Pfiesteria: Background Information and Contingency Plan

May 24, 2000

Prepared for:
New Jersey Department of Environmental Protection (DEP)
New Jersey Department of Health and Senior Services (DHSS)

By:
DEP - Division of Fish & Wildlife (DF&W)
DEP - Division of Responsible Party Site Remediation (DRPSR)
DEP - Division of Science, Research & Technology (DSRT)
DEP - Division of Watershed Management (DWM)
DHSS - Division of Epidemiology, Environmental and Occupational Health (DEEOH)

Table of Contents

Background 1
Summary 1
Possible Human Health Effects. 2
Current New Jersey Situation 2

Response To Potential *Pfiesteria* Events 5
Operational Guidelines and Notification Procedures. 5
Sampling Criteria 5
Water Sampling Safety Protocol. 6
Water Sampling Protocol 9

Protocol for Closing and Reopening Waterways 11
Affected by *Pfiesteria* or *Pfiesteria*-Like Events
Waterways 11
A Waterway Closure will be Recommended When. 11
A Waterway will be Recommended for Reopening When 11
A Temporary Advisory will be Issued When 11
Comments to Consider 12
Who to Call 12
Internal Communications 13
External Communications, Public Education, Outreach 14
Epidemiologic Investigation 14

Areas of Responsibility (Lead Agencies) 15

Background

Summary

- *Pfiesteria,*(pronounced "fee-steer-ee-uh"), *and Pfiesteria* - like species are microscopic aquatic life forms known as a dinoflagellates; a group of single celled organisms which have the ability to swim in the water column. This group of organisms is known to exhibit characteristics of both plants and animals.

- *Pfiesteria piscicida* (*Pfiesteria*) is sometimes referred to as the "phantom dinoflagellate," or "ambush algae," because it can stay dormant in the sediment for long periods, suddenly emerge en masse to prey upon fish, and then vanish just as quickly from the water column. This characteristic makes it very difficult to effectively monitor for the organism in the environment.

- *Pfiesteria* was first identified and characterized in 1991. It has been found in coastal waters and tributaries of the East Coast and the Gulf of Mexico. It is assumed that the organism has existed for thousands of years. It has not been found in freshwater lakes or streams.

- The organism is not a disease-causing pathogen (e.g. the organism does not multiply within the body, nor is it contagious to other persons); rather it is the toxin produced by the organism that causes the adverse health effects observed.

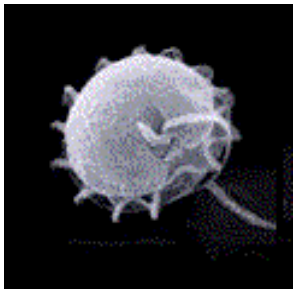


Figure 1

- *Pfiesteria piscicida* is a polymorphic organism (see Figures 1 & 2) with as many as twenty-four (24) different stages in its life cycle, only a few of which produce toxins. *Pfiesteria piscicida* possesses a wide temperature and salinity tolerance, ranging from nearly freshwater (2 psu) to full-strength seawater (35 psu), and temperatures between 50°F and 90°F. However, toxic outbreaks occur most frequently when water temperature is about 75°F or greater, and at a salinity of around 15 psu. Additional environmental conditions suspected to trigger toxic outbreaks of *Pfiesteria* include calm - slow flowing waters and large amounts of fresh fish secreta/excreta.

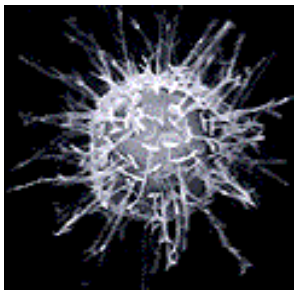


Figure 2

- Besides preying upon fish, *Pfiesteria piscicida* is known to be a predator of other estuarine microorganisms, such as bacteria, algae, and ciliates. Under laboratory conditions, *Pfiesteria* has demonstrated toxicity to every finfish and shellfish species tested, including blue crabs, young eastern oysters, littleneck clams, bay scallops, striped bass, mullet, croakers, spot, eel, menhaden, flounder, and largemouth bass. In waters where *Pfiesteria* is known to occur under natural conditions, the organism is associated with a finfish commonly referred to as

menhaden, or "moss bunker." Menhaden are a small fish, up to fourteen (14) inches in length, that are commonly used for bait or animal feed.

- The exact structure of the two (2) known *Pfiesteria* toxins is not as yet understood; however, it is known that one toxin depresses the central nervous system while the other dissolves the skin mucus layers.

- *Pfiesteria* has been linked both to massive fish kills in North Carolina's Albemarle-Pamlico estuary, starting in the early 1990's, and to recent (1997) fish kills in the Pocomoke and Chicamacomico Rivers, and King's Creek, three tributaries to the Chesapeake on Maryland's Eastern Shore (see Figure 3).

- Presently, testing for the presence of *Pfiesteria* is complicated, time -consuming, and requires highly specialized facilities, equipment, and training. No short term, simple laboratory tests for confirmation of the presence of the organism or the toxins exist to determine exposure, although ongoing research is promising.

Figure 3

Possible Human Health Effects

- *P. piscicida* has been linked to serious human health effects among laboratory workers exposed to either water or aerosols from *Pfiesteria* cultures in the toxic stage. Effects include epidermal lesions, respiratory distress, stomach cramping, disorientation, behavioral changes, erratic heart beat, short-term memory loss and/or severe cognitive impairment, and compromised immune systems. Most of these effects reverse over time.

- Recently, commercial watermen working in the affected areas in Maryland, have exhibited many of the symptoms linked to *Pfiesteria* exposure in the laboratory, namely skin lesions, memory loss, respiratory problems, stomach cramps and vomiting.

Current New Jersey Situation

- By correlating the known environmental condition preferences of *Pfiesteria* and *Pfiesteria*-like organisms with the Department of Environmental Protection (DEP) ambient water monitoring database, a map demonstrating an estimation of the level of potential risk for *Pfiesteria* outbreaks has been developed (see Figure 4).

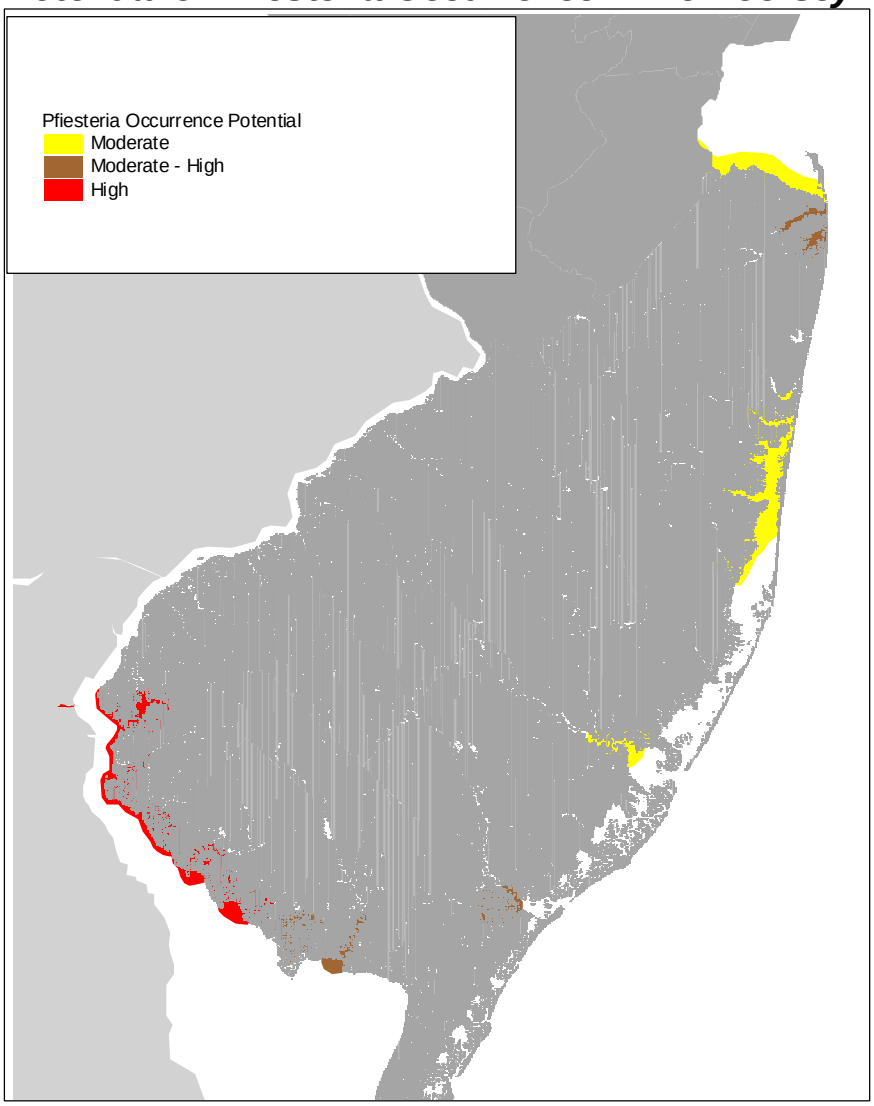
- This estimation of the potential for a *Pfiesteria* outbreak could be used in a variety of ways. For instance, fisheries biologists and public health personnel could be informed of areas of potential concern. Furthermore, if a decision were made to initiate any ambient water monitoring, the information could be used to prioritize sampling.

- Between August and November, 1999, the DEP, Division of Science, Research and Technology collected 38 water and 18 sediment samples from 32 estuarine sites within most of the shaded areas of figure 4. These samples were tested by Dr. Parke Rublee, University of North Carolina at Greensboro, for *Pfiesteria piscicida* and 2 other species, using a DNA-based assay. *Pfiesteria piscicida*-specific DNA was detected at one of the estuary areas. This test cannot tell if live *Pfiesteria* organisms are present, how many are present, or whether or not the organisms are or were toxic. Despite this testing, the geographic distribution of *Pfiesteria* in NJ estuaries has not been adequately characterized. Thus, additional sampling is anticipated. For current information on *Pfiesteria* research in NJ, contact the DEP, Division of Science, Research and Technology, (609) 984-6070.

- In September, 1999, the DEP responded to a report of a fish kill in the Tuckahoe River at Corbin City, Atlantic County. A water sample was collected and submitted to the laboratory of Dr. JoAnn Burkholder, North Carolina State University, for analysis to determine if toxic *Pfiesteria* complex (TPC) organisms were present. Toxic *Pfiesteria* organisms were not found in this sample nor was *Pfiesteria* DNA found in the sample. The day after DEP responded to this fish kill, Tropical Storm Floyd arrived in the region and ended the conditions that contributed to the fish kill. No further action was initiated.

- This contingency plan is a work in progress, subject to change and enhancement as more is learned about *Pfiesteria*, as practical experience with components of the plan is obtained, and as the experiences of the other mid-Atlantic and Southeastern States can be incorporated. The plan has been jointly developed by the DEP (Division of Science, Research and Technology, Division of Fish and Wildlife, Division of Watershed Management, Division of Responsible Party Site Remediation) and the DHSS (Division of Epidemiology, Environmental and Occupational Health) as both a training aid for internal monitoring staff (boat captains, fisheries biologists, ambient monitoring staffs, etc.), and as the blueprint for the State of New Jersey response should a *Pfiesteria*-like fish kill occur in New Jersey. The contingency plan describes criteria for the collection of water samples designed to confirm the presence of *Pfiesteria*, safety protocols for the collection of samples, QA/QC protocols for the collection of the samples, and policies for the closure and opening of coastal waters to primary contact activities (e.g. bathing, waterskiing, etc.) and other recreational activities such as fishing.

Potential of Pfiesteria Occurrence in New Jersey



New Jersey Department of Environmental Protection
Water Monitoring Management

Figure 4

Response To Potential *Pfiesteria* Events

Operational Guidelines and Notification Procedures:

The DEP, Division of Fish & Wildlife (DF&W) will use the following guidelines when presented with a possible *Pfiesteria* caused fish kill:

1. A fish kill should be reported to the toll-free DEP Action Line, (877) WARNDEP, or Trenton Dispatch.

2. A Conservation Officer (CO) or biologist (depending on availability) will go to the scene and make an assessment as to whether or not *Pfiesteria* samples should be collected based on the protocol given in this document. (See next section)

3. The responder will contact Chief, Bureau of Law Enforcement (DF&W) or designee, who in turn will contact both, Administrator, Marine Fisheries Administration and the Bureau of Emergency Response (DRPSR). If the responding CO is equipped with a boat and a sample collection kit, he/she will assume the responsibility for assisting the Emergency Response team, for shipping the samples to North Carolina State University Department of Botany (NCSU) and for the follow-up phone call as prescribed in the protocol below.

4. If the responder does not have a boat and/or sample collection kit, Marine Fisheries will make the necessary arrangements for the transport of a boat (if necessary) and sample containers to the Emergency Response Team at the designated location.

5. If the fish kill has the outward appearance of a *Pfiesteria*-related event, the Emergency Response Coordinator will notify and update the DEP Commissioner's Office and Press Office, and the DHSS Division of Epidemiology, Environmental and Occupational Health.

6. Emergency Response Team members will collect, preserve and label sample containers as prescribed in the protocol.

7. The designated DF&W support staff member will receive the boxed samples, seal and label the shipping containers, affix postage to each sample and send via overnight mail to NCSU as prescribed in the protocol.

8. The individual mailing the samples will then telephone NCSU and advise of the shipment of samples.

Sampling Criteria:

Water samples should be collected from one (1) site within the area of dead or dying fish when one or more of the following criteria are met:

- A large fish kill for which no readily apparent cause, such as low dissolved oxygen, can be identified.
- Fish kill occurs in estuarine or near-shore coastal waters.
- Fish kill takes place over a period of several days.
- Fish kill involves either menhaden or another species traveling in large schools.
- Fish display erratic swimming behavior, sporadic movements, disorientation, and lethargy.
- Fish are exhibiting characteristic lesions, namely shallow, bleeding ulcers of the skin (see Figures 5 and 6). However, since the absence of lesions does not rule out the presence of *Pfiesteria*, failure to observe lesions should not negate the collection of samples. This would be particularly true if large numbers of menhaden, observed to be displaying lethargic or erratic swimming behavior, are dying in the absence of any apparent cause.

Figure 5

Figure 6

It is important to keep in mind that sores and lesions occur naturally at a low frequency every year in all fish communities, and that there are many types of skin abnormalities in fish that are not lesions from *Pfiesteria piscicida.* These abnormalities fall into two major categories:

Abrasions: Abrasions are scrapes, patches of missing scales, or other superficial anomalies. These may be mechanical injuries caused by nets, handling by fishermen, rubbing against other fish or the water bottom, etc.

Other Lesions: Other lesions may take the form of ulcers, swellings, reddening, discoloration and bleeding. *Pfiesteria* toxins are among the known causes for lesions, but are not the only causes. The most typical lesions caused by *Pfiesteria* are often large, round, deep (usually into the muscle), bleeding, and are usually near the anus. It is important to note that even in the presence of *Pfiesteria* toxins, lesions are not always visible to the observer.

Water Sampling Safety Protocol:

DEP-BER SOP FOR *PFIESTERIA* SAMPLING

The DEP Bureau of Emergency Response (BER) of the Division of Responsible Party Site Remediation has entered into an agreement to provide response personnel to work in conjunction with the DF&W for the purpose of obtaining water samples in suspected *Pfiesteria* tainted waters. This sampling will take place on an emergency basis when DF&W personnel suspect a possible *Pfiesteria* toxin exposure. The toxins released by the *Pfiesteria* dinoflagellate have been shown to be dermal and respiratory irritants as well as causes of memory difficulties and behavioral changes. These symptoms have been documented primarily in watermen who are subject to prolonged, repeated exposure to contaminated waters. With respect to these recognized hazards, only properly trained personnel using the appropriate protective equipment should sample known or suspected contaminated waters. BER personnel, who are already trained, fit-tested, medically monitored, and properly equipped, will be able to perform this duty and meet the requirements of the PEOSH respiratory protection standard and the personal protection equipment standards. The following are the standard operating procedures which will be followed for the sampling operation:

1. A team of two Responders will be deployed to a sampling request.

2. The Responders will provide the required Personal Protective Equipment (PPE), Personal Flotation Device, and communications equipment. The BER Responders will have successfully completed the Boaters Safety Course.

3. The DF&W will transport the boat, sampling equipment, and a supply of cleaning water to the sampling site. The boat will have a First aid kit.

4. BER Responders, wearing personal flotation devices and PPE will travel by boat to the sampling site, take the samples, record all pertinent observations, and return to the departure point. The samples will be relinquished to DF&W personnel for eventual lab analysis. Sample locations will be fixed using two separate handheld GPS units or LORAN and coordinates will be recorded.

PPE PROTOCOL FOR *PFIESTERIA* SAMPLING

1. A Personal Flotation Device (Type II PFD Vest/Float Coat) will be worn at all times.

2. PPE will consist of Level C or Level B gear.

Respiratory:	Minimum of full-face respirator with combo cartridges. SCBA/Escape bottle may be used at the discretion of responders, keeping in mind the increased safety hazard of boat work.
Skin:	Minimum of Coated Tyvek or Vinyl Acid Suit, with hood to prevent contact with water or spray.
Hands:	Forearm-length nitrile glove(s).

PPE PROCEDURES DURING *PFIESTERIA* SAMPLING

1. The nature of the *Pfiesteria* toxins requires respiratory and dermal protection, as well as attention to proper contaminant decontamination.

2. Prior to embarking on the boat, responders will don personal floatation device, protective suit, nitrile gloves, and boots. To enhance protection from contaminated water, the suit will be taped at glove and boot junctions. The storm flap on suit will be secured or taped.

3. Boat travel through non-contaminated waters may be made without a respirator or Lance-gloves. When nearing suspected contamination area, respiratory equipment and suit hood will be donned.

4. When transiting suspected contaminated waters the boat will be operated so as to minimize water spray and aerosolization of the toxin.

5. Lance-gloves will be donned and taped for the sampling procedure. The samples will be taken from one foot below the water surface attitude (open, invert, and right under water). The boat will be placed in a secure attitude (zero headway, safe boating procedures) during sampling. The attending responders will act as a safety spotter for the sampling responder, watching for boat wakes, and other hazardous conditions.

6. Subsequent to sampling, the samples will be preserved (when required), closed, wiped down, and secured for transit. Responders will remove outer gloves and contaminated towels, etc. and deposit and secure in a trash bag. Suit areas contacted by contaminated water will be cleaned using water spray.

7. Departure from the contaminated area will be made minimizing water spray. Upon arrival at a dock or in clean water, respirators and hoods may be removed, keeping in mind proper doffing procedures. An extra pair of nitrile gloves should be maintained for handling sample jars or potentially contaminated equipment. All such equipment should be decontaminated at the dock using a bleach/water solution. Disposable PPE and equipment should be bagged for disposal. Respirators should be thoroughly cleaned using the standard respirator decontamination solution. Upon removal from the water, the boat should be decontaminated.

8. Throughout the entire *Pfiesteria* operation, all personnel should maintain a heightened awareness for **heat stress** and take appropriate precautions (fluid intake, rest breaks, emergency cooling). In addition, boating safety techniques become more important due to the use of PPE and taking of samples. Donning of full PPE can be done just prior to entering contaminated area to minimize heat stress; however, if contamination possibility is unknown then a judgement must be made as to when to don personal protection.

BOAT EQUIPMENT LIST FOR *PFIESTERIA* SAMPLING

1. Cooler containing sampling jars, preservative, and other equipment.
2. Lance-gloves, nitrile gloves, duct tape.
3. Sprayer containing bleach/water.
4. First aid kit.
5. Paper towels.
6. Garbage bags.
7. Cell phone and/or radio.
8. Handheld GPS units.

Water Sampling Protocol:

- Water samples are to be collected from one site within the area where dying fish are observed.

- All sampling locations shall be accurately determined using either GPS or LORAN.

- Each water sample is to be collected from approximately one (1) foot below the surface of the water.

- At each collection site, two (2) samples, a preserved and an unpreserved water sample, are to be collected.

  - 500 ml unpreserved sample volumes should be collected in either a plastic or glass container.

  - 250 ml sample volumes preserved with acidic Lugol's solution (a 0.01% solution to roughly a golden-orange color) should be collected in either a plastic or glass container.

- Samplers are to record on the sample record sheets the location, date and time of both the sample collection and the fish kill which prompted the sampling. A copy of the sample sheet is to be kept with the samples during transport to the analytical laboratory.

- All sample containers should be clearly marked with the sample number, the sampling date, the sampling time, and the sampling location.

- On either the sample record sheets or in a sampling log book, record observations of fish behavior, fish appearance, the presence of any external lesions, the species of fish involved in the event, and approximate numbers affected, time and date of the onset of the fish kill, and any other observations thought to be pertinent.

- Samples are to be packaged in a sturdy shipping container with an inner plastic liner[1]. Include the sample record sheet with the samples and the name, address, and phone number of the person to be contacted with the results of analysis.

- Samples are to be shipped, by overnight Express[2] mail, to:

  Dr. JoAnn Burkholder
  Department of Botany
  North Carolina State University
  Box 7612
  Raleigh, N.C. 27695
  and;
  Contact Dr. Burkholder by telephone at (919) 515-2726 or (919) 515-3421 when shipping samples to let her know (usually by a voice-mail message) that samples have been forwarded. If your office has an Internet connection, an e-mail notification can also be made to the NCSU Aquatic Botany Laboratory at the following addresses:

  howard_glasgow@ncsu.edu
  FaganJohns@aol.com

---

[1] Nalgene® produces a packaging system complete with sample bottle, absorbent material and inner plastic liner. Catalog number 9214-0500 contains a 500 ml sample bottle and catalog number 9214-0250 contains a 250 ml plastic bottle. Each sample can be packaged and shipped separately.

[2] A package containing samples from three (3) sites (three 500 ml unpreserved and three 250 ml preserved samples) should weigh between six (6) and seven (7) pounds.

Protocol for Closing and Reopening Waterways Affected by *Pfiesteria* or *Pfiesteria*-Like Events

Waterways

A Waterway Closure will be Recommended When:

1. A fish kill of a significant nature is confirmed and the affected fish display *Pfiesteria* or *Pfiesteria*-like sores; or
2. A significant number of fish are confirmed to be acting erratically without apparent explanation for the behavior (such as low dissolved O_2) ; or
3. There is evidence of increased *Pfiesteria* or *Pfiesteria*-like activity as reflected by an increase in the number of fish with *Pfiesteria* or *Pfiesteria*-like lesions.

Procedures for Waterway Closure:

1. Based upon the presence of one or more of the above conditions, the DHSS will recommend closure of the affected area to the local or county health authority.
2. The DHSS will consult with and coordinate recommended closure activities with the DEP.
3. Closure boundaries will be determined through visual observations.
4. Waterways that are closed will be visually inspected and assessed for *Pfiesteria* or *Pfiesteria*-like activity.
5. Notification of waterway closures will be posted by the local or county emergency response coordinator to protect the public from possible health complications which may result from direct water contact while the *Pfiesteria* toxin is active.
6. The DHSS will notify the New Jersey State Police Marine Law Enforcement Troop and the US Coast Guard of a waterway closure.

A Waterway will be Recommended for Reopening When:

1. Analytical results of water sampling for toxic *Pfiesteria* or *Pfiesteria*-like organisms are negative; or
2. The conditions that initiated the closure have abated for 14 days.

Procedures for Waterway Reopening:

1. The DHSS will recommend the reopening of the waterway to the local health authority after coordinating this action with the DEP.
2. Notification postings will be removed immediately by the local health authority upon reopening of the waterway.
3. The local health authority will notify the New Jersey Police Marine Law Enforcement Troop and the Coast Guard upon reopening of the waterway.

A Temporary Advisory will be Issued When:

1. A fish kill of a significant nature is confirmed; and
2. The fish involved do not display *Pfiesteria* or *Pfiesteria*-like sores; and
3. No other explanation for the fish kill is apparent. (ex - low dissolved O_2)

Procedures During the Issuance of a Temporary Advisory:

1. A preliminary water analysis for *Pfiesteria* of *Pfiesteria*-like organisms will be done to rule out *Pfiesteria* or *Pfiesteria*-like organisms as a cause of the fish kill.
2. If the preliminary water analysis reveals that the cause is *Pfiesteria* or *Pfiesteria*-like organisms, the DHSS will coordinate the appropriate amendment of the temporary advisory to recommended waterway closure with the DEP. The amended status will be communicated to the local health authority.
3. If the preliminary water analyses reveal that *Pfiesteria* of *Pfiesteria*-like organisms are not the cause, the DHSS will recommend that the advisory be removed.

Comments to Consider:

1. Current knowledge indicates that toxins emitted by *Pfiesteria* and *Pfiesteria*-like organisms break down in less than 48 hours. Investigation is ongoing to identify the nature and activity of the toxins. As new information becomes available this protocol may be amended to reflect current knowledge.
2. The behavior of *Pfiesteria* and *Pfiesteria*-like organisms is seasonal and episodic in nature. Therefore, recommended closures and reopenings may be repeated as necessary.
3. This protocol is subject to continuous evaluation and modification.

Who to Call:

To report fish kills or fish lesions call:

Toll-free NJDEP Action Line
(877) WARNDEP

or,

Regional Offices:
Marine Region - Nacote Creek Research Station: (609) 748 - 2050
(Atlantic, Cape May, Cumberland, Middlesex, Monmouth, Ocean and Salem Counties)
Southern Region: (856) 629 - 0555
(Atlantic, Camden, Cape May, Cumberland, Gloucester and Salem Counties)
Central Region: (609) 259 - 2120
(Burlington, Mercer, Middlesex, Monmouth and Ocean Counties)
Northern Region: (908) 735 - 8240
(Bergen, Essex, Hudson, Union, and non-estuary counties)

or

The New Jersey Department of Environmental Protection
Division of Fish and Wildlife
Marine Fisheries Administration
Mr. Tom McCloy, Acting Administrator
(609) 292-7794

To report possible adverse health effects on persons in contact with fish with lesions or *Pfiesteria* toxin in a waterway call:

The New Jersey Department of Health and Senior Services
Division of Epidemiology, Environmental and Occupational Health
Mr. Ronald S. Ulinsky, Program Manager
Public Health Sanitation and Safety Program
Office: 609-588-3124

or,

Mr. John E. Sharp, Coordinator - Environmental Health Hazards
Public Health Sanitation and Safety Program
Office: 609-588-3124

or,

Mr. James A. Brownlee, M.P.H., Director
Consumer and Environmental Health Services
Office: 609-588-3120

Evening and weekends, please contact the Department's Answering Service at (609) 392-2020 or (609) 888-1900.

Internal Communications

1. DHSS contacts, Messrs. James A. Brownlee, Ronald S.Ulinsky or John E. Sharp, will notify supervisory and subordinate staff members upon receipt of notification of a toxic outbreak, fish lesion or fish kill event.
2. Notification shall be transmitted from the program level to the Office of Commissioner through established organizational channels.
3. Confirmation of events will be conducted by the DHSS, Public Health Sanitation and Safety Program through collaboration with the DEP, local health authorities, laboratories, health care providers, and field observations and interviews, as necessary.

4. Sampling events of the impacted waterway will be conducted cooperatively among the DHSS, DEP and the local health authorities. Analytical results will be evaluated by the DHSS and DEP. Appropriate amendments to the course of action will be issued

based upon sample results and field observations.

5. Upon notification of a *Pfiesteria* event, DHSS will notify regional federal contacts.

Mr. Robert Dieterich
USEPA Region II
290 Broadway
New York, NY 10007-1866
212-637-3794

6. A recommended action plan for sampling, issuance of a temporary advisory and possible waterway closure shall be developed among the DHSS, DEP and the local health authorities.
7. Notification of waterway closure will be issued by the DHSS to:

N J State Police, Troop F
Marine Law Enforcement
Lt. Walter Schwatka
(609) 882-2000 ext. 6171
After hours operator
(609) 882-2000

US Coast Guard
MSO Phildelphia
1 Washington Street
Philadelphia, PA 19147-4395
(215) 271-4992

External Communications, Public Education, Outreach

Upon arriving at a recommended course of action to close a waterway, the DHSS, Public Health Sanitation and Safety Program will inform the local health authorities. Fact sheets will be distributed to the local health authorities as well as health care providers and all health care facilities in the impacted area. Fact sheets will provide information on *Pfiesteria* and the health-related signs and symptoms that have been attributed to the organism. A Fact Sheet entitled "What You Should Know About *Pfiesteria piscicida*", developed by the USEPA, NOAA, USDA, USGS, USDHHS, and the Association of State and Interstate Water Pollution Control Administrators, is available on-line at http://www.epa.gov/owow/estuaries//pfiesteria. A second [draft] Fact Sheet entitled "The Facts on Pfiesteria", by the USEPA and States that are either currently or potentially impacted by *Pfiesteria*, is under development. These fact sheets will be key references in communicating risks to the public.

In cooperation with the DEP, information will be made available to the public through the respective state agency communications office. To obtain information related to *Pfiesteria*, individuals should contact their local health department or access information at the New Jersey Department of Health and Senior Services Web site: **www.state.nj.us/health** .

Epidemiologic Investigation

1. A *Pfiesteria*-Related Illness Report Form will be developed by the DHSS and provided to local health departments and local medical care providers. The form will request

information on basic demographics, signs and symptoms of illness experienced, results of laboratory testing, and exposure to affected waterways.

2. Completed forms will be relayed to the DHSS for compilation and analysis.
3. The need for further studies and analysis will be determined according to results of the preliminary analysis and consultation with *Pfiesteria* medical experts at the Centers for Disease Control and Prevention (CDC) and elsewhere.

Areas of Responsibility (Lead Agencies)

In order to ensure the most efficient and effective use of resources, the following lead agencies and corresponding areas of responsibility are delineated:

- Investigation and monitoring of fish kills suspected to be caused by *Pfiesteria* or *Pfiesteria* - like organisms.....

  NJDEP- Division of Fish and Wildlife

- Evaluation of human health effects and issuance of health advisories, and waterway closures.....

  NJDHSS - Division of Epidemiology, Environmental and Occupational Health

- Communications with the press and general public.....

  NJDEP - Office of Communications
  NJDHSS - Office of Communications

- Explanation and description of ambient water quality leading to potential for *Pfiesteria* outbreaks.....

  NJDEP - Division of Watershed Management - Water Monitoring Management

Pfiesteria Monitoring in New Jersey 1998 – 2000

NJ Department of Environmental Protection

Fourth Update: June 8, 2007
(www.state.nj.us/dep/dsr)

Thomas Atherholt and Bruce Ruppel
Division of Science, Research & Technology
P.O. Box 409
Trenton, New Jersey 08625-0409
(609) 984-6070

Note: No additional data has been generated since the update of April 15, 2001. New information on *Pfiesteria* published since the last update is incorporated in this update. Some of this information necessitated a change in background information and in the interpretation of some of the *Pfiesteria*-related data.

Contents

I. Background Information 1
II. Public Health Information 3
III. The Pfiesteria Monitoring Test and its Strengths and Limitations 4
IV. NJ Pfiesteria Monitoring Results 5
V. The Pfiesteria Toxicity Test: Pfiesteria Did Not Cause a Suspicious Fish Kill in 1999 .. 6
VI. NJ Pfiesteria Contingency Plan 7
VII. References 7
VIII. Figures 11
Appendix 16
A. Initial NJ survey for Pfiesteria using a molecular assay (June-September 1998). 16
B. Initial NJDEP survey for Pfiesteria using the molecular assay (August 1999). 16
C. Pfiesteria analysis during a Tuckahoe River fish kill (September 1999)… 17
D. Tuckahoe River Pfiesteria sampling at the 9/99 fish kill site (October 1999)..... 18
E. Second NJDEP Pfiesteria survey (November 1999). 19
F. Second Tuckahoe River Pfiesteria survey (September 2000). 20

I. Background Information

- *Pfiesteria* (pronounced "fee-STEER-ee-uh") are microscopic aquatic life forms. They are single cell organisms that live in marine estuary areas such as back bays and tidal tributaries. They spend a portion of their life in the water and a portion in a dormant state in the bottom sediment. They are not found in fresh water areas (USEPA, 1999; USEPA *et al*, 1999).

- *Pfiesteria* appear to be a natural part of the marine environment (Rublee *et al*, 2001; Seahorn *et al*, 1999). *Pfiesteria* are found more often in bottom sediments than in overlying waters (Rublee *et al*, 2001). In sediments, the organisms appear to be unevenly distributed (Magnien *et al*, 2002), even in areas historically subject to multiple *Pfiesteria*-associated fish kills.

- *Pfiesteria* are not normally pathogenic but under certain environmental conditions, some species have the ability to prey upon and kill fish and other marine animals. *Pfiesteria* are capable of directly attacking fish (Burkholder *et al*, 2001a; Burkholder *et al*, 2001c; Cancellieri *et al*, 2001; Berry *et al*, 2002; Vogelbein *et al*, 2002; Drgon *et al*, 2005). Some types of *Pfiesteria* may also cause death through the release of one or more toxic chemicals (Burkholder *et al*, 2001a; Burkholder *et al*, 2001c; Gordon *et al*, 2002). Some aspects of toxin production and life cycle morphology of *Pfiesteria* are currently unclear (Kaiser, 2002a; Litaker *et al*, 2002; Drgon *et al*, 2005).

- Two toxin-producing species, *Pfiesteria piscicida* (*P. piscicida*; "pis-kih-SEED-uh"; Burkholder *et al*, 1992; Steidinger *et al*, 1996) and *Pseudopfiesteria shumwayae* (*P. shumwayae*; "shum-WAY-eye"; Litaker *et al*, 2005) have caused or contributed to several large fish kills in the coastal waters of North Carolina and Maryland between 1991 and 1998 (Burkholder *et al*, 2001c; Glasgow *et al*, 2001b). *Pseudofiesteria shumwayae* was formerly known as *Pfiesteria shukmwayae* (Glasgow *et al*, 2001a) and before that, *P. piscicida* species B (Oldach *et al*, 2000).

- Toxic forms of these organisms have been identified in states other than North Carolina and Maryland and in other countries but not as causative agents of fish kills (Burkholder *et al*, 2001c).

- The environmental conditions that allow an outbreak of fish-killing *Pfiesteria* to develop are not fully understood. However, *Pfiesteria*-associated fish kill events have always been associated with the presence of high densities of fish (almost always Atlantic menhaden [*Brevoortia tyrannus*]) and warm, brackish, shallow, poorly flushed waters with high levels of nutrients (Cancellieri *et al*, 2001; Glasgow *et al*, 2001b; Magnien *et al*, 2002; Mallin *et al*, 2002).

- In addition to toxic or potentially toxic strains of *Pfiesteria*, there are closely-related microorganisms such as *Cryptoperidiniopsis* (Litaker *et al*, 1999) and yet others ("Lucy" and "Shepherd's crook") within the family Pfiesteriaceae (Litaker *et al*, 2005), that look like *Pfiesteria*, but which are not able to produce toxins under any known conditions

("noninducible"; Burkholder *et al*, 2001a & 2001c). *Cryptoperidiniopsis* has been frequently found in estuary waters of some states (Marshall *et al*, 1999; Seaborn *et al*, 1999; Marshall *et al*, 2000).

- Interestingly, one of the first possible sightings of a *Pfiesteria*-related fish kill may have occured in Stowe Creek, NJ (Barker, 1997).

II. Public Health Information

- The toxic forms of *P. piscicida* and *P. shumwayae* appear capable of causing adverse human health effects. These effects include respiratory, skin, eye and gastrointestinal problems and memory loss and confusion (Glasgow *et al*, 1995; Grattan *et al*, 2001). Exposure routes include direct skin contact with *Pfiesteria*-containing water and/or inhalation of toxin-containing vapors emanating from *Pfiesteria*-related fish kill areas. Hence, swimming, watersport activities, fishing, shellfish harvesting and boating should not take place in waterways that are closed due to a *Pfiesteria* fish kill. Fish or water from such areas should be avoided (USEPA *et al*, 1999).

- Adverse health symptoms have occurred in laboratory workers working with *Pfiesteria*. Toxin exposure in these workers may have occurred by skin contact (on hands and wrists) during the cleaning of, or removing dead fish from aquaria containing *Pfiesteria*. Exposure may have also occurred by breathing toxin-containing vapors emanating from these aquaria which were located in a humid, enclosed room (Schmechel and Koltai, 2001). Adverse health symptoms have also occurred in bay fisherman and state response personnel exposed in similar ways (from boats) during a fish kill (Grattan *et al*, 1998; Haselow *et al*, 2001; Morris, 2001). Adverse health effects may have also occurred in citizens living close to *Pfiesteria*-affected waterways or in those individuals fishing, shellfish harvesting or boating on such waterways (Backer *et al*, 2001; Shoemaker, 2001).

- The toxin(s) attributed to *Pfiesteria* has been partially characterized (Moeller *et al*, 2003; Moeller et al, 2007). *Pfiesteria* genetic loci or genetic elements within the organism responsible for toxin production have not yet been elucidated (Fairey *et al*, 1999; Doucette *et al*, 1998). An exotoxin from *Pfiesteria piscicida* cells has been isolated and partially chemically characterized. The toxin is an unstable, copper (and iron)-containing organic compound that appears to act through a light-induced free-radical formation mechanism (Kaiser, 2002b; Moeller et al, 2007). Multiple toxin congeners are apparent. A second alleged *Pfiesteria* toxin was previously found to be di(2-ethylhexyl)phthalate (DEHP), a plasticizer and a contaminant of the salt mix used to create aquaria seawater (Moeller *et al*, 2001).

  Note: Several menhaden fish kills in State of Delaware, Rehoboth Bay tributaries during the summer of 2000 were caused by a novel brevetoxin-producing alga, *Chatonella cf. verruculosa* (Bourdelais *et al*, 2002). This alga has caused fish kills in other countries

but the Delaware fish kills are the first reports of this toxin-producing organism in temperate US waters. The menhaden were free of lesions during these fish kill events.

- Fish or shellfish should not be harvested or consumed from waterways closed due to a *Pfiesteria*-associated fish kill (USEPA *et al*, 1999). There is no evidence to indicate that finfish from *Pfiesteria*-affected areas are unsafe to eat (USEPA *et al*, 1999; Grattan *et al*, 2001) but "common sense" dictates caution until additional safety data are available. Springer (2000) [from Burkholder *et al*, 2001c] has shown that juvenile and subadult eastern oysters have the capacity to ingest toxic forms of *Pfiesteria* (conversely, toxic *Pfiesteria* can kill the larval stage of oysters and scallops). Hence edible-size oysters may have the potential to concentrate viable, toxic *Pfiesteria* organisms. Therefore, the safety of shellfish harvested from *Pfiesteria*-related fish kill areas, during or immediately following an event, is uncertain.

III. The *Pfiesteria* Monitoring Test and its Strengths and Limitations

- The *Pfiesteria* monitoring test is a molecular test that detects the DNA of *Pfiesteria piscicida* and *Pfiesteria shumwayae* as well as the DNA of *Cryptoperidiniopsis* (Oldach *et al*, 2000). The test was developed by Dr. Parke Rublee at the University of North Carolina at Greeensboro (UNCG; for *P. piscicida*) and Dr. David Oldach at the University of Maryland (for *P. shumwayae* and *Cryptoperidiniopsis*).

- Water sample volumes of between 100 and 250 ml are collected at each site, filtered through glass fiber filters, immersed in a cell lysis buffer in small containers, shipped overnight to the analytical laboratory at UNCG and analyzed for *Pfiesteria*-specific DNA. After October 1999, sediment samples of approximately 10 grams were also collected at each site in separate containers (without a lysis buffer).

- The test can distinguish *P. piscicida* and *P. shumwayae* from other "look-alike" strains (however see next paragraph) but **the test is not able to distinguish toxic and nontoxic varieties of these species nor can it tell whether or not the organisms it detects are alive or dead.** Hence, detecting *Pfiesteria* DNA is not indicative of the presence of toxin-producing *Pfiesteria*.

- It is possible that the molecular test is not totally specific for *Pfiesteria piscicida* or the other two target organisms. Field samples may contain unknown or uncultured, possibly nontoxic *Pfiesteria* species that also contain the same target DNA sequence as *P. piscicida* (Rublee *et al*, 1999; Marshall *et al*, 1999; Bowers *et al*, 2000).

- The test is a "presence-absence" test. That is, the test can determine if *Pfiesteria* is present in a sample but it cannot determine the number of *Pfiesteria* organisms present in a "positive" sample.

- The sensitivity of the test for field samples is not known, but the test appears to be fairly sensitive. The sensitivity limit when testing dilutions of pure dinospore (zoospore)

cultures of *P. piscicida* in the lab is ~ 0.6 organisms for unpreserved cultures and about 6 organisms for preserved cultures (Bowers *et al*, 2000). Single cell isolates (unpreserved) are routinely detected. The sensitivity limit is not appreciably altered by the presence of an excess of other microbes prior to DNA extraction. The sensitivity limit for detecting *Pfiesteria* in other life cycle stages, such as cysts and amoebae (this stage may not exist; see Litaker *et al*, 2002), is not yet known. The sensitivity limit in estuarine water samples is likely higher (less) than that for pure cultures for several reasons, but Dr. Rublee "is confident that the assay will detect *Pfiesteria* at concentrations well below those found during *Pfiesteria* fish kill events."

IV. NJ *Pfiesteria* Monitoring Results

- Between 1998 and 2000 the NJDEP and other investigators tested a number of estuary waters and sediments in NJ for *Pfiesteria* using the molecular monitoring test. All analyses were conducted by Dr. Parke Rublee and his co-workers. The results are summarized briefly below and are the same as reported in two previous *Pfiesteria* updates (May 24, 2000 and April 15, 2001). **Sampling and analysis details are provided in the Appendix.**

- A total of 46 water column samples and 26 sediment samples from 35 estuarine sites in NJ have been tested. *Pfiesteria* DNA was found on one occasion only (October 1999), in one estuary (the Tuckahoe River estuary). *Pfiesteria* DNA was not detected in multiple samples collected from the same estuary one month later and the following summer. NJ estuaries were sampled and analyzed on 5 separate occasions as follows:

  1. Four estuarine water samples were collected in the summer of 1998 by Rublee *et al* (1999). *Pfiesteria* were not found.

  2. Water samples were collected from 20 estuary sites by NJDEP in August 1999 (see Figure 1; Appendix, Table 1). *Pfiesteria* were not found.

  3. Three water samples and three sediment samples were collected in the Tuckahoe River estuary in October 1999, three weeks following a fish kill event (see Figure 3; Appendix, Table 2; see additional discussion below). *Pfiesteria* were detected in 1 of the 3 water samples and all sediment samples.

  4. Fifteen water and 15 sediment samples were collected from 7 estuary locations in November 1999 (see Figure 2; Appendix, Table 3). *Pfiesteria* were not found.

  5. Eight water and 8 sediment samples were collected from the Tuckahoe River estuary in September 2000 (see Figure 4; Appendix, Table 4). *Pfiesteria* were not found.

- **The *Pfiesteria* test is used for screening purposes only**. As stated above, the test can reveal the presence of *Pfiesteria* but cannot tell if live organisms are present, how many organisms are present or whether or not the organisms are toxin-producing strains. Not

all *Pfiesteria* organisms are capable of producing toxins. Therefore, detecting *Pfiesteria* DNA is not indicative of the presence of *Pfiesteria* toxins.

- Because *Pfiesteria* are found more often in bottom sediments than in overlying waters, Rublee *et al* (2001) concluded, "routine monitoring of water is not the optimal method to detect *Pfiesteria*." Therefore, due to the test limitations, the uneven spatial and temporal distribution of the organism, and because NJ appears to have few estuary areas with the combination of environmental conditions associated with *Pfiesteria*-related fish kill events, the NJDEP has elected not to **routinely** monitor NJ's estuary waters or sediments for *Pfiesteria*. Test improvements in the future or other unforseen factors may alter this decision.

V. The *Pfiesteria* Toxicity Test: *Pfiesteria* Did Not Cause a Suspicious Fish Kill in 1999

- Toxic forms of *Pfiesteria* can be identified in estuary water samples using a laboratory fish bioassay (Burkholder *et al*, 2001b). This test was used to show that *Pfiesteria* did not cause a fish kill that occurred in the Tuckahoe River in September 1999 at Corbin City, NJ (see Figure 3). During this fish kill the fish displayed ulcerative lesions which were similar in appearance to fish lesions that had been observed during earlier fish kills in other states in which *Pfiesteria* was implicated in the fish kill. However, recent research has shown that ulcerative lesions on Atlantic menhaden fish are not caused by *Pfiesteria* (Kiryu *et al*, 2002). Hence the presence of ulcerative lesions on fish during a fish kill event is no longer considered to be an indication of *Pfiesteria* involvement.

- The sampling and analysis portion of the NJ *Pfiesteria* Contingency Plan (NJDEP/NJDHSS, 2000; see below) was implemented by state personnel. Samples of water and fish were collected at the site of the fish kill.

- The water samples were analyzed by Dr. JoAnn Burkholder, North Carolina State University, for toxic *Pfiesteria* or *Pfiesteria*-like organisms. **Toxic *Pfiesteria* organisms were not observed.** In addition, *Pfiesteria*-specific DNA was not observed in the toxicity test aquarium water. Thus, according to the criteria established by Dr. Burkholder and her colleagues (Burkholder *et al*, 2001b; Glasgow *et al*, 2001b; Magnien, 2001) ***Pfiesteria* did not cause this fish kill.** Dr. Burkholder and her colleagues are experts in this field. Detecting *Pfiesteria* DNA or observing fish with ulcerative lesions at a fish kill site is not indicative of the presence of *Pfiesteria* toxins. It is possible that the fish died due to low oxygen levels. It is also possible that the fish lesions began developing when the fish were in another location.

- It is interesting to note however that the only place in NJ where *Pfiesteria* DNA has been found to date is at and near the Tuckahoe River fish kill site, 3 weeks after the fish kill event (and after the passage of Tropical Storm Floyd resulted in considerable flushing of NJ's estuaries). However, *Pfiesteria* appear to be normal inhabitants of some estuarine sediments (Rublee *et al*, 2001) and the detection of *Pfiesteria* DNA in the Tuckahoe River water and sediment samples may have been unrelated to the earlier fish kill. As

stated above, it is also possible that the molecular test is not totally specific for *Pfiesteria piscicida*.

VI. NJ *Pfiesteria* Contingency Plan

- NJ has a *Pfiesteria* Contingency Plan to be followed in the event of a fish kill in which there is evidence that *Pfiesteria* may be involved or there is no obvious alternative explanation such as, for example, low dissolved oxygen or a chemical spill. (Note: fish kills occur from time to time in NJ and other states for a variety of reasons not related to the presence of *Pfiesteria*). The Plan was adopted on May 24, 2000 and is available at the websites of both the NJ Departments of Environmental Protection (www.state.nj.us/dep/dsr) and Health and Senior Services (www.state.nj.us/health/eoh/phss).

- The Plan dictates the roles of various federal, state, and local personnel during a suspicious fish kill. The Plan will be used by the NJDEP and the NJDHSS to protect the public as well as state sampling personnel. NJDHSS personnel have the responsibility to close and reopen affected water bodies. The Plan also contains guidance for NJDHSS personnel on when to close and reopen affected water bodies.

VII. References

Backer, L.C., A.S. Niskar, C. Rubin, K. Blindauer, D. Christianson, L. Naeher, and H.S. Rogers. 2001. Environmental public health surveillance: possible estuary-associated syndrome. Environ. Health Pers. 109 [Suppl. 5]: 797-801.

Barker, R. 1997. *And the Waters Turned to Blood*. Touchstone Books, NY, part 4, chap. 15. pp. 218-219.

Berry, J.P., K.S. Reece, K.S. Rein, D.G. Baden, L.W. Haas, W.L. Ribeiro, J.D. Shields, R.V. Snyder, W.K. Vogelbein, and R.E. Gawley. 2002. Are *Pfiesteria* species toxicogenic? Evidence against production of ichthyotoxins by *Pfiesteria shumwayae*. Proc. Nat. Acad. Sci. 99: 10970-10975.

Blazer, V.S., W.K. Vogelbein, C.L. Densmore, E.B. May, J.H. Lilly, and D.E. Zwerner. 1999. *Aphanomyces* as a cause of ulcerative skin lesions of menhaden from Chesapeake Bay tributaries. J. Aquatic Animal Health 11 (4): 340-349.

Bowers, H.A., T. Tengs, H.B. Glasgow, Jr., J.M. Burkholder, P.A. Rublee, and D.W. Oldach. 2000. Development of real-time PCR assays for rapid detection of *Pfiesteria piscicida* and related dinoflagellates. Appl. Environ. Microbiol. 66 (11): 4641-4648.

Bourdelais, A.J., C.R. Tomas, J. Naar, J. Kubanek, and D.G. Baden. 2002. New fish-killing alga in coastal Delaware produces neurotoxins. Environ. Health Persp. 110 (5): 465-470.

Burkholder, J.M., E.J. Noga, C.H. Hobbs, H.B. Glasgow, Jr. and S.A. Smith. 1992. New 'phamtom' dinoflagellate is the causative agent of major estuarine fish kills. Nature 358: 407-410 *et errata* 360: 768.

Burkholder, J.M. and H.B. Glasgow, Jr. 1997. *Pfiesteria piscicida* and other *Pfiesteria*-like dinoflagellates: behavior, impacts, and environmental controls. Limnol. Oceanogr. 42 (5, part 2): 1052-1075.

Burkholder, J.M. 1998. Links Teleconference: minutes of Dr. Burkholder's talk on *Pfiesteria piscicida*. Western Center for Environmental Decision-making, July 9.

Burkholder, J.M. 1999. The lurking perils of *Pfiesteria*. Sci. Am. 281 (2): 42-49.

Burkholder, J.M., H.B. Glasgow, N.J. Deamer-Melia, J. Springer, M.W. Parrow, C. Zhang, and P.J. Cancellieri. 2001a. Species of the toxic *Pfiesteria* complex, and the importance of functional type in data interpretation. Environ. Health Pers. 109 [Suppl. 5]: 667-679.

Burkholder, J.M., H.G. Marshall, H.B. Glasgow, D.W. Seaborn, and N.J. Deamer-Melia. 2001b. The standardized fish bioassay procedure for detecting and culturing actively toxic *Pfiesteria*, used by two reference laboratories for Atlantic and Gulf Coast States. Environ. Health Pers. 109 [Suppl. 5]: 745-756.

Burkholder, J.M., H.B. Glasgow and N. Deamer-Melia. 2001c. Overview and present status of the toxic *Pfiesteria* complex (Dinophyceae). Phycologia 40 (3): 186-214.

Cancellieri, P.J., J.M. Burkholder, N.J. Deamer-Melia, and H.B. Glasgow. 2001. Chemosensory attraction of zoospores of the estuarine dinoflagellates, *Pfiesteria piscicida* and *P. shumwayae*, to finfish mucus and excreta. J. Exp. Mar. Biol. Ecol. 264: 29-45.

Doucette, G.J., M. Kodama, and S. Gallacher. 1998. Bacterial interactions with harmful algal bloom species: bloom ecology, toxigenesis, and cytology. pp. 619-647. In: *Physiological Ecology of Harmful Algal Blooms*. D.M. Anderson, A.D. Cembella, G.M. Hallegraeff, eds. Berlin, Springer-Verlag.

Drgon, T., K. Saito, P.M. Gillevet, M. Sikaroodi, B. Whitaker, D.N. Krupatkina, F. Argemi, and G.R. Vasta. 2005. Characterization of ichthyocidal activity of *Pfiesteria piscicida*: dependence on the dinospore cell density. Appl. Environ. Microbiol. 71: 519-529.

Dykstra, M.J. and A.S. Kane. 2000. *Pfiesteria piscicida* and ulcerative mycosis of Atlantic menhaden - current status of understanding. J. Aquatic Animal Health, 12(1): 18-25.

Fairey, E.R., J. Stewart, G. Edmunds, N. Deamer-Melia, H. Glasgow, Jr., F.M. Johnson, P.R. Moeller, J.M. Burkholder, and J.S. Ramsdell. 1999. Reporter gene assay for fish-killing activity produced by *Pfiesteria piscicida*. Environ. Health Pers. 107 (9): 711-714.

Haselow, D.T., E. Brown, J.K. Tracy, R. Magnien, L.M. Grattan, J.G. Morris, Jr., and D.W. Oldach. 2001. Gastrointestinal and respiratory tract symptoms following brief environmental exposure to aerosols during a *Pfiesteria*-related fish kill. J. Tox. Environ. Health, Part A, 63: 553-564.

Glasgow, H.B., Jr., J.M. Burkholder, D.E. Schmechel, P.A. Tester, and P.A. Rublee. 1995. Insidious effects of a toxic estuarine dinoflagellate on fish survival and human health. J. Toxicol. Environ. Health, 46: 501-522.

Glasgow, H.B., J.M. Burkholder, S.L. Morton, and J. Springer. 2001a. A second species of ichthyotoxic *Pfiesteria* (Dinamoebales, Dinophyceae). Phycologia 40 (3): 234-245.

Glasgow, H.B., J.M. Burkholder, M.A. Mallin, N.J. Deamer-Melia, and R.E. Reed. 2001b. Field ecology of toxic *Pfiesteria* complex species and a conservative analysis of their role in estuarine fish kills. Environ. Health Pers. 109 [Suppl. 5]: 715-730.

Gordon, A.S., B.J. Dyer, D. Seaborn, and H.G. Marshall. 2002. Comparative toxicity of *Pfiesteria* spp., prolonging toxicity of *P. piscicida* in culture and evaluation of toxin(s) stability. Harmful Algae 1: 85-94.

Grattan, L.M., D. Oldach, T.M. Perl, MH Lowitt, D.L. Matuszak, C. Dickson, C. Parrott, R.C. Shoemaker, C.L. Kauffman, M.P. Wasserman, J.R. Hebel, P. Charache, and J.G. Morris, Jr. 1998. Learning and memory difficulties after environmental exposure to waterways containing toxin-producing *Pfiesteria* or *Pfiesteria*-like dinoflagellates. Lancet 352: 532-539.

Grattan, L.M., D. Oldach, and J.G. Morris. 2001. Human health risks of exposure to *Pfiesteria piscicida*. BioScience 51 (10): 853-857.

Kaiser. J. 2002a. The science of *Pfiesteria*: elusive, subtle, and toxic. Science 298: 346-349.

Kaiser. J. 2002b. Is sugary toxin the smoking gun?. Science 298: 945-946.

Kiryu, Y., J.D. Shields, W.K. Vogelbein, D.E. Zwerner and H. Kator. 2002. Induction of skin ulcers in Atlantic menhaden by injection and aqueous exposure to the zoospores of *Aphanomyces invadans*. J Aquatic Animal Health 14: 11-24.

Law, M. 2001. Differential diagnosis of ulcerative lesions in fish. Environ. Health Pers. 109 [Suppl. 5]: 681-686.

Litaker, R.W., P.A. Tester, A. Colorni, M.G. Levy and E.J. Noga. 1999. The phylogenetic relationship of *Pfiesteria piscicida*, Cryptoperidiniopsoid sp. *Amyloodinoum ocellatum* and a *Pfiesteria*-like dinoflagellate to other dinoflagellates and Apicomplexans. J. Phycol. 35: 1379-1389.

Litaker, R.W., M.W. Vandersea, S.R. Kibler, V.J. Madden, E.J. Noga, and P.A. Tester. 2002. Life cycle of the heterotrophic dinoflagellate *Pfiesteria piscicida* (Dinophyceae). J. Phycol. 38: 442-463.

Litaker, R.W., K.A. Steidinger, P.L. Mason, J.H. Landsberg, J.D. Shields, K.S. Reece, L.W. Haas, W.K. Vogelbein, M.W. Vandersea, S.R. Kibler, and P.A. Tester. 2005. The reclassification of *Pfiesteria shumwayae* (Dinophyceae): *Pseudopfiesteria*, gen. nov. J. Phycol. 41: 643-651.

Magnien, R.E. 2001. The dynamics of science, perception, and policy during the outbreak of *Pfiesteria* in the Chesapeake Bay. BioScience, 51 (10): 843-852.

Magnien, R., M. Trice, B. Michael, D. Goshorn, D. Oldach, H. Bowers, and J. Halka. 2002. Distribution of *Pfiesteria* sp. in Chesapeake and Delamarva coastal bays sediments. In: Proc. Xth Int. Conf. on Harmful Algae, October 21-25, 2002, St. Pete Beach, FL, p. 181.

Mallon, M.A., M.R. McIver, S.H. Ensign, J.M. Burkholder, and P.A. Rublee. 2002. Eutrophication and the distribution of *Pfiesteria* in urbanized tidal creeks. In: Proc. Xth Int. Conf. on Harmful Algae, October 21-25, 2002, St. Pete Beach, FL, p. 183.

Marshall, H.G., D. Seaborn, and J. Wolny. 1999. Monitoring results for *Pfiesteria piscicida* and *Pfiesteria*-like organisms from Virginia waters in 1998. Virginia J. Sci. 50 (4): 287-298.

Marshall, H.G., A.S. Gordon, D.W. Seaborn, B. Dyer, W.M. Dunstan, and A.M. Seaborn. 2000. Comparative culture and toxicity studies between the toxic dinoflagellate *Pfiesteria piscicida* and a morphologically similar cryptoperidiniopsoid dinoflagellate. J. Exp. Mar. Biol. Ecol. 255: 51-74.

Moeller, P.D.R., S.L. Morton, B.A. Mitchell, S.K. Sivertsen, E.R. Fairey, T.M. Mikulski, H. Glasgow, N.J. Deamer-Melia, J.M. Burkholder, and J.S. Ramsdell. 2001. Current progress in isolation and characterization of toxins isolated from *Pfiesteria piscicida*. Environ. Health Pers. 109 [Suppl. 5]: 739-743.

Moeller. P.D.R., J.S. Ramsdell, S.L. Morton, B.A. Mitchell, S. Eaker, J.M. Burkholder, H.B. Glasgow, and N.J. Deamer-Melia. 2002. Isolation, characterization and current chemical structural information on a water soluble toxin derived from *Pfiesteria piscicida*. In: Proc. Xth Int. Conf. on Harmful Algae, October 21-25, 2002, St. Pete Beach, FL, p. 201.

Moeller, P.D.R., K.R. Beauchesne, K.M. Huncik, W.C. Davis, P. Riggs-Gelasco, and A.K. Gelasco. 2007. Metal complexes and free radical toxins produced by *Pfiesteria piscicida*. Environ. Sci. Technol. 41: 1166-1172.

Morris, J.G., Jr. 2001. Human health effects and *Pfiesteria* exposure: a synthesis of available clinical data. Environ. Health Pers. 109 [Suppl. 5]: 787-790.

Oldach, D.W., C.F. Delwiche, K.S. Jakobsen, T. Tengs, E.G. Brown, J.W. Kempton, E.F. Schaefer, H.A. Bowers, H.B. Glaskow, Jr., J.M. Burkholder, K.A. Steidinger and P.A. Rublee. 2000. Heteroduplex mobility assay-guided sequence discovery: elucidation of the small subunit (18S) rDNA sequences of *Pfiesteria piscicida* and related dinoflagellates from complex algal culture and environmental sample DNA pools. Proc. Natl. Acad. Sci. 97 (8): 4303-4308.

NCSU. 2000. January 14, 2000 letter and laboratory data from Nora Deamer, North Carolina State University, Aquatic Botany Laboratory, to William Andrews, Principal Biologist, NJ Bureau of Marine Fisheries and January 14, 2000 E-mail from Dr. JoAnn Burkholder to William Andrews.

NJDEP. 1999. Internal memorandum [undated] from the NJ Division of Fish and Wildlife and NJ Division of Fish, Game and Wildlife, Office of Fish and Wildlife, Health and Forensics Diagnostic Consultation Report. 27-IB, September 14.

NJDEP/NJDHSS. 2000. *Pfiesteria: Background Information and Contingency Plan*. NJ Department of Environmental Protection and the NJ Department of Health and Senior Services, May 2000.

Noga, E.J. and M.J. Dykstra. 1986. Oomycete fungi associated with ulcerative mycosis in menhaden, *Brevoortia tyrannus* (Latrobe). J. Fish Dis. 9 (1): 47-53.

Noga, E.J. 2000. Skin ulcers in fish: *Pfiesteria* and other etiologies. Toxicol. Pathol. 28 (6): 807-823.

Rublee, P.A., J. Kempton, E. Schaefer, J.M. Burkholder, H.B. Glasgow, Jr., and D. Oldach. 1999. PCR and FISH detection extends the range of *Pfiesteria piscicida* in estuarine waters. Virginia J. Sci. 50 (4): 325-335.

Rublee, P.A., J.W. Kempton, E.F. Schaefer, C. Allen, J. Harris, D.W. Oldach, H. Bowers, T. Tengs, J.M. Burkholder, and H.B. Glasgow. 2001. Use of molecular probes to assess geographic distribution of *Pfiesteria* species. Environ. Health Pers. 109 [Suppl. 5]: 765-767.

Samet, J., G.S. Bignami, R. Feldman, W. Hawkins, J. Neff, and T. Smayda. 2001. *Pfiesteria*: review of the science and identification of research gaps. Report for the National Center for Environmental Health, Centers for Disease Control and Prevention. Environ. Health Pers. 109 [Suppl. 5]: 639-659.

Seaborn, D.W., A.M. Seaborn, W.M. Dunston and H.G. Marshall. 1999. Growth and feeding studies on the algal feeding stage of a *Pfiesteria*-like dinoflagellate. Virginia J. Sci. 50 (4): 337-343.

Schmechel, D.E. and D.C. Koltai. 2001. Potential human health effects associated with laboratory exposures to *Pfiesteria piscicida*. Environ. Health Pers. 109 [Suppl. 5]: 775-779.

Shoemaker, R.C. 2001. Residential and recreational acquisition of possible estuary-associated syndrome: a new approach to successful diagnosis and treatment. Environ. Health Pers. 109 [Suppl. 5]: 791-796.

Springer, J.J. 2000. *Interactions between two commercially important species of bivalve molluscs and the toxic estuarine dinoflagellate,* Pfiesteria piscicida*.* MS thesis, North Carolina State University, Raleigh, NC.

Steidinger, K.A., J.M. Burkholder, H.B. Glasgow, E. Truby, J. Garrett, E.J. Noga, and S.A. Smith. 1996. *Pfiesteria piscicida* (Pfiesteriaceae, fam. nov.), a new toxic dinoflagellate with a complex life cycle and behavior. J. Phycol. 32: 157-164.

USEPA, NOAA, USDA, USGS, USDHHS, ASIWPCA. 1999. What you should know about *Pfiesteria piscicida*. U.S. Environmental Protection Agency, US Department of Commerce - National Oceanic and Atmospheric Administration, US Department of Agriculture, United States Geological Survey, US Department of Health and Human Services, Association of State and Interstate Water Pollution Control Administrators, June 10.

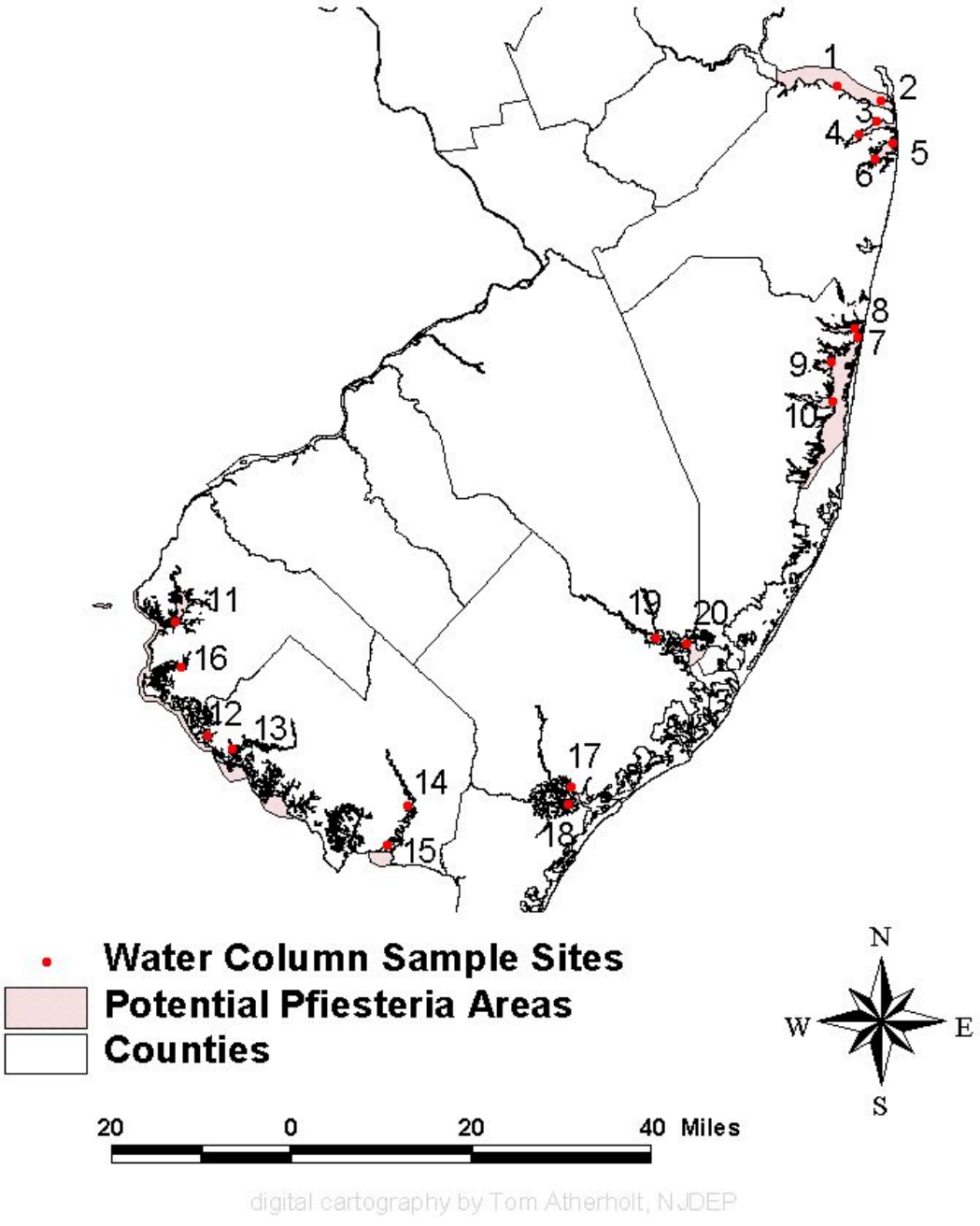
USEPA. 1999. Draft: The facts on Pfiesteria. U.S. Environmental Protection Agency, Office of Water, June 10.

Vogelbein, W.K., J.D. Shields, L.W. Haas, K.S. Reece, and D.E. Zwerner. 2001. Skin ulcers in estuarine fishes: a comparative pathological evaluation of wild and laboratory-exposed fish. Environ. Health Pers. 109 [Suppl. 5]: 687-693.

Vogelbein, W.K., V.J. Lovko, J.D. Shields, K.S. Reece, P.L. Mason, L.W. Haas, and C.C. Walker. 2002. *Pfiesteria shumwayae* kills fish by micropredation not exotoxin secretion. Nature 418: 967-970.

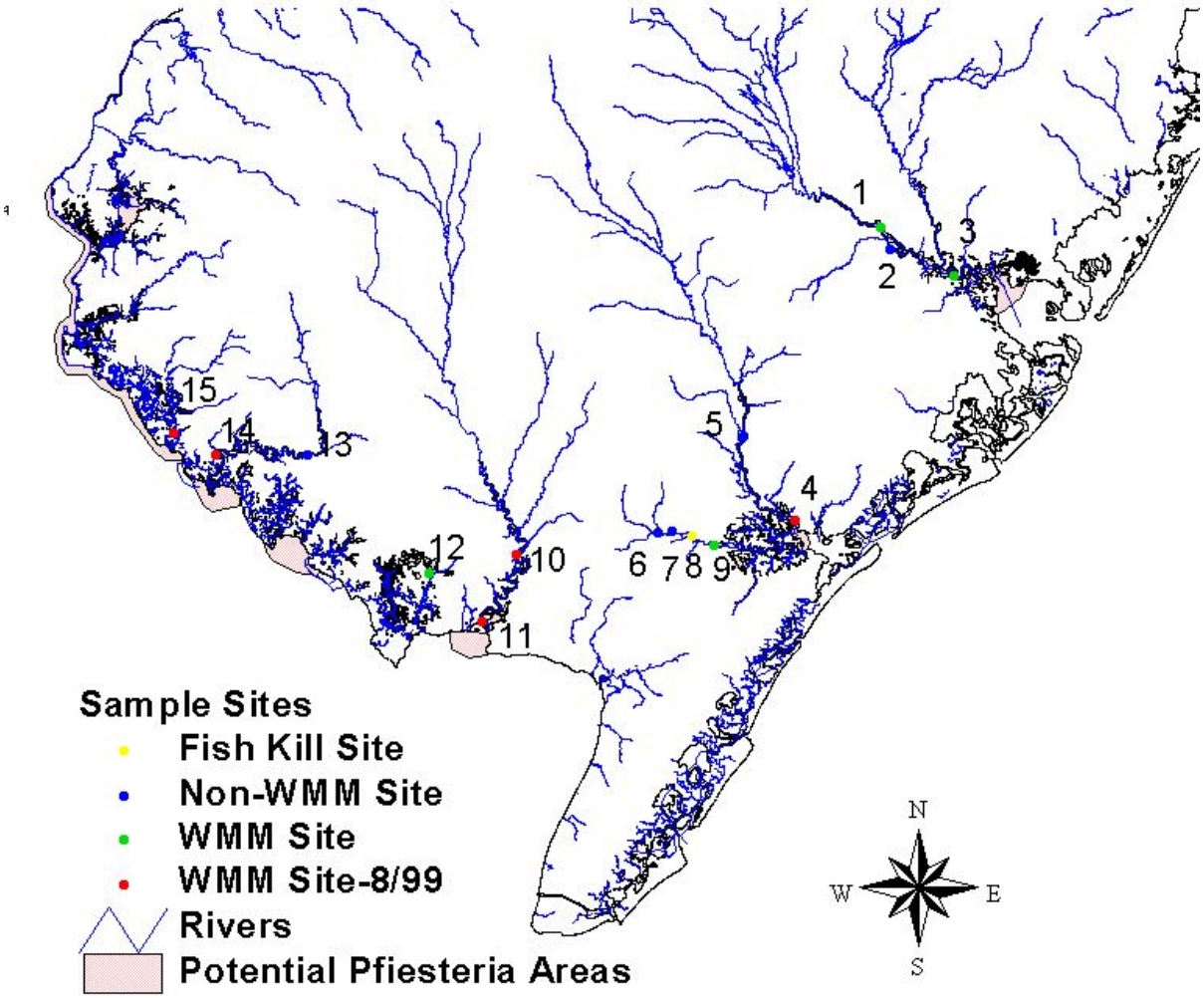
VIII. Figures

Figure 1
Initial Pfiesteria Survey
August 1999



digital cartography by Tom Atherholt, NJDEP

Figure 2
Second Pfiesteria Survey
November 1999



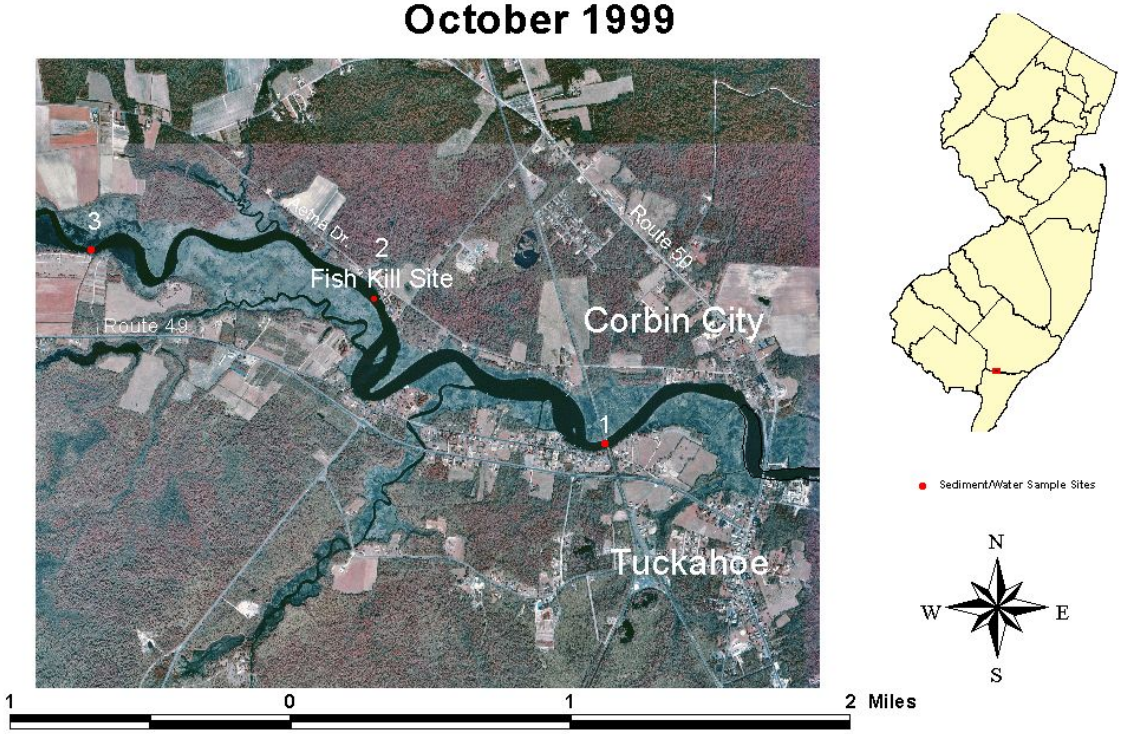
WMM = Office of Water Monitoring Management
Nutrient Biomonitoring Network site.

8/99 = Also sampled in August 1999.

10 0 10 20 Miles

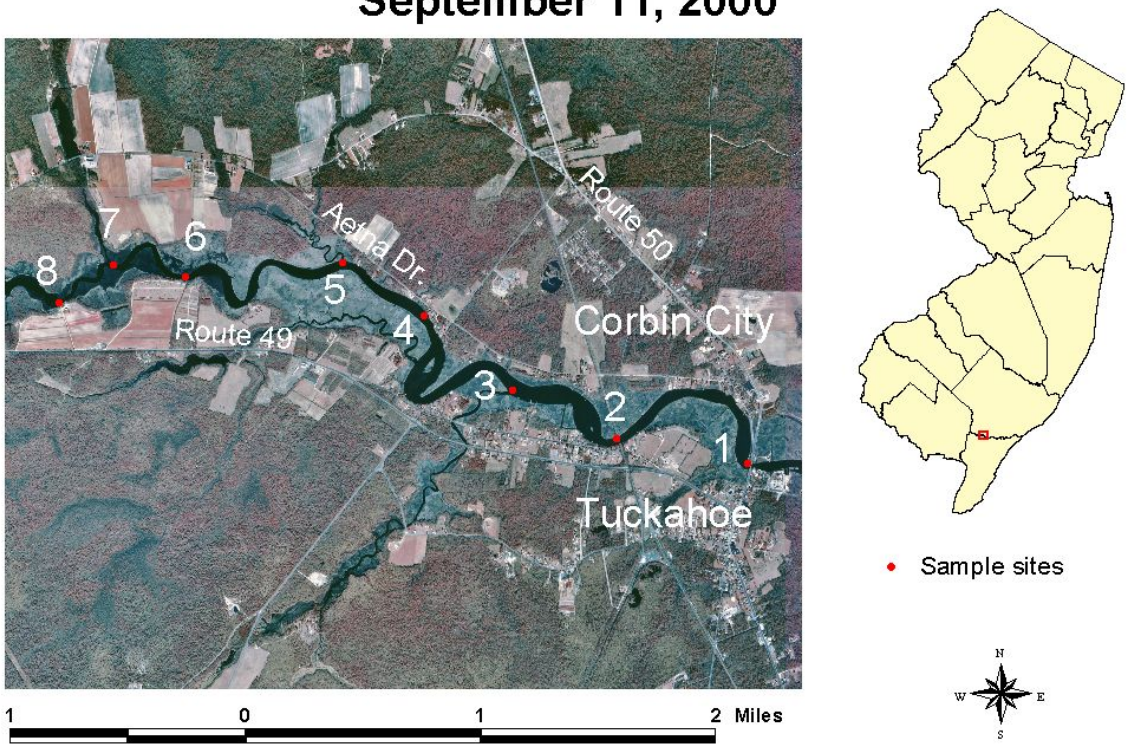
digital cartography by Tom Atherholt, NJDEP

Figure 3
Tuckahoe River Pfiesteria Sampling
October 1999



digital cartography by Tom Atherholt, NJDEP

Figure 4
Tuckahoe River Pfiesteria Sampling
September 11, 2000



digital cartography by Tom Atherholt, NJDEP

Appendix

Chronology of *Pfiesteria* monitoring in New Jersey

A. Initial NJ survey for *Pfiesteria* using a molecular assay (June-September 1998).

Water column samples from four NJ estuary sites were sampled by Dr. Parke Rublee, University of North Carolina at Greensboro, and his colleagues between June and September 1998 (Rublee *et al*, 1999). The samples were analyzed for *Pfiesteria piscicida* using a new gene probe assay developed by Dr. Rublee. *Pfiesteria* were not observed in any of the four NJ samples even though *Pfiesteria* were observed in 20% of the 170 samples collected between New York and Florida for this study (including 8 positives out of 26 samples collected from the Long Island, New York area).

B. Initial NJDEP survey for *Pfiesteria* using the molecular assay (August 1999).

In August 1999, the Division of Science, Research and Technology, NJDEP, collected water column samples from 20 estuary sites in NJ (see Figure 1; Table 1) and sent these samples to Dr. Rublee for analysis for *Pfiesteria piscicida*, *Pfiesteria shumwayae*, and *Cryptoperidiniopsis* using the monitoring test described on page 4.

The sites sampled were a subset of NJDEP, Office of Water Monitoring Management, Bureau of Marine Water Monitoring's 260 Nutrient Biomonitoring Stations. The sites were selected using a map of two GIS coverages: the Nutrient Biomonitoring Network coverage and a coverage created by the Bureau of Marine Water Monitoring, showing estuary areas that have a combination of environmental conditions (salinity, nitrogen, phosphate, flushing, etc.) that would have a higher-than-average potential in NJ of being conducive to *Pfiesteria* growth. Sampling took place toward the end of a multi-month period of drought. The test results from Dr. Rublee's lab were received on September 28, 1999 (Table 1). None of the three organisms were found in any of the samples.

One of the samples was collected from the Tuckahoe River, approximately 9 miles east ("downstream") from the site of a later fish kill (see III). When Dr. Rublee was later made aware of the fish kill, he re-analyzed the archived sample to make sure he did not miss any *Pfiesteria* that might have been present. The re-analysis was also negative for these organisms.

Table 1. Initial NJDEP Bay/Estuary *Pfiesteria* Survey - August 17, 23 - 25, 1999. *

Site	DSRT # (DEP #)**	Bay or Estuary Location	Water Temp. (^oC)	Salinity (Ppt.)	Test Result		
					P. piscicida	*P. shumwayae*	*Cryptop.*
1	NJ-1 (RB-3)	Raritan Bay	25.0	17.0	N	N	N
2	NJ-2 (906A)	Raritan Bay @ Sandy Hook	26.0	18.0	N	N	N
3	NJ-3 (1014)	Navesink River @ Claypit Creek jct.	26.5	18.0	N	N	N
4	NJ-4 (1006B)	Navesink River	26.0	18.0	N	N	N
5	NJ-5 (1104B)	Shrewsbury River	26.0	19.0	N	N	N
6	NJ-6 (1127A)	Shrewsbury River	27.0	18.0	N	N	N
7	NJ-7 (1605A)	Metedeconk River @ Route 528	22.0	13.5	N	N	N
8	NJ-8 (1600D)	Metedeconk River	24.0	11.5	N	N	N
9	NJ-9 (1618A)	Barnegat Bay @ Silver Bay jct.	23.5	13.5	N	N	N

10	NJ-10 (1632B)	Barnegat Bay @ Toms River jct.	23.0	14.5	N	N	N
11	NJ-11 (R-57)	Salem River @ Pennsville-Salem Rd.	26.0	5.0	N	N	N
12	NJ-12 (R-50)	Stow Creek @ Wheaton Island Rd.	25.5	9.5	N	N	N
13	NJ-13 (R-48)	Cohansey River @ Hannah Taylor Rd.	25.5	9.5	N	N	N
14	NJ-14 (R-43)	Maurice River @ Mauricetown bridge	26.0	5.0	N	N	N
15	NJ-15 (3900A)	Maurice River @ Bivalve	26.0	10.0	N	N	N
16	NJ-16 (R-56)	Alloways Creek	26.0	4.5	N	N	N
17	NJ-17 (2801)	Egg Harbor River @ Jeffers Landing	26.0	12.0	N	N	N
18	NJ-18 (2902A)	Tuckahoe River	26.0	13.5	N	N	N
19	NJ-19 (2011A)	Mullica River @ Wading River jct.	26.0	10.0	N	N	N
20	NJ-20 (2002A)	Mullica River	27.5	15.0	N	N	N

* Water column samples only. No sediment samples were collected.
** Office of Water Monitoring Management, Bureau of Marine Water Monitoring, Nutrient Biomonitoring Station identification number.
Cryptop. = *Cryptoperidiniopsis*. N = negative. P = positive.
Samples 1-6 collected 8/17/99; samples 7-10 collected 8/23/99; samples 11-16 collected 8/24/99; samples 17-20 collected 8/25/99.

C. *Pfiesteria* analysis during a Tuckahoe River fish kill (September 1999).

On September 14, 1999, NJDEP's Division of Fish and Wildlife (F&W) was first made aware of a fish kill on the Tuckahoe River, at Corbin City, Cape May County, NJ, by a citizen who owns a home on the river (see Figure 3). The citizen had been noticing dead and dying Atlantic menhaden fish off of his dock "for the past two weeks" [9/1-14/99] and stated that about 80% of the fish he observed had lesions.

On 9/15/99, F&W notified NJDEP's Law Enforcement Office, who dispatched a Conservation Officer (CO) to investigate. Distressed fish displaying erratic swimming behavior and fish with lesions were observed. The CO then notified Law Enforcement, who contacted Emergency Response personnel as per the draft *Pfiesteria* Contingency Plan protocol. Emergency Response (ER) personnel collected two samples (one preserved, one not preserved) in the middle of the river at the fish kill location. The samples were sent to Nora Deamer in the laboratory of Dr. JoAnn Burkholder at the North Carolina State University for analysis for "toxic *Pfiesteria* complex" (TPC) organisms (Burkholder *et al*, 2001b). The sampling occurred one day before Tropical Storm Floyd passed over NJ, bringing 6-12 inches of rain.

Dr. Burkholder's laboratory examined the preserved sample (shipped via an overnight courier) and made a "presumptive" identification of a low concentration of *Pfiesteria* of about 60 organisms per milliliter. Such a low concentration is not typically associated with fish kills. Estuarine waters contain a myriad of different microorganisms. Even if TPC organisms were present, they are often a minor component (1% or even less) of the total plankton population (Burkholder, 1998). The presumptive test (microscopic examination) cannot determine whether or not **toxic** *Pfiesteria* are present.

The concentration of presumptive *Pfiesteria* was low enough that Dr. Burkholder's lab personnel would not normally process the unpreserved sample in their toxicity bioassay, but since this was the first sample from NJ, they proceeded

to culture the unpreserved sample. The fish toxicity bioassay is now completed in 21 days to determine *Pfiesteria* causality during a fish kill event (Burkholder *et al*, 2001b). Previous information generated in Dr. Burkholder's laboratory has shown that adverse effects on fish have been observed at "toxic zoospore" concentrations of > 100 per milliliter and lethal effects at > 250 per milliliter (Burkholder and Glasgow, 1997; Burkholder *et al*, 2001c). On the other hand, field concentrations of toxic stages less than 100 per milliliter, shortly after fish kill events, have been observed (Burkholder and Glasgow, 1997; Glasgow *et al*, 2001b). The result of the toxicity test was negative (NCSU, 2000). That is, under laboratory conditions conducive to toxic *Pfiesteria* complex growth and activity, TPC organisms were not detected by DNA molecular probing of the water nor were any organisms in the sample able to grow and consume target algae or adversely affect target fish following 15 weeks incubation in culture.

On the day of water sampling, two personnel from the Bureau of Marine Fisheries (MF) observed about 100 distressed fish and fish with lesions at the fish kill location. MF personnel netted five dying Atlantic menhaden fish (*Brevoortia tyrannus*) near the shore line. All five fish displayed bleeding ulcerated lesions along their posterior near the anal vent. Such lesions have been observed during fish kills in other states in which *Pfiesteria* were implicated as a cause of the kill. These fish were preserved in formalin and these fish, and 100 other fish that had been frozen by the concerned citizen, were taken by F&W personnel on 9/21/99 to the F&W fish pathology laboratory. Necropsies were performed and histologic slides were prepared and examined on 7 of the 100 frozen fish and 3 of the 5 formalin-preserved fish. The specimens were prepared and examined by F&W's fish pathologist. A diagnosis of ulcerative mycosis was made (NJDEP, 1999). Lesions 5 to 12 mm in diameter were observed. Bacteria and fungal hyphae were observed in the lesions. The pathologist described the clinical finding as consistent with that described by Noga & Dykstra (1986) in fish samples from a *Pfiesteria* fish kill on the Pamlico River, NC.

The role of *Pfiesteria* in the formation of ulcerative lesions has been questioned (Noga and Dykstra, 1986; Blazer *et al*, 1999; Dykstra and Kane, 2000; Noga, 2000; Law, 2001; Vogelbein *et al*, 2001). The presence of fungal hyphae in the lesions of the fish taken during this fish kill (NJDEP, 1999) is evidence that a fungus, specifically *Aphanomyces invadans*, may have been the cause or one of several causes of the lesions. Nevertheless, *Pfiesteria* toxin(s) is(are) capable of destroying fish epidermis and causing bleeding lesions (Burkholder *et al*, 2001c). Because *Pfiesteria* also depress fish white blood cell counts (Glasgow *et al*, 2001b), it may be that fungi are able to invade and multiply within the fish lesions only after the *Pfiesteria* toxin has depressed immune system function and perhaps damaged the epithelium. "Extreme caution is needed when attributing particular fish kills, especially fish lesions, to *Pfiesteria*" (Samet *et al*, 2001). Glasgow *et al* (2001b), however, observed a positive correlation between the percentage of fish with lesions and concentrations of *Pfiesteria*-like zoospores in a 3-week period leading up to a large *Pfiesteria*-related fish kill in the Neuse River estuary in 1998.

D. Tuckahoe River *Pfiesteria* sampling at the site of the 9/99 fish kill (October 1999).

At the request of Dr. Rublee, on 10/6/99 DSRT personnel sampled river bottom sediment using a ponar grab sampler and water column samples at the site of the 9/99 fish kill and from two additional sites located about 1 mile upstream and 1 mile downstream of this site (see Figure 3; Table 2). The molecular assay, prior to this time, had only been used on water column samples, but protocol modifications were made that enabled Dr. Rublee to examine sediment samples. One of the 3 water column samples (the east or "downstream" site) and 3 of the 3 sediment samples tested positive for *Pfiesteria piscicida*-specific DNA (Table 2). This test is not quantitative and cannot tell how many *Pfiesteria* cells are in the sample or if the cells are toxic.

Table 2. Tuckahoe River *Pfiesteria* Water Column (W) and Sediment (S) Sample Sites - October 6, 1999.

Site	DSRT #	Location	Water Temp. (°C)	Salinity (ppt)	Test Result		
					P. piscicida	*P. shumwayae*	*Cryptop.*
1	NJ-21 (W)	Railroad bridge		3.5	P	N	N
	NJ-1 (S)				P	N	N
2	NJ-22 (W)	9/99 Fish kill site		1.5	N	N	N
	NJ-2 (S)				P	N	N
3	NJ-23 (W)	Campground		1.0	N	N	N
	NJ-3 (S)				P	N	N

Cryptop. = *Cryptoperidiniopsis*. N = negative. P = positive.

E. Second NJDEP *Pfiesteria* survey (November 1999).

On November 10 and 17, 1999, DSRT personnel collected 15 water column and 15 sediment samples from 7 estuary locations in New Jersey including the Tuckahoe River, site of the 9/99 fish kill. Five sample sites were Nutrient Biomonitoring Station sites that had been previously sampled in August. Four sites were Nutrient Biomonitoring sites that had not been previously sampled, and six sites, including the previously sampled fish kill site, were not Nutrient Biomonitoring sites. The estuary locations sampled are shown in Figure 2 and Table 3. The test results from Dr. Rublee were received on March 8, 2000. None of the three organisms was found in any of the samples.

Many of the sediment samples collected were sandy mixtures in nature. *Pfiesteria* have been found mostly, but not exclusively, in sediments with higher levels of organic matter but it is not yet known what types of sediments are best associated with the presence of *Pfiesteria* (Dr. Rublee, 3/14/00 E-mail communication). It is possible that *Pfiesteria* were not found because the chosen sample sites were not "*Pfiesteria*-permissive" locations.

Table 3. Second NJDEP *Pfiesteria* Survey - November 10 & 17, 1999.

Site	DSRT # (DEP #)**	Location	Water Temp. (°C)	Salinity (ppt)	Test Result		
					P. piscicida	*P. shumwayae*	*Cryptop.*
1	NJ-46 (W)	Mullica River	11.0	0.0	N	N	N
	NJ-31 (S)	@ Lower Bank			N	N	N
	(R-28)	bridge					
2	NJ-47 (W)	Mullica River	11.0	2.0	N	N	N
	NJ-32 (S)	@ Clarks Landing			N	N	N
3	NJ-48 (W)	Mullica River	11.5	10.0	N	N	N
	NJ-34 (S)	@ Chestnut Neck			N	N	N
	(R-29)						
4	NJ-49 (W)	Egg Harbor River	14.0	14.0	N	N	N
	NJ-34 (S)	@ Jeffers Landing			N	N	N
	(2801)						
5	NJ-50 (W)	Egg Harbor River	12.0	2.0	N	N	N
	NJ-35 (S)	@ Sandy Marina			N	N	N
6	NJ-51 (W)	Tuckahoe River	12.0	0.0	N	N	N
	NJ-36 (S)	@ Rt. 49 bridge			N	N	N
7	NJ-52 (W)	Tuckahoe River	14.0	0.5	N	N	N
	NJ-37 (S)	@ Lords Lane			N	N	N
8	NJ-53 (W)	Tuckahoe River	14.0	1.5	N	N	N
	NJ-38 (S)	@ 9/99 fish kill			N	N	N
9	NJ-54 (W)	Tuckahoe River	13.0	4.0	N	N	N
	NJ-39 (S)	@ Rt. 50 bridge			N	N	N
	(R37)						

10	NJ-55 (W)	Maurice River	n/a	n/a	N	N	N
	NJ-40 (S)	@ Mauricetown			N	N	N
	(R-43)	bridge					
11	NJ-56 (W)	Maurice River	n/a	n/a	N	N	N
	NJ-41 (S)	@ Bivalve			N	N	N
	(3900A)						
12	NJ-57 (W)	Dividing Creek	n/a	n/a	N	N	N
	NJ-42 (S)	@ Rt. 553 bridge			N	N	N
	(R-44)						
13	NJ-58 (W)	Cohansey River	n/a	n/a	N	N	N
	NJ-43 (S)	@ Tindall's Wharf			N	N	N
14	NJ-59 (W)	Cohansey River	n/a	n/a	N	N	N
	NJ-44 (S)	@ Hannah Taylor			N	N	N
	(R-48)	Rd.					
15	NJ-60 (W)	Stow Creek	n/a	n/a	N	N	N
	NJ-45 (S)	@ Wheaton Island			N	N	N
	(R-50)	Rd.					

** Office of Water Monitoring Management, Bureau of Marine Water Monitoring, Nutrient Biomonitoring Stations.
W = Water column sample. S = Sediment sample.
Cryptop. = *Cryptoperidiniopsis*. N = negative. P = positive.
Samples 1-9 collected 11/10/99. Samples 10-15 collected 11/17/99.
n/a = data not available.

F. Second Tuckahoe River *Pfiesteria* survey (September 2000).

The following summer, on September 11, 2000, DSRT personnel collected 8 water column and 8 sediment samples in the Tuckahoe River at and near the site of the September 1999 fish kill (see Figure 4; Table 4). Rather than sampling fixed NJDEP sampling locations as was done (for the most part) in past surveys, sampling was targeted at locations with high levels of organic matter in the sediments, as determined by visible inspection of ponar grab samples. The sediment samples at all of these sites consisted of fine-grained organic material. None of the three organisms were found in any of the samples (Table 4). Salinity levels at all of the sites were low due to the higher level of precipitation in 2000 compared to 1999. Salinity levels were well below the optimum salinity for *Pfiesteria* (15 ppt), *Aphanomyces* (2-10 ppt), and juvenile Atlantic menhaden growth (Blazer, 1999; Dykstra and Kane, 2000).

Table 4. Second Tuckahoe River *Pfiesteria* Survey - September 11, 2000.

Site	DSRT # (DEP #)	Location	Water Temp. (^oC)	Salinity (ppt)	Test Result		
					P. piscicida	*P. shumwayae*	*Cryptop.*
1	NJ-73 (W)	NJ Route 50 bridge	25.0	1.0	N	N	N
	NJ-57 (S)				N	N	N
	(R37)						
2	NJ-72 (W)	Railroad bridge	25.0	1.0	N	N	N
	NJ-56 (S)				N	N	N
3	NJ-71 (W)	Mill Creek junction	25.0	0.5	N	N	N
	NJ-55 (S)				N	N	N
4	NJ-70 (W)	Sept. 1999 fish kill site	25.0	0.0	N	N	N
	NJ-54 (S)				N	N	N
5	NJ-69 (W)	Gravelly Run junction	24.0	0.0	N	N	N
	NJ-53 (S)				N	N	N
6	NJ-68 (W)	Campground	24.0	0.0	N	N	N
	NJ-52 (S)				N	N	N
7	NJ-67 (W)	Warners Mill Stream	24.0	0.0	N	N	N
	NJ-51 (S)	jct. (@ Lords Lane)			N	N	N
8	NJ-66 (W)	"Upstream"	22.5	0.0	N	N	N
	NJ-50 (S)	(near Becket Drive)			N	N	N

W = Water column sample. S = Sediment sample.

Cryptop. = *Cryptoperidiniopsis*. N = negative. P = positive.