Pfiesteria: Background Information and NJ Status

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What is Pfiesteria? "fee-STEER -ee-uh"

- Single-celled dinoflagellate microorganism found in back bays & tidal tributaries.
  - not found in fresh water.
- Dinoflagellates:
  - flagella (usually 2), diverse group (> 1000 species; > 55 toxin-producing species), unusual shapes common, prominent component of plankton, phosphorescence common, largely marine, some parasites.

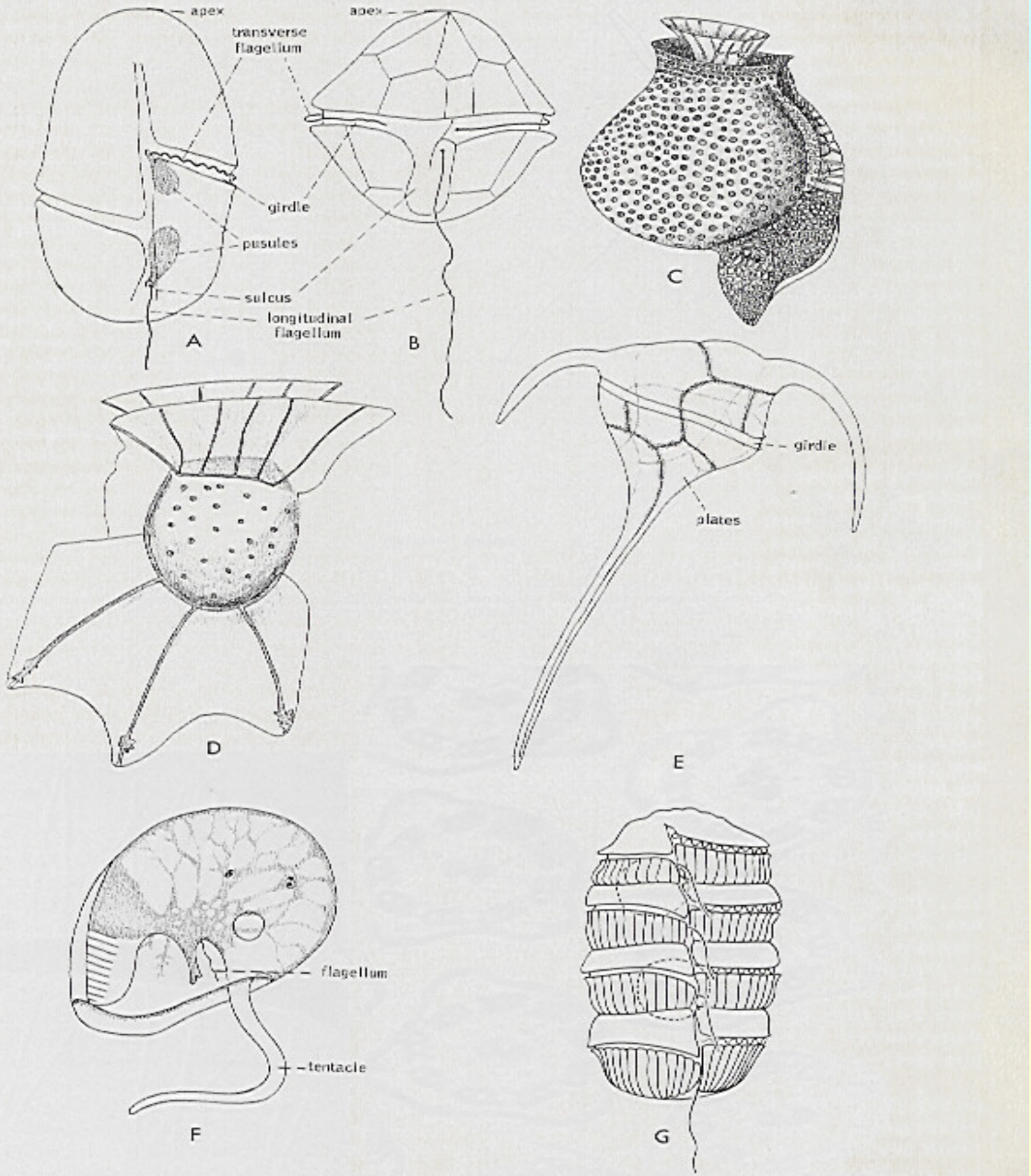


Figure 2-4. Dinoflagellates. *A.* A naked dinoflagellate, *Gymnodinium.* (After Kofoid and Swezy from Hall.) *B.* A freshwater armored dinoflagellate, *Glenodinium cinctum.* (After Pennak.) *C. Histophysis rugosa.* Flagella are hidden. *D. Ornithocercus.* (*C* and *D*, after Kofoid and Skogsberg from Chatton.) *E. Ceratium.* (After Jörgenson from Hyman.) *F. Noctiluca.* An aberrant phosphorescent dinoflagellate. Only one of the small flagella is visible within the "oral" depression. (After Robin from Kudo.) *G. Polykrikos kofoidi.* A colonial dinoflagellate composed of four individuals. (After Kofoid and Swezy from Kudo.)

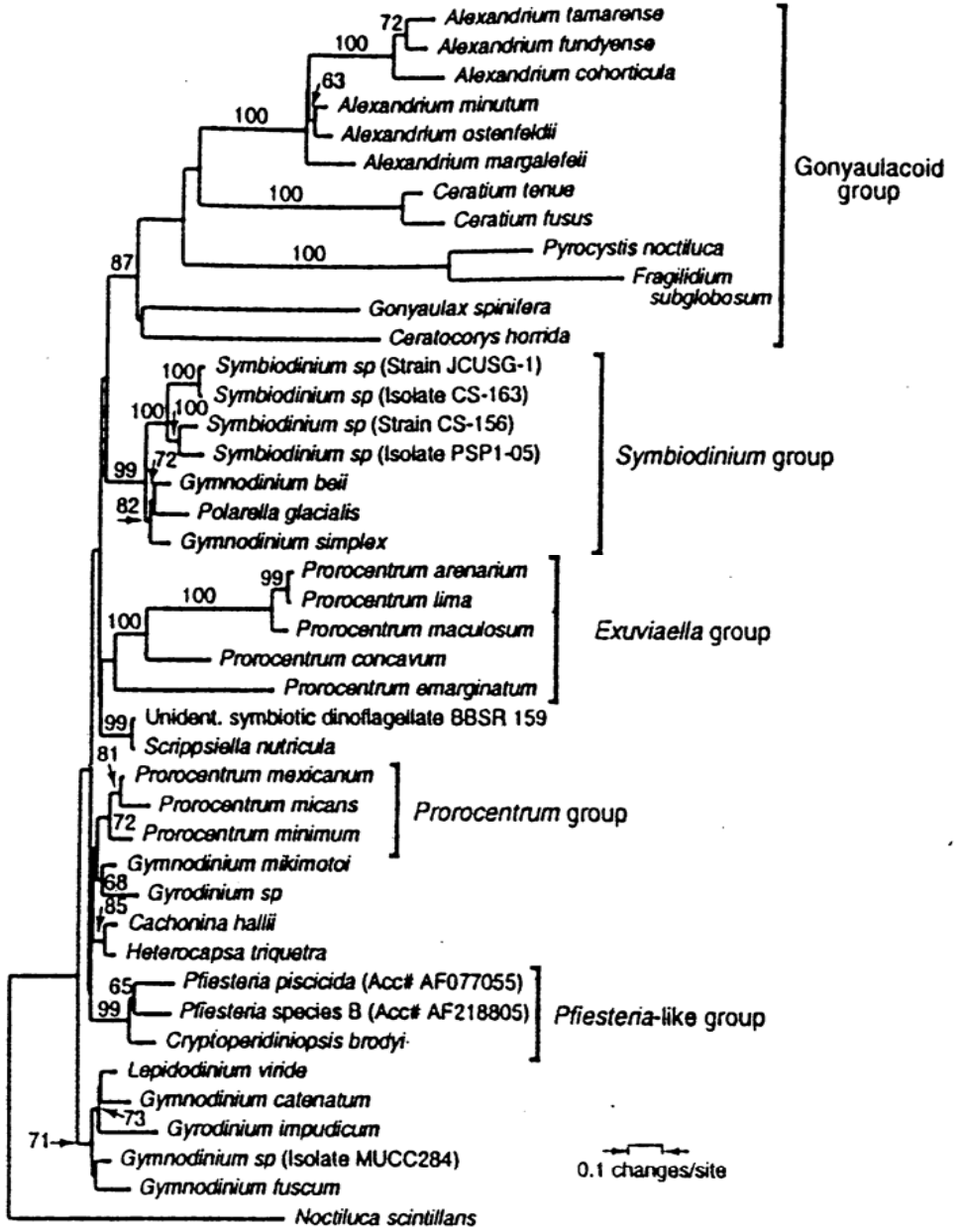


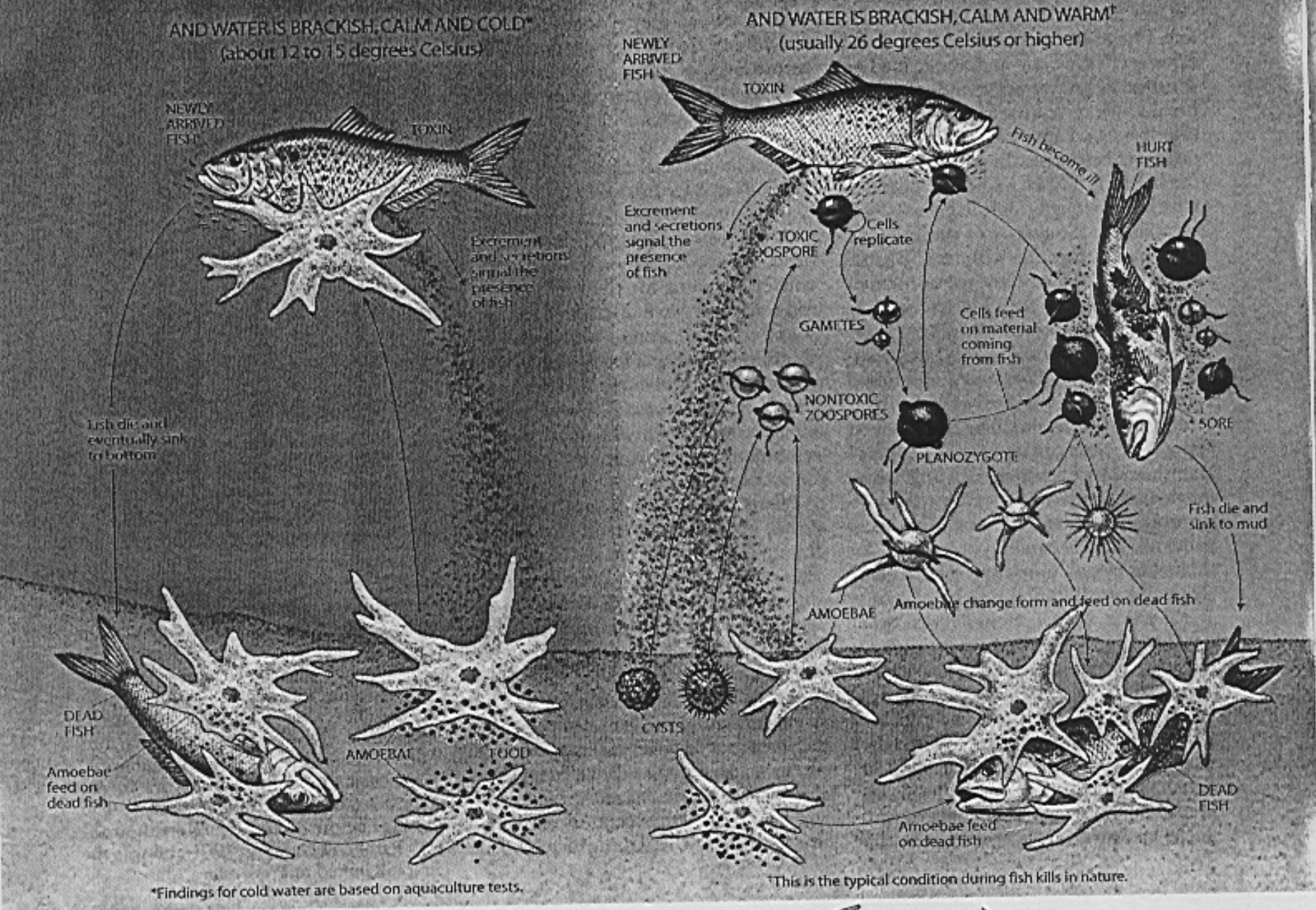
Fig. 5. Minimum evolution phylogenetic tree showing the placement of *P. piscicida* and *Pfiesteria* species B among dinoflagellates. *P. piscicida* (GenBank

- Not toxic in the absence of fish. Feed on algae and other microbes.
- In presence of large numbers of fish, some species produce toxins that can adversely affect fish, shellfish and humans.
- Very low concentrations can kill fish (250-300 zoospores/ml). Fish death (from higher concentrations) can occur in minutes to hours.
- Very complex life-cycle.

- 2 toxin-producing species:
  - *Pfiesteria piscicida* (“pis-kih-SEED-uh”)
  - *Pseudofiesteria shumwayae*
  - may be others
- Non-toxic species also.
- First identified in 1987 by JoAnn Burkholder, NC State, in contaminated laboratory aquaria water.
- First observed in environment during a fish kill in 1991 in the Pamlico River estuary, NC.

- Dormant in sediment in cyst form.
- In the presence of large numbers of fish, emerges as a toxin-producing amoeba or zoospore (TZ).
- After fish kill event, becomes non toxin-producing zoospore or amoeba.

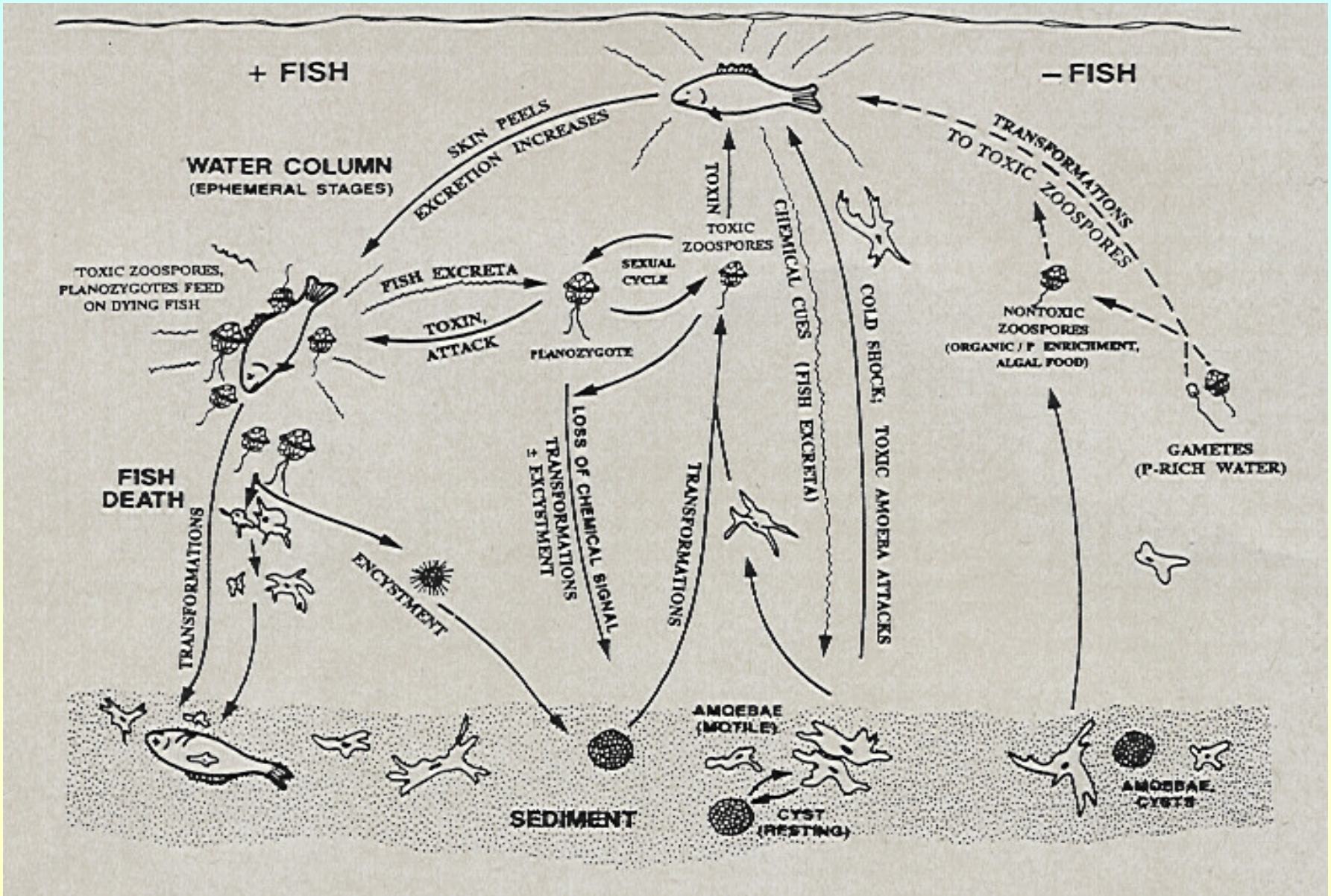
WHEN FISH ARE PRESENT . . .



*Findings for cold water are based on aquaculture tests.

†This is the typical condition during fish kills in nature.

Complex Life Cycle



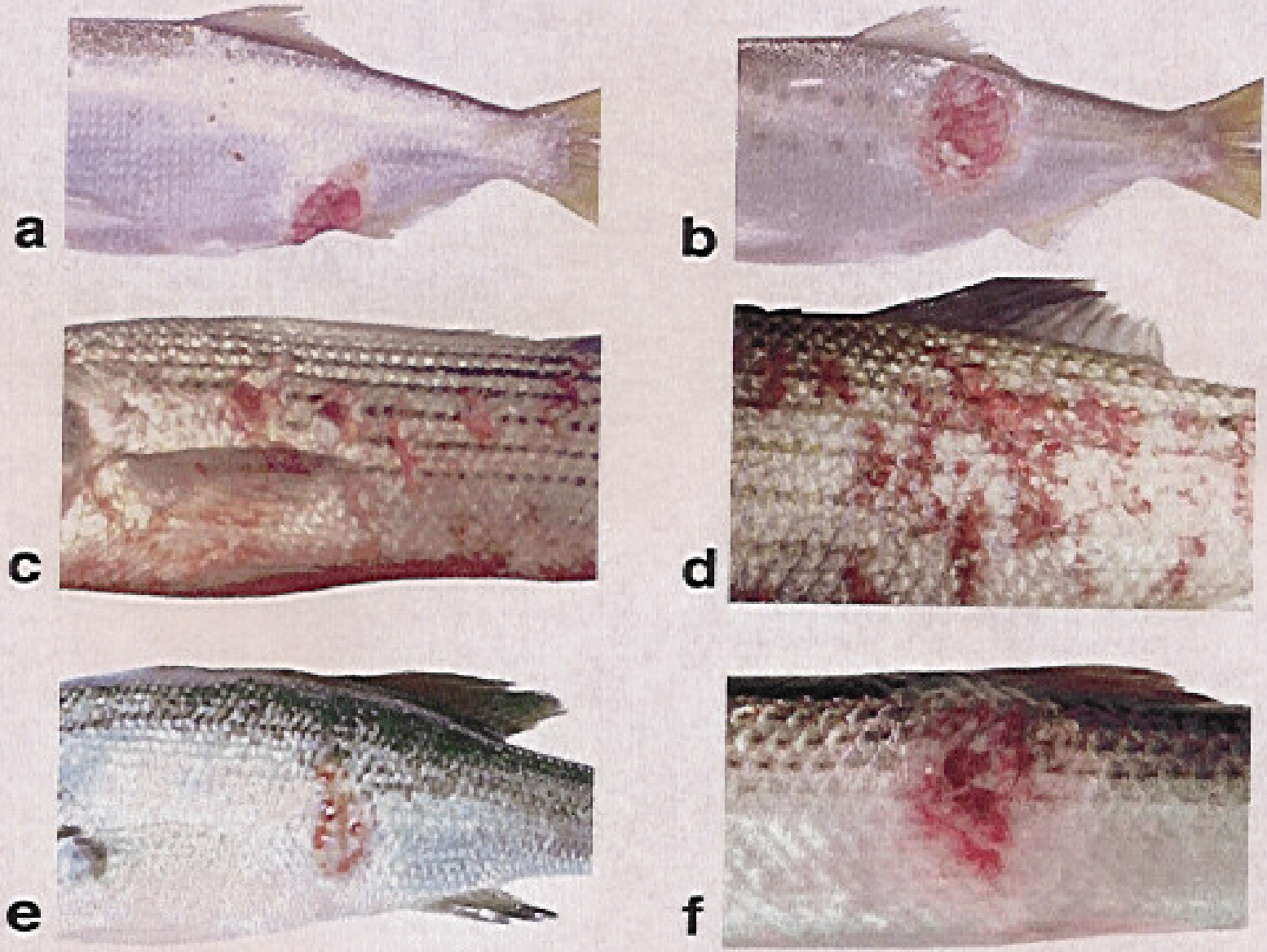


FIGURE 1.—Chronic ulcerative lesions in finfish associated with (**a, b**) fungus-like Oomycetes in Atlantic menhaden, (**c**) *Mycobacterium* in striped bass *Morone saxatilis*, (**d**) *Edwardsiella* bacteria in striped bass, (**e**) microsporidian parasites in largemouth bass *Micropiera salmoides*, and (**f**) viral hemorrhagic septicemia virus in Pacific herring *Clupea pallasi*. Photographs (c), (d), and (e) are courtesy of A. Baya; photograph (f) is courtesy of G. Marty (reproduced with permission from Inter-Science Publishing).

Pfiesteria Toxin(s)

- Toxin has been partially characterized.
  - An unstable organic compound containing copper (and iron).
  - Acts through light-induced free-radical formation.
  - May be one of a family of similar toxins (congeners).
- The genetic components of Pfiesteria responsible for toxin production have not yet been characterized.
- A cell culture assay can detect the presence of the toxin(s) in water or blood.

Pfiesteria toxins (con't)

- Do not last long in the environment (2-5 days before all activity is lost).
- Do not accumulate in fish tissues or organs.
- No evidence that eating fish or shellfish from a fish kill area causes illness, but other states advise not eating fish with sores or from fish kill areas.
- Role of Pfiesteria toxins in fish lesions in environment is unclear.
  - Fungus-like *Aphanomyces invadans* always found in the lesions, often with bacteria.

History of Pfiesteria

- First identified by JoAnn Burkholder in laboratory aquaria of Smith and Noga at NC State in 1987.
  - tilapia fish started dying from unknown cause (had introduced Pfiesteria-contaminated NC estuary water).
- First observed in environment during a fish kill in 1991 in the Pamlico River estuary, NC.

Environmental conditions favorable to toxic Pfiesteria

- Presence of high density of fish (Atlantic menhaden)
- Warm, brackish, shallow, poorly flushed waters with high levels of nutrients.
- A potential indicator of a polluted estuary.

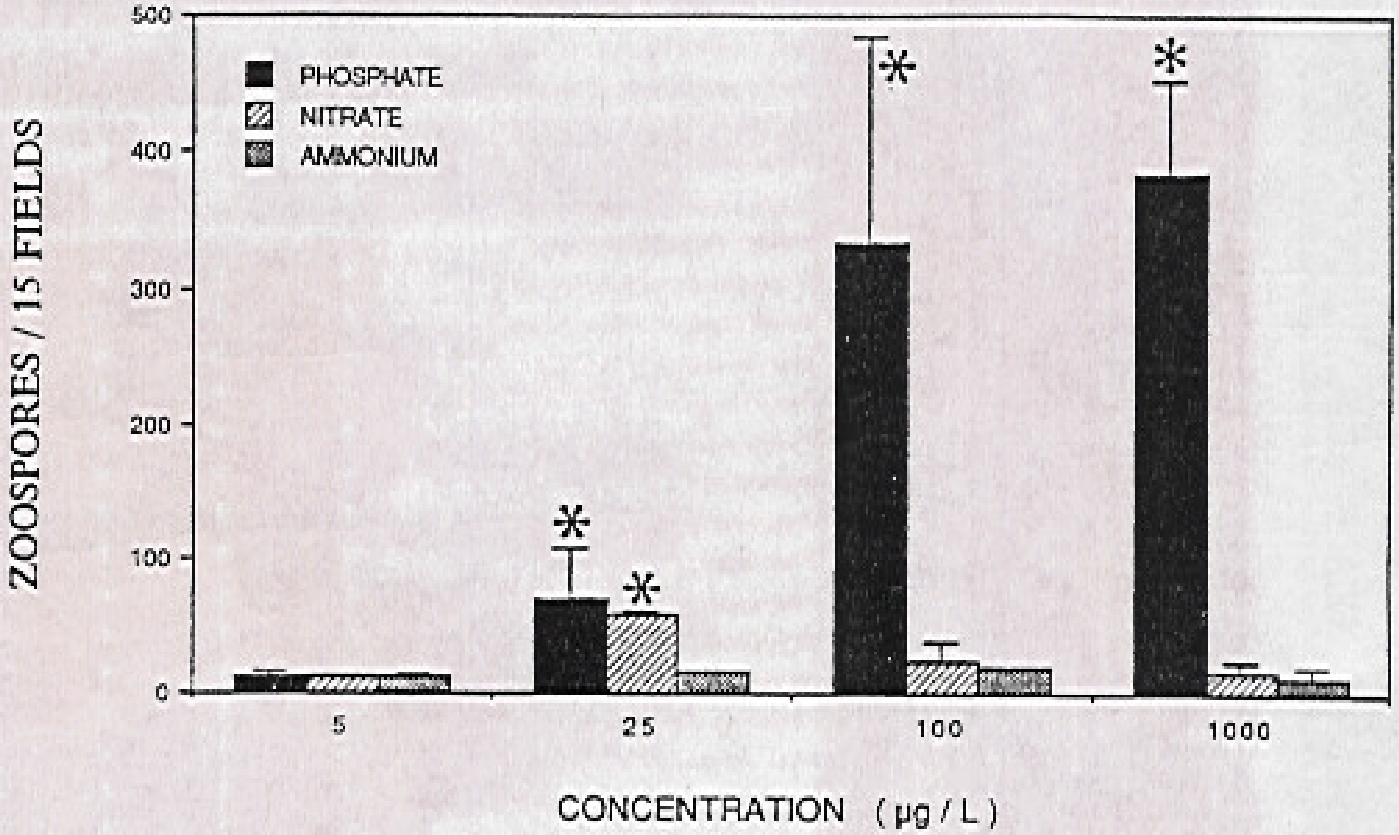


FIGURE 2. Response of *P. piscicida* amoeboid zoospores (reverted from gametes, and transformed/transforming into amoebae that were taken from actively killing cultures) to gradients of phosphate ($PO_4^{-3}P$), nitrate (NO_3^-N), and ammonium (NH_4^+N) enrichment after 4 d in batch cultures without finfish (means ± SE, $n = 3$). Note: The 5 µg/L concentration (background nutrient levels in the Instant Ocean media) represented the response in controls without nutrient additions. Asterisks indicate significant differences (tests for homogeneity of variance [Hartley's test; Gill, 1978], followed by one-way ANOVAs and comparison of means using Fisher's protected least significant difference test, with a comparison-wise error rate; $\alpha = 0.05$ [SAS, Inc., 1987; Day & Quinn, 1989]). Also note that in three of four experiments, we were able to repeat these results. The fourth experiment involved a culture inoculum that had not been exposed to live fish for 5 d, and the culture did not show P stimulation. These results suggest that the response of this animal-like dinoflagellate to nutrient enrichment depends, at least in part, on its recent food supplies, and that nutrient controls on growth of various stages likely are more complex than typically considered for plant-like dinoflagellates and other algae.

Toxic Pfiesteria in the Environment

- Have caused fish kills in Albemarle-Pamlico estuary (NC) and Chesapeake Bay (MD).
  - Large economic losses can result (MD 1997: $60 million)
- Toxic forms also found in VA, DE and other countries but not during fish kill events.
  - Pfiesteria of unknown toxicity observed as far north as Long Island, NY (not associated with fish kills).

TABLE 1. Environmental Conditions and Fish Affected at Kills Linked to *Pfiesteria piscicida* in North Carolina Estuaries, Coastal Waters, and Aquaculture Facilities During 1991–1993

Habitat	Temperature (°C)	Salinity (‰)	Nutrients (μg/L)	*Pfiesteria* (cells/ml)[a]	Fish affected[a]
Estuaries					
Neuse	28 ± 1	10 ± 1	TP 200 ± 35 TKN 1015 ± 295	810 ± 100	Atlantic menhaden, blue crab, catfish, mullet, spot, white perch (>1 × 10^9)
Pamlico	29 ± 1	7 ± 1	TP 290 ± 4 TKN 580 ± 45	4300 ± 2510	Atlantic menhaden, American eel, blue crab, croaker, hog-choker, southern flounder, spot (>2 × 10^6)
Coastal					
Near WWTP, inner channel	15	30	NA	11,960	Southern flounder, American eel, sheepshead (2 × 10^4)
Open Atlantic	13 ± 4	35	NA	1400[a]	Atlantic menhaden (>6 × 10^3)
Aquaculture	17 ± 2	22 ± 3	NA	820 ± 340	Atlantic menhaden, bay scallop, hybrid striped bass, littleneck clam, naked goby, sheepshead, southern flounder, tilapia, white perch (>4 × 10^4; >$100,00 loss)

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FIGURE 1.—Map of lower Chesapeake Bay indicating locations in which menhaden with lesions were collected in 1997. Symbols indicate closure of river due to fish kill (■), closure of river due to menhaden lesion event (●) and no closures but high prevalence of lesions (▲).

Dundalk
Glen Burnie
Severn
Severna Park
Annapolis
See map at left
Bowie
Chestertown
Rock Hall
QUEEN ANNES
Centreville
Queenstown
Stevensville
KENT ISLAND
Dover
KENT
Delaware Bay
DELAWARE
MARYLAND
Greensboro
Denton
Harrington
Milford
TALBOT
Easton
St. Michaels
Tilghman
CAROLINE
Federalsburg
Bridgeville
Seaford
Georgetown
SUSSEX
Lewes
Rehoboth Beach
Dewey Beach
Millsboro
Laurel
Hurlock
Cambridge
DORCHESTER
Vienna
CALVERT
Prince Frederick
Salisbury
WICOMICO
Ocean City
Berlin
WORCESTER
Snow Hill
Pocomoke City
SOMERSET
Princess Anne
Crisfield
Lexington Park
ST. MARYS
Leonardtown
ASSATEAGUE ISLAND NATIONAL SEASHORE
VIRGINIA
F
G
H
I
J
K
L
M
N

Locations of samples for *Pfiesteria piscicida* in 1998

Native Fish & Shellfish Affected (lethal to all tested fish and shellfish)

- Mostly Atlantic menhadin (Brevoortia tyrannus).
- Other finfish:
  - American eel, Atlantic croaker, black grouper, channel catfish, hogchoker, killifish, mosquitofish, naked goby, pinfish, red drum, sheepshead, southern flounder, spot, spotted sea trout, striped bass, striped mullet, white perch.
- Shellfish: bay scallops, blue crab, eastern oyster, littleneck clams.

Adverse Human Health Effects

- First observed in laboratory personnel working with Pfiesteria cultures.
- Reports from numerous fisherman exposed during fish kill events.
- H.B. Glasgow *et al*. 1995. J. Toxicol. Environ. Health 46:501-522.

TABLE 3. Symptoms in Laboratory Exposures to Toxic Cultures of *Pfiesteria piscicida*

Symptom	Case A	Case B	Case C	Others ($n = 7$)
Extremity paresthesias (tingling burning sens.)	+	+	–	–
Circumoral paresthesias	?	+	–	–
Paradoxical paresthesias	–	–	–	–
Arthralgia (joint pain)	–	–	+	2
Myalgia (muscle pain)	+	–	–	2
Diarrhea	–	–	–	–
Asthenia (loss of strength)	+	+	+	2
Headache	–	+	+	–
Pruritus (itching, irritation)	–	–	–	–
Nausea	–	+	+	1
Abdominal pain	–	+	+	1
Vomiting	–	+	–	–
Perspiration	–	+	–	–
Tearing/eye irritation	–	+	+	4
Dyspnea/respiratory problems	–	+	+	3
Paresis (partial paralysis)	–	–	–	–
Memory problems	+	+	+	1
Emotional changes	+	+	+	–
Skin lesions	+	+	+	–

Note. Adapted from Swift and Swift (1993), with rank order following that of ciguatera poisoning.

Adverse Human Health Effects (con't)

- Disorientation with a sense of depersonalization and of being compelled to continue. Lacking ability to recognize that "something was wrong".
- Short term memory loss: lasting 1-8 weeks.
- Heavily exposed persons: residual symptoms, following vigorous exercise, lasting for years afterward.

Pfiesteria Detection Assays

- "TPC" (toxic Pfiesteria complex) assay for water samples ("gold standard"):
  - Pfiesteria capable of killing target fish (typically tilapia) in test aquaria.
  - Burkholder's lab and perhaps 1 or 2 others only; P3 safety conditions employed.
  - Takes 8 weeks or more for low concentration samples.
- Toxin assay for water or blood samples:
  - cell culture assay (rat pituitary cells)
  - cytotoxicity and gene induction endpoints.
  - developed by John Ramsdell *et al*, NOAA, Charleston, SC.

JEFFREY J. SPRINGER N.C.S.U. (left); HOWARD B. GLASGOW JR. N.C.S.U. (right)

HIGHLY PROTECTIVE EQUIPMENT is now de rigueur for researchers studying *Pfiesteria* and its close relatives. People can be harmed not only by having contaminated water touch their skin but also by inhaling *Pfiesteria* toxins from the air.

Pfiesteria Detection Assays (con't)

- Species-specific detection assays for water and sediments:
  - detects ribosomal DNA specific to *P. piscicida, Ps. shumwayae*, or *Cryptoperidiniopsis*.
  - shows if DNA of these organisms is present.
  - **can't tell if cells dead or alive or capable of producing toxins.**
  - developed by David Oldach (U. Md.) and Parke Rublee (UNC - Greensboro).

Figure 1
Initial Pfiesteria Sampling Sites
August, 1999

Water Column Sample Sites
Potential Pfiesteria Areas
Counties

N
W
E
S

20 0 20 40 Miles

Figure 2
Tuckahoe River
September, 1999 Fish Kill Site &
October, 1999 Pfiesteria Sampling Sites

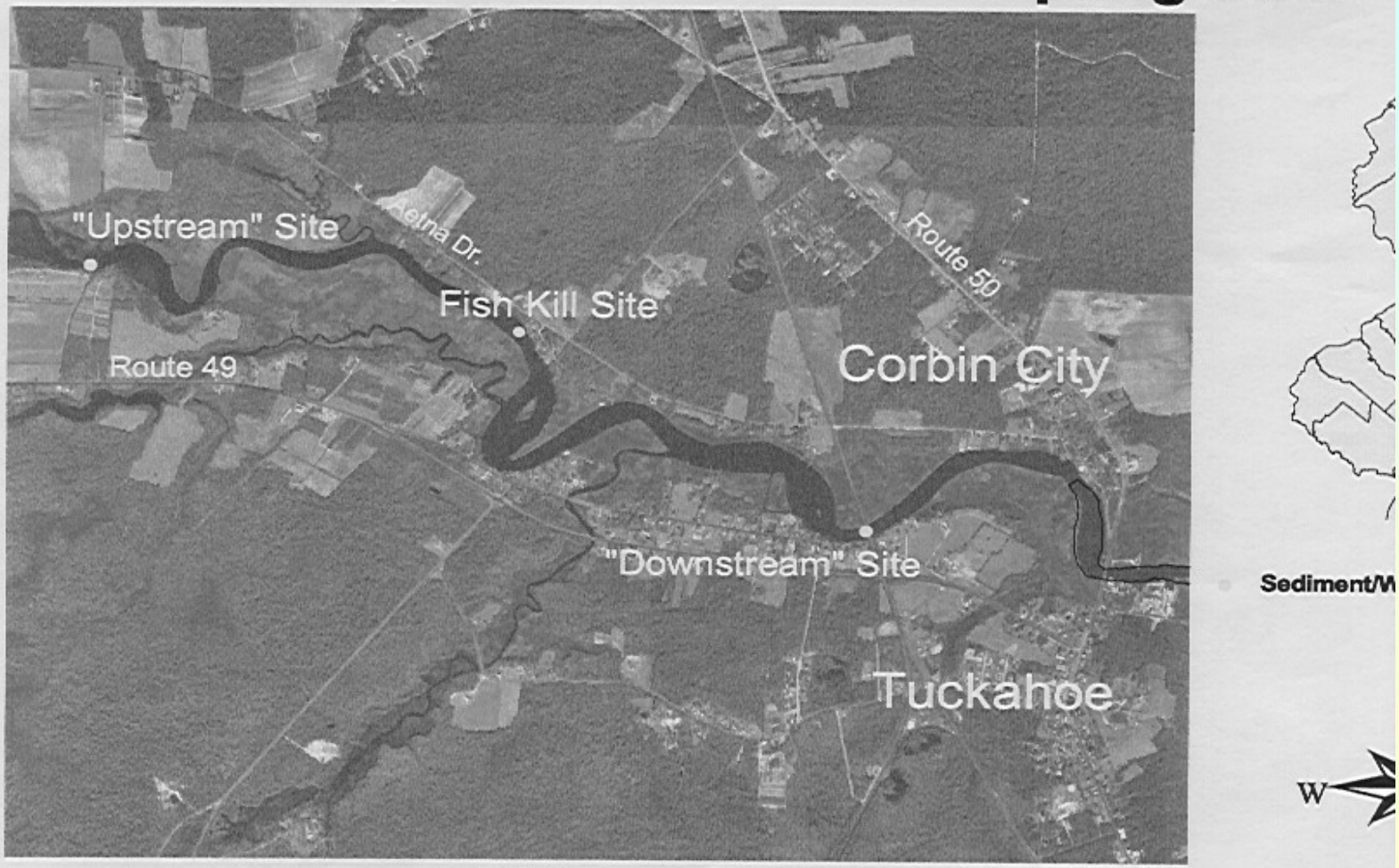
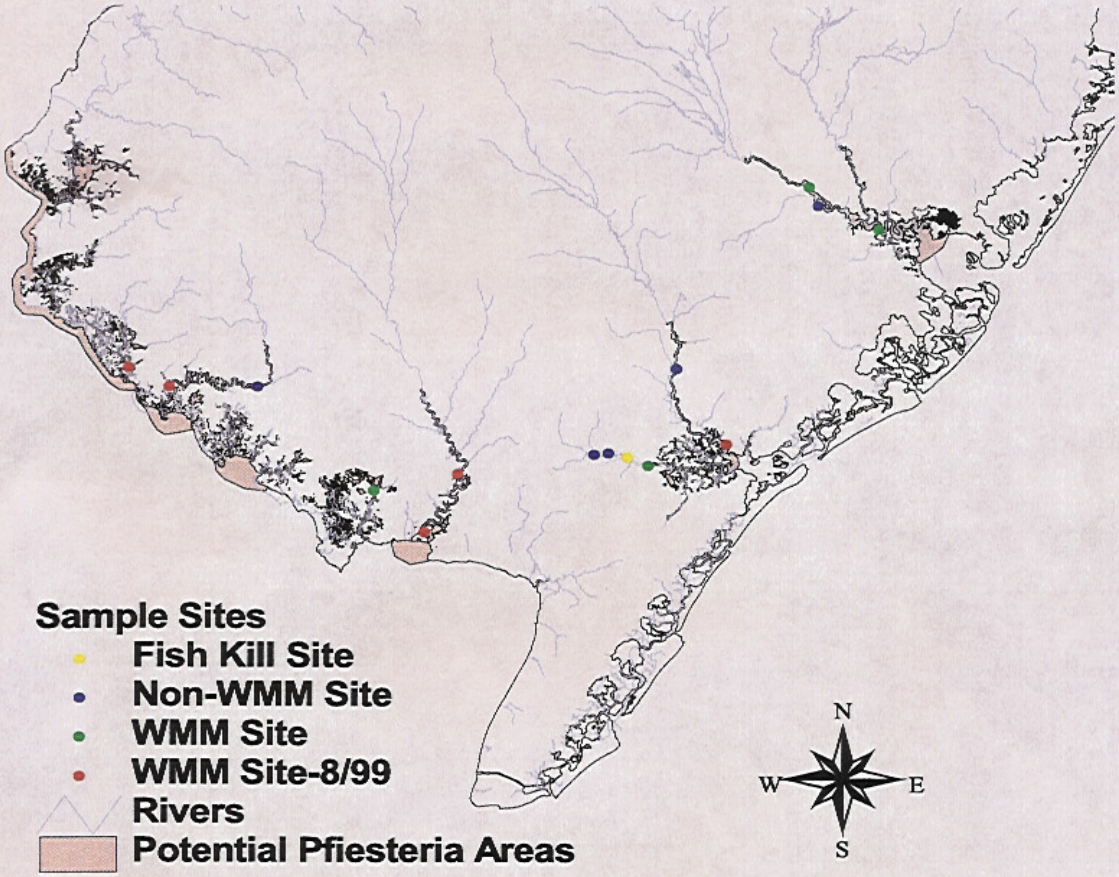


Figure 3
Pfiesteria Sampling Sites
November, 1999



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

8/99 = Also sampled in August, 1999.

digital cartography by Tom Atherholt, NJDEP