agreed that there was a need to standardize monitoring protocols. The National Oceanic & Atmospheric Administration convened two workshops that brought together state, federal, and academic resource managers and scientific experts to *a*) seek consensus on responding to and monitoring potential toxic *Pfiesteria* outbreaks; *b*) recommend standard parameters and protocols to characterize water quality, fish health, and plankton at historical event sites and potentially susceptible sites; and *c*) discuss options for integrating monitoring data sets from different states into regional and national assessments. Workshop recommendations included the development of a three-tiered TPC monitoring strategy: Tier 1, rapid event response; Tier 2, comprehensive assessment; and Tier 3, routine monitoring. These tiers correspond to varying levels of water quality, fish health, and plankton monitoring frequency and intensity. Under the strategy, sites are prioritized, depending upon their history and susceptibility to TPC events, and assigned an appropriate level of monitoring activity. Participants also agreed upon a suite of water quality parameters that should be monitored. These recommendations provide guidance to state and federal agencies conducting rapid-response and assessment activities at sites of suspected toxic *Pfiesteria* outbreaks, as well as to states that are developing such monitoring programs for the first time. *Key words:* dinoflagellate, fish bioassay, fish kill, harmful algal bloom, lesion, monitoring, *Pfiesteria, Pfiesteria*-like organism, *Pfiesteria piscicida, Pfiesteria shumwayae*, PLO, presumptive count, toxic *Pfiesteria* complex, toxic *Pfiesteria* outbreak, TPC. — *Environ Health Perspect* 109(suppl 5):707–710 (2001).
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In 1997, when outbreaks of toxic *Pfiesteria* spp. [*Pfiesteria piscicida* Steidinger & Burkholder (*1*), *Pfiesteria shumwayae* Glasgow & Burkholder (*2*)] in North Carolina and Maryland threatened public health and local economies, federal and state agencies initiated a strong cooperative effort to ensure public and environmental safety. This effort combined federal and state resources to monitor environmental conditions and evaluate immediate watershed land use and loadings as potential contributing factors for poor fish health and fish kills. Public health and seafood safety teams were mobilized to ensure public safety, document potential illnesses associated with the events, and assay seafood for toxicity. The National Oceanic & Atmospheric Administration (NOAA), the U.S. Environmental Protection Agency, the Centers for Disease Control and Prevention, and the U.S. Geological Survey contributed to the response effort. This effort included medical diagnoses, epidemiology, fish toxicity studies, and assessments of water quality, fish lesions and mortality, watershed land use, and nutrient and pollution loads.

Although this response was effective, it was only the first step toward addressing the potential threats posed by toxic *Pfiesteria* complex (TPC) species. TPC species are those organisms that resemble *P. piscicida* and have demonstrated toxicity through the production of bioactive compounds that cause erratic behavior, adverse health effects, or kill fish as evidenced in toxic bioassays (*3*). The TPC thus far includes *P. piscicida* and *P. shumwayae*, although other similar species may exist that have not yet been detected. These species are strongly attracted to live fish, which stimulate them to produce bioactive substances that cause fish disease and death, as confirmed in standardized fish bioassays (*1–6*). TPC species can have both toxic and benign strains; therefore, their presence alone does not indicate a problem (*4–7*). Toxic strains are only a threat under certain conditions, when they are stimulated to actively make toxin in response to their detection of the presence of live fish. Long-term monitoring of water quality, fish health, and plankton communities in affected and susceptible waters is crucial to identify the environmental factors that contribute to the presence of toxic strains and the expression of toxicity that occurs during toxic *Pfiesteria* outbreaks.

In the aftermath of toxic *Pfiesteria* outbreaks in North Carolina and Maryland, a number of states initiated or expanded programs to monitor waters believed to be susceptible to toxic *Pfiesteria* outbreaks. To facilitate the intercomparison of data collected by different states, the federal and state agencies agreed there was a need to standardize monitoring protocols.

NOAA Workshops to Standardize *Pfiesteria* Monitoring Protocols

NOAA convened the Workshop to Standardize *Pfiesteria* Monitoring Protocols 14–15 December 1998 in Silver Spring, Maryland. A second workshop that focused on fish health monitoring protocols was held 22–23 June 1999 in Silver Spring, Maryland. Administrators from Atlantic and gulf coast states from New Jersey to Texas were asked to designate up to two people in their state who had responsibility for monitoring water quality, fish health, and plankton communities. Appropriate representatives from federal agencies were also invited, as were academic experts. These workshops had three goals: *a*) to bring together resource managers and scientific experts to seek consensus on responding to and monitoring potential toxic *Pfiesteria* outbreaks; *b*) to recommend standard parameters and protocols to characterize water quality, fish health, and plankton at fish kill/fish disease sites and potentially susceptible sites; and *c*) to discuss options for integrating monitoring data sets from different states into regional and national assessments.

Before the workshop, states sent information to NOAA, describing their current monitoring practices and the storage of the resulting data. This information was compiled by NOAA and distributed to the participants to form the basis for discussion. The workshop was deliberately structured both to facilitate in-depth discussion and to enable the

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group to arrive at consensus. Over the course of 2 days, there were three 2-hr working sessions, one for each topic. Each working session comprised an overview presentation followed by a breakout period during which the attendees divided into three smaller groups for discussion. Working with an expert facilitator, each of the breakout groups explored the questions related to the topic at hand and came up with their own recommendations. The facilitators later integrated the recommendations of the three independent breakout groups into a single set of recommendations. These recommendations were presented to the group during the final plenary session and were unanimously accepted.

The second workshop focused on fish collection procedures, laboratory analyses, and the storage, management, and sharing of data. The recommendations of the second workshop were combined with those of the first and circulated to all the attendees for review and comment. Highlights of these results are briefly summarized below. For a full description, see the full report "Standardized Protocols for Monitoring Toxic *Pfiesteria* Complex Organisms and Associated Environmental Conditions"(*8*). Note that since the NOAA report for this workshop was finalized, a working group comprising federal, state, and academic scientists and managers has developed a consensus glossary of *Pfiesteria*-related terms to provide consistent and scientifically correct definitions of words and phrases used to describe events and activities related to toxic *P. piscicida* and toxic *Pfiesteria*-like species. According to this glossary, "*Pfiesteria*-complex organisms" (PCOs) is an incorrect term that should no longer be used. "*Pfiesteria*-like organisms" (PLOs) is the term used to describe dinoflagellates that look similar to *P. piscicida* under high-power conventional light microscopy (*3*).

Recommendations for Standard Monitoring

The workshop participants proposed a standard suite of water quality parameters, outlined a three-tier monitoring program, and agreed to make agency data available for an integrated electronic database.

Water Quality Parameters

To fully characterize water quality during potential toxic *Pfiesteria* outbreaks at historical toxic *Pfiesteria* outbreak sites and at potentially susceptible sites, the following water quality parameters should be measured. (Detection limits are specified where appropriate.)

- Tidal stage and water depth
- Meteorological conditions
- Current speed and direction
- Light penetration/turbidity (i.e., Secchi depth)
- Temperature (±1°C)
- Salinity (±0.1 ppt)
- pH (±0.2)
- Dissolved oxygen (±0.5 ppm)
- Dissolved ammonia
- Dissolved organic nutrients (carbon, nitrogen, phosphorus)
- Dissolved nitrate plus nitrite
- Dissolved phosphate
- Dissolved silicate
- Chlorophyll *a*

Three-Tiered Monitoring Strategy

Participants developed a three-tiered monitoring strategy. These tiers correspond to varying levels of water quality, fish health, and plankton monitoring frequency and intensity. Under the strategy, sites are prioritized depending upon their history and susceptibility to toxic *Pfiesteria* outbreaks and assigned an appropriate level of monitoring intensity.

Tier 1: Rapid-event response. The goal of Tier 1 monitoring is to characterize water quality, fish health, and the phytoplankton community during fish kills and/or disease events that may be associated with actively toxic *Pfiesteria*. "Toxic *Pfiesteria* outbreak" refers to a fish kill or fish disease event in which an actively toxic TPC species has been collected, identified, and tested with fish bioassays as actively toxic (*3*).

When conducting rapid-event response monitoring during a potential toxic *Pfiesteria* outbreak, it is important to use safety precautions, including protective clothing (e.g., North Carolina's prescribed protocols) and respirators, until all samples are sealed and all equipment decontaminated. Nitrile gloves (not latex) should be worn when touching the equipment, water, or bottles. Bottles and equipment can be decontaminated fairly well by washing in a dilute Clorox solution (30% bleach, i.e., 30% of commercially available bleach as 5% hypochlorite, for a final concentration of 1.5% hypochlorite) (*9*).

WATER QUALITY. To characterize the water quality of a fish kill site or disease event possibly associated with activity by TPC species, participants recommended that water samples be collected along transects outward from the epicenter to beyond the kill zone. The full suite of recommended water quality parameters listed above be measured. In addition, water samples should be collected for bacterial, viral, and fungal pathogens; organic chemicals (pesticides and herbicides); and toxic blue-green algal (cyanobacterial) species, as well as other potentially harmful algae.

FISH HEALTH. Rapid-response teams monitoring fish health during fish kill or disease events should determine the extent of the event, the overall environmental conditions, and the condition of the fish present. When possible, response personnel knowledgeable about fish health, including a fish pathologist or fish disease specialist, should be on site to respond to the event. American Fisheries Society procedures should be used to determine the geographic extent of the event. Fish behavior and overall environmental conditions should be noted. The response team should estimate the mortality and prevalence of lesions and the location of lesions on each species of fish affected. Fish should be collected and necropsied for histopathology, parasitology, and microbiology (bacteriology, mycology, virology). Diseased, fresh dead, and healthy fish should be subsampled for pathogen analyses. If there are pathologists or other trained personnel on site, samples should be field processed and preserved with the appropriate fixative and transport media. If no such trained personnel are available, fish should be individually bagged and transported on ice to the appropriate laboratories for analysis. For toxicological analyses, fish must be flash frozen. Tissue, blood, and whole fish should be archived in a freezer for retrospective analyses.

PHYTOPLANKTON MONITORING. During an in-progress fish kill or fish disease event, or when fish are acting erratically even without signs of disease, phytoplankton samples should be collected from the immediate vicinity and preserved with acidic Lugol's solution (*10*). Unpreserved water should also be collected. Presumptive counts should be conducted on acidic Lugol's-preserved plankton samples using light microscopy to determine the presence and abundance of PLOs. Presumptive counts are counts of the number of PLOs in a given volume of water and conducted as the first step in determining whether TPC was involved in a fish distress event (*3*). If presumptive counts are high (approximately 100 cells/mL or more), standardized fish bioassays should be conducted with the unpreserved water samples to test for toxicity (*6*). This standardized fish bioassay is currently the only accepted technique for the confirmation of actively toxic strains of TPC species. In replicated fish bioassays, fish are exposed to the unpreserved water samples collected during the fish kill/disease event (*4,6,11*). If these fish become stressed, behave erratically, become ill, or die in <21 days in the presence of an active population of *Pfiesteria*-like zoospores, absent other apparent cause (determined from intensive monitoring of physical/chemical conditions and other potentially harmful microorganisms), the test is considered positive for a toxic PLO. If the fish bioassay is positive, *Pfiesteria*-like zoospores should be isolated from the water in clonal cultures (usually fed benign algal prey for 1–3 weeks), then added to a second

set of fish bioassays to test for toxicity of the cloned dinoflagellates. A set of control fish bioassays is similarly maintained for comparative purposes with both sets of test fish bioassays. All fish bioassays are intensively monitored to ensure that other conditions or organisms were not involved in fish death. If fish death occurs in the test bioassays with clonal *Pfiesteria*-like zoospores at ≥300 cells/mL and no other apparent cause but not in the control bioassays, the zoospores are reisolated, cloned, and identified to species level with scanning electron microscopy on suture-swollen or membrane-stripped cells (*1,2,12*).

Laboratory samples for presumptive counts and for fish bioassays should be analyzed "blind" to guard against bias. When presumptive counts in fish bioassays reveal PLOs in concentrations of potential concern in association with fish disease or death (≥100 or ≥300 zoospores/mL, respectively) samples should be split and analyzed by two different laboratories to enable cross-corroboration of findings about the presence of actively toxic strains of TPCs. Whenever possible, those molecular probes that have been field-tested, cross-corroborated by independent laboratories with appropriate expertise, and shown to be reliable for the detection of TPC species should be used. Also, if assays to detect possible TPC toxins become available and reliable, they should be incorporated into monitoring (*13*).

Tier 2: Comprehensive surveys and assessments. The goal of Tier 2 is to monitor the distribution and abundance of TPC species and assess fish health and environmental conditions at sites that have historically supported or are believed to be susceptible to toxic *Pfiesteria* outbreaks.

In addition to characterizing historical and/or susceptible sites, Tier 2 monitoring may provide managers with early warning of toxic *Pfiesteria* outbreaks. Therefore, this level of monitoring should be conducted during the primary event season, i.e., summer and early fall, the time of year when toxic *Pfiesteria* outbreaks have most frequently occurred.

WATER QUALITY. The measurements and procedures listed under "Tier 1: rapid-event response" should be conducted to characterize the water quality at historical or susceptible event sites.

FISH HEALTH MONITORING. The measurements and procedures listed under "Tier 1: rapid-event response" should be conducted to assess the health of fish at sites that have historically supported or are believed to be susceptible to toxic *Pfiesteria* outbreaks. In addition, fish health should be monitored both before and after the primary event season to establish a baseline for those sites.

PLANKTON MONITORING. The measurements and procedures listed under "Tier 1: rapid-event response" should be conducted to assess the plankton community, including the presence of TPC species, at sites that have historically supported or are believed to be susceptible to toxic *Pfiesteria* outbreaks.

Tier 3: Routine monitoring. The goal of Tier 3 monitoring is to characterize the long-term dynamics of water quality, fish health, and plankton communities in less-susceptible coastal waters.

Routine monitoring can be added to existing monitoring activities. This level of monitoring can help managers understand the relationship between TPC species and water quality, fish health, and other phyto- and zooplankton.

WATER QUALITY. The measurements and procedures listed under "Tier 1: rapid-event response" should be conducted to characterize long-term water quality dynamics.

FISH HEALTH MONITORING. General fish health, behavior, and any external abnormalities should be noted when routinely monitoring fish health. The locations of any lesions should be noted on fish diagrams. In addition, fish populations should be sampled with cast nets to capture small fish (1–2 inches). A subsample of healthy and lesioned fish should be necropsied to examine gross pathology.

PLANKTON MONITORING. Integrated water-column samples and surficial sediment samples should be collected to characterize plankton populations. The plankton community should be identified and quantified to the species level, if possible. When available, molecular probes that have been cross-corroborated by independent specialists should be used, as indicated above, to help detect the presence of TPC species.

Data Integration

The participants agreed that quality-assured, consistent state monitoring data would facilitate decision making related to human health and natural resources and the development of regional assessments. These data would be most useful in a database that provided user-friendly access, links to geographical information system applications, the ability to integrate with other national databases, and the ability to facilitate data summary and retrieval. The state managers agreed to make their monitoring data available for such a database.

Other Results and Recommendations

Participants identified the need to work together to respond to suspected toxic *Pfiesteria* outbreaks and to characterize the conditions conducive to such events. When asked to identify how their monitoring efforts could be improved, many participants mentioned the need for continuous, *in situ* monitoring of environmental conditions. They strongly called for the development and testing of new technologies such as probes and toxin assays and encouraged the federal government to support to these efforts.

In response to these recommendations, NOAA developed the intensive Harmful Algal Bloom (HAB) Monitoring Program in 1999. This program funds small teams of federal, state, tribal, and academic researchers to develop pilot monitoring programs that focus on the environmental conditions that may be conducive to HABs, including TPC species. These pilot projects enable researchers and managers to experiment with and develop new monitoring methods and incorporate new technologies, when appropriate. In 1999, projects were initiated in Maryland and Florida to study *Pfiesteria* and *Pfiesteria*-like organisms. In 2000, the program was expanded to include HABs other than TPC species; a pilot project was initiated in Washington State to study blooms of the potentially toxic diatom *Pseudo-nitzschia*. This program will be expanded in the future, as funding allows, to support other projects on a competitive basis.

Summary

The NOAA Workshops to Standardize *Pfiesteria* Monitoring Protocols filled a need expressed by both federal and state agency managers for consistent protocols to monitor suspected toxic *Pfiesteria* outbreaks. More than 60 managers and scientists who participated in these workshops reached consensus on the need for consistency in the parameters measured, the types of monitoring needed, and the need to share data to further understand toxic *Pfiesteria* outbreaks. All recommendations put forth by this group were unanimously agreed upon. The participants called for concurrent collection of phytoplankton, fish health, and water quality samples for each tier of their program. They outlined a three-tiered monitoring program that prioritized sites depending upon their history and susceptibility to toxic *Pfiesteria* outbreaks and assigned an appropriate level of monitoring intensity. They identified the need for new, innovative monitoring methods and called on the federal government to encourage this kind of research and development. These recommendations provide guidance to state and federal agencies conducting rapid-response and assessment activities at sites of toxic *Pfiesteria* outbreaks as well as to those states developing such monitoring programs for the first time.

References and Notes

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