Pfiesteria Monitoring in New Jersey 1998 – 2000

NJ Department of Environmental Protection

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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

- *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.

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VIII. Figures

Figure 1
Initial Pfiesteria Survey
August 1999

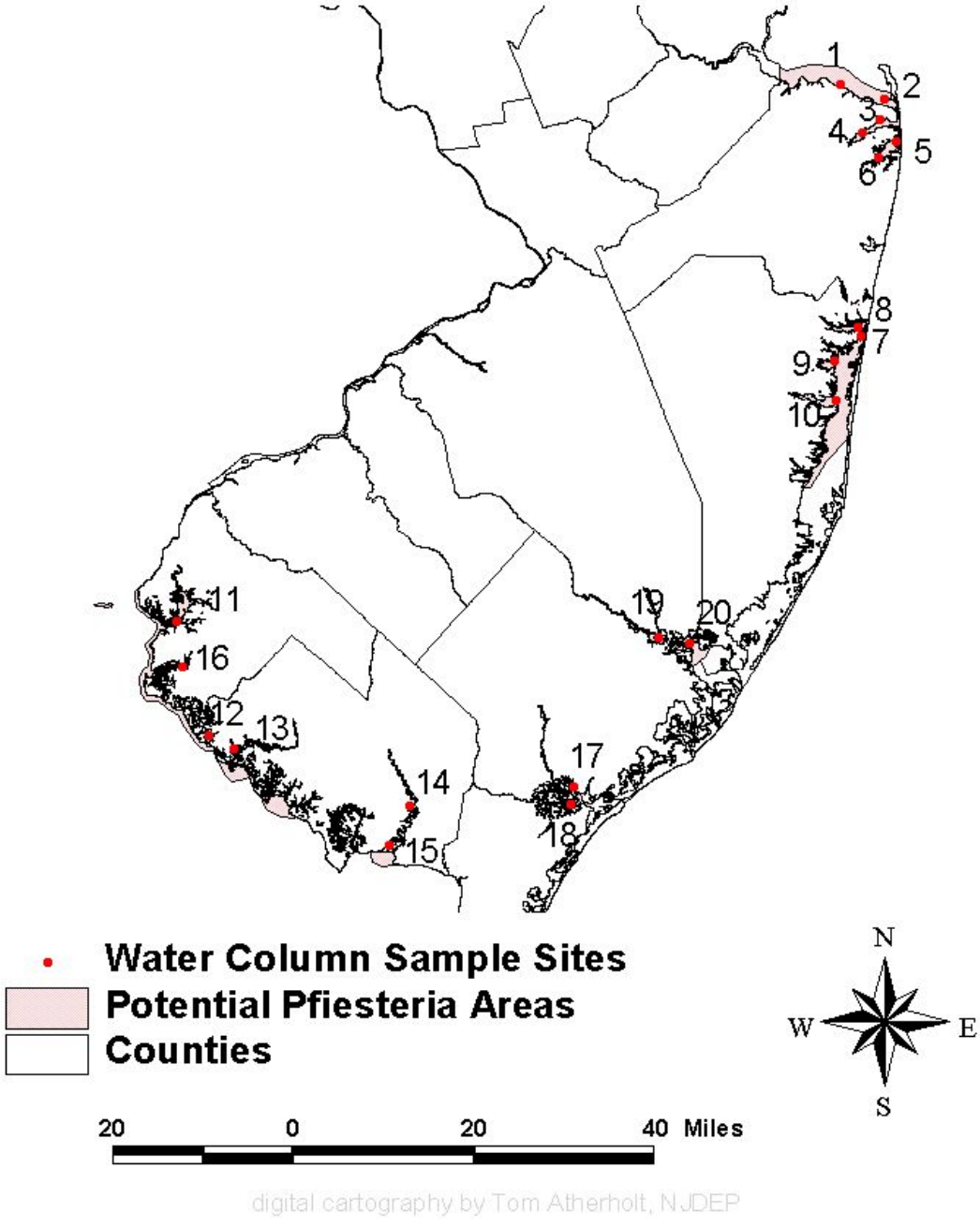
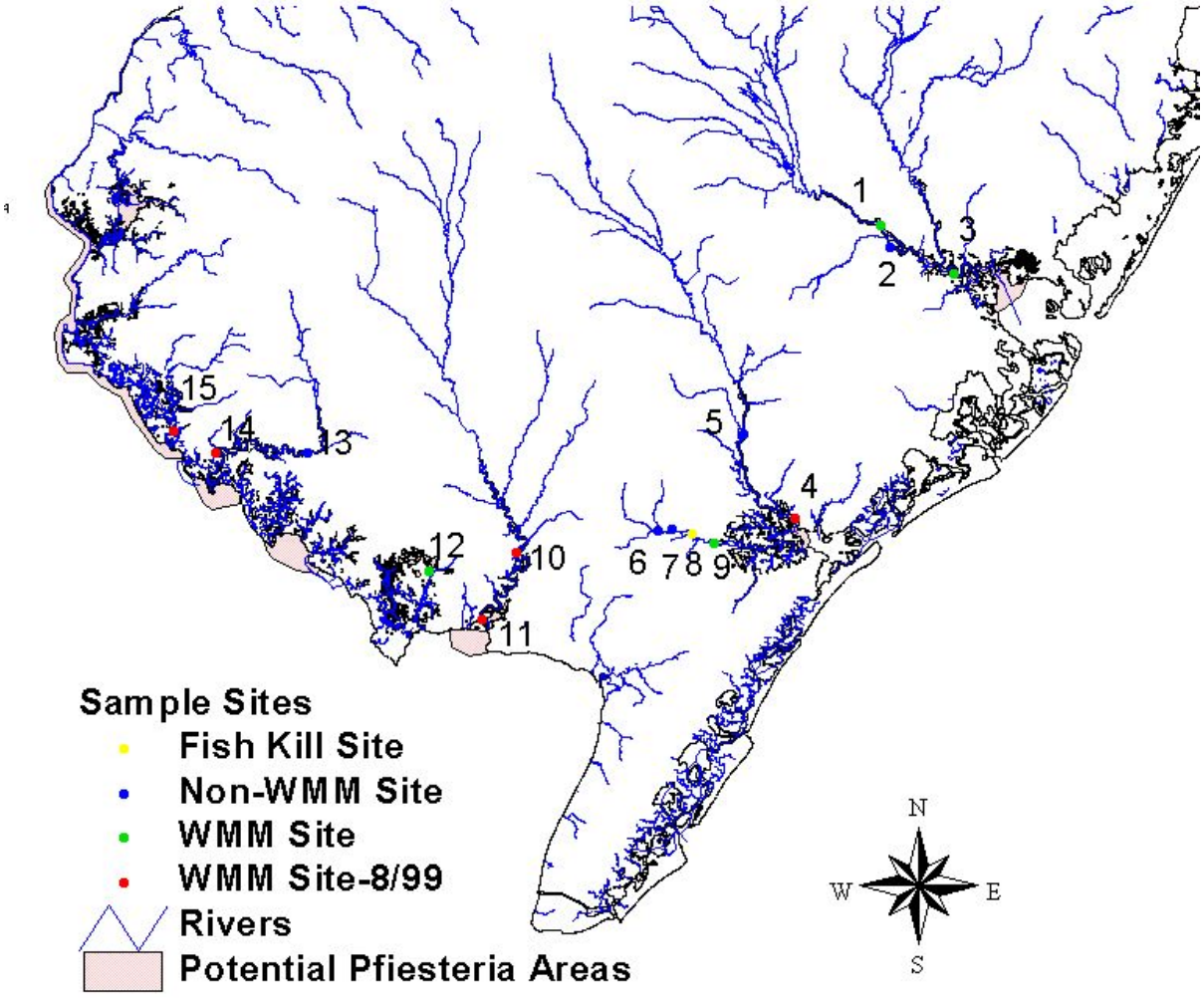


Figure 2
Second Pfiesteria Survey
November 1999



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

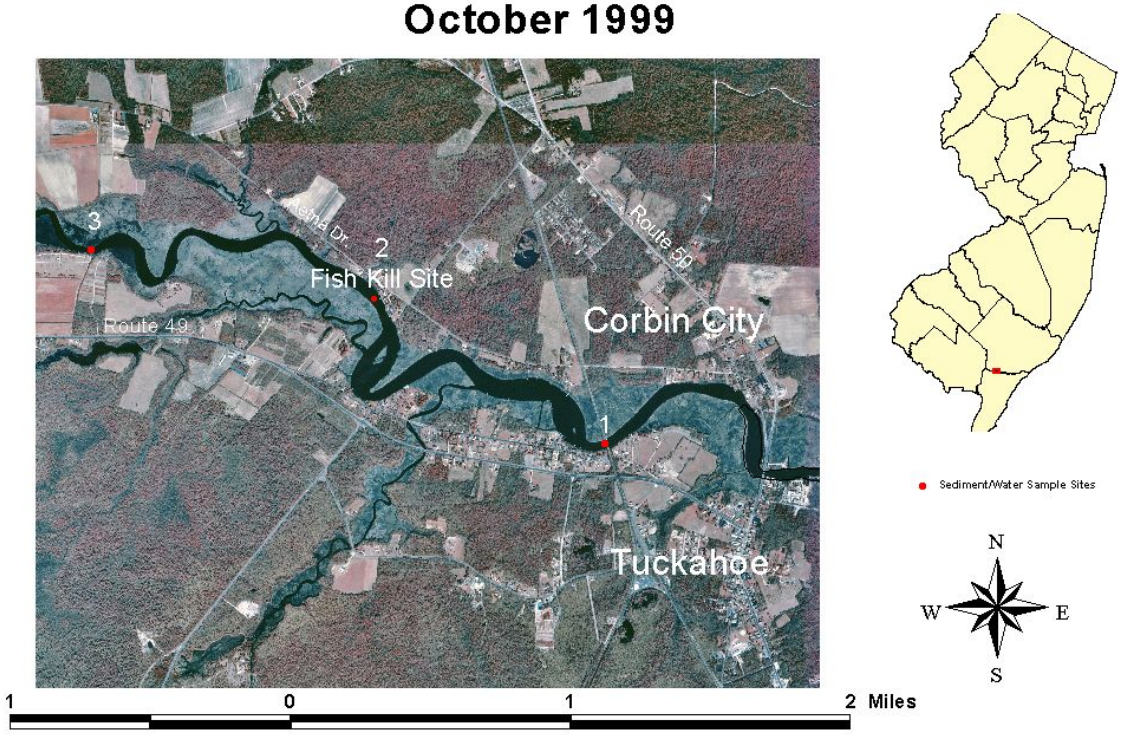
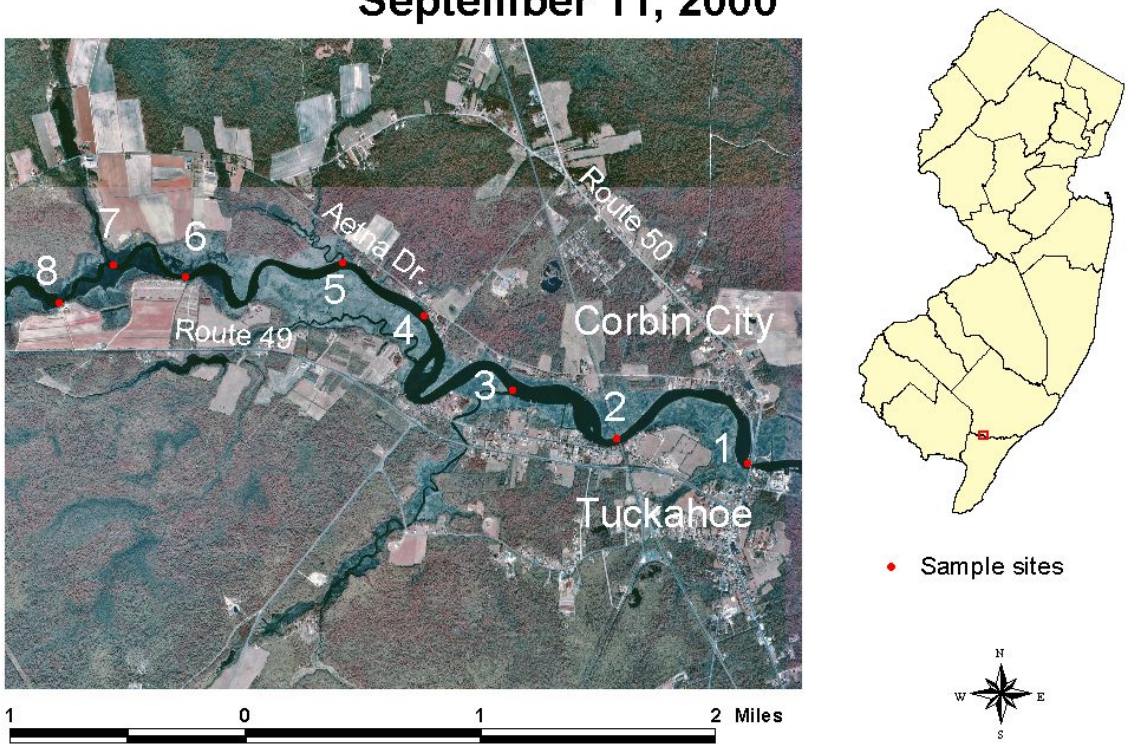


Figure 4
Tuckahoe River Pfiesteria Sampling
September 11, 2000



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. (^{o}C)	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) (R-28)	@ Lower Bank bridge			N	N	N
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) (R-29)	@ Chestnut Neck			N	N	N
4	NJ-49 (W)	Egg Harbor River	14.0	14.0	N	N	N
	NJ-34 (S) (2801)	@ Jeffers Landing			N	N	N
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) (R37)	@ Rt. 50 bridge			N	N	N

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. (^{o}C)	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.