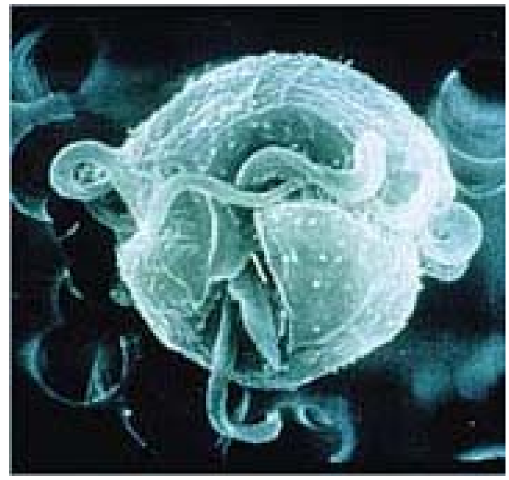
ISSUES – FIGURE SET



Pfiesteria piscicida
© Howard Glasgow,
JoAnn Burkholder, NCSU

Human Alteration of the Global Nitrogen Cycle

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Figure Set 4: What is the ecology and biology of *Pfiesteria*?

Purpose: Help students better understand the ecotoxicological effects of nitrogen loading to estuaries.
Teaching Approach: "jigsaw"
Cognitive Skills: (see Bloom's Taxonomy) — comprehension, interpretation, application, evaluation
Student Assessment: newspaper article

BACKGROUND

The *Pfiesteria* story is fascinating from many points of view - ecological, political, personal. Its life history is so bizarre that students think you are telling them a science fiction story. The politics center around JoAnn Burkholder, a microbial ecologist from North Carolina State University, who is either admired or chided, depending on who you are talking to. The politics are those related to the chicken and hog industry in North Carolina. Federal politics also come into play because funding for *Pfiesteria* research was limited until the dinoflagellate was linked to fish kills in the Chesapeake Bay (Washington D.C. is of course very close to the Bay).

Burkholder's first paper on *Pfiesteria piscicida* was published in Nature in 1992. Since then researchers have learned a great deal about its highly complex life cycle (more than 20 different stages), its mode of attack on fish and other animals, the action and structure of the *Pfiesteria* toxin, factors that stimulate the toxic forms to grow in nature, and its presence in other estuaries in the U.S. and globally. There are many websites about *Pfiesteria* and numerous references are listed below. BioScience devoted a whole issue (vol. 51, no. 10; 2001; Magnien 2001, Grattan et al. 2001, Staver and Brinsfield 2001) to *Pfiesteria* outbreaks - the history, the policy, human health risks, and agricultural contamination of receiving waters. See also numerous www sites in the resource section.

Pfiesteria piscicida was discovered by accident when a colleague of Burkholder noticed that the fish tilapia held in tanks suddenly died several days after being exposed to water collected in the Pamlico River, NC. The scientists observed that density of a small dinoflagellate increased before fish death and declined rapidly in number unless live fish were introduced. The dinoflagellate produced resting cysts or non-toxic amoeboid forms in the absence of fish (Burkholder et al. 1992). When Burkholder and her colleagues looked for dinoflagellates during massive fish kills in local estuaries *Pfiesteria* was abundant, but only when fish were dying. In the Nature paper they name *Pfiesteria* as the causative agent of the many unexplained fish kills in NC estuaries, they identify a neurotoxin from the dinoflagellate as the lethal agent, and they link fish kill events to nutrient enriched *Pfiesteria* blooms (Burkholder et al. 1992).

In subsequent studies, Burkholder reported that toxic forms of *Pfiesteria* were stimulated by inorganic and organic phosphate (e.g. Burkholder and Glasgow 1997). She became embroiled in arguments with NC officials about the role of the state's hog farm effluents in stimulating *Pfiesteria* growth. Nitrogen entered the story in the late 1990's when the incredible nutritional versatility of the dinoflagellate became clearer. In addition to consuming dissolved organic compounds (such as in animal waste), *Pfiesteria* retains chloroplasts from algae that it consumes when fish are not available. *Pfiesteria* zoospores with these inclusions, called kleptochloroplasts, are stimulated by N and P. In addition to stimulating the growth of *Pfiesteria* zoospores directly, N indirectly contributes to growth of the dinoflagellate because N is a limiting nutrient for phytoplankton in estuaries. Therefore, N loadings stimulate phytoplankton growth which in turn contribute to proliferation of *Pfiesteria*.

The neurotoxin excreted by *Pfiesteria* deserves special mention. Until the discovery of *Pfiesteria*, estuarine ecologists often had a difficult time explaining to legislators and the public how too many nutrients, which sound like good things, could be bad. In addition to the vocabulary problem, nutrient loading does not directly give people diseases or make them sick. For both these reasons, explaining that nutrient loading to estuaries is a severe environmental problem has been an uphill battle.

But *Pfiesteria* does make people sick. Both Burkholder and Howard Glascow, who works with her, suffered serious neurological effects when they unknowingly inhaled the *Pfiesteria* toxin. The scientists were hospitalized and affected for months.

Pfiesteria is the first reported dinoflagellate that produces aerosols that seriously harm human nervous systems. Symptoms include acute respiratory problems blurred vision, nausea, vomiting, extreme headaches, and severe memory dysfunction. According to Burkholder and Glasgow (2001) "For days to weeks following exposure, several laboratory personnel could recognize words individually but could not form sentences, perform simple arithmetic, or remember more than the last words of a sentence directed to them. The most seriously affected person in our laboratory, who is a highly intelligent researcher, managed only a 7-year-old's reading level for 3 months after exposure and required reading lessons at first to help regain reading ability … two others … could not remember their names or where they had lived." One of the many controversial aspects of the *Pfiesteria* story is the degree to which fishermen were harmed by neurotoxin aerosols during *Pfiesteria* blooms.

A note about the book *And The Water Turned To Blood* by Rodney Barker (see resources below for websites): this is a fascinating read, written like a biological thriller. Barker is far from neutral in the book; he sides with Burkholder and sensationalizes the story. My students loved the book and were quite conscious of the sensational journalism; discussion of the book could stimulate a lively discussion about objectivity in science and journalists as science reporters.

Literature Cited

Burkholder, J. M., E. J. Noga, C. H. Hobbs, and H. B. Glasgow, Jr. 1992. New 'phantom' dinoflagellate is the causative agent of major estuarine fish kills. Nature 358: 407-410.

Burkholder, J. M., and H. B. Glasgow, Jr. 2001. History of toxic *Pfiesteria* in North Carolina estuaries from 1991 to the present. BioScience 51: 827-841.

Burkholder, J. M., and H. B. Glasgow, Jr. 1997. *Pfiesteria piscicada* and other *Pfiesteria*-like dinoflagellates: Behavior, impacts, and environmental controls. *Limnology and Oceanography* 42: 1052-1075.

Grattan, L. M., D. Oldach, and J. G. Morris. 2001. Human health risks of exposure to *Pfiesteria piscicida*. BioScience 51: 853-858.

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

Staver, K. W. 2001. Agriculture and water quality on the Maryland eastern shore: where do we go from here? BioScience 51: 859-868.

STUDENT INSTRUCTIONS

Jigsaw

For this jigsaw follow your professor's instructions about which group you are initially in (A, B, or C) and which Figures you are responsible for understanding and explaining. In this jigsaw students first become "experts" in the figures assigned to group A, B, or C. In the second step of the jigsaw students regroup so that A, B, and C experts teach each other.

Remember to use the step one- step two approach to understanding figures and tables that you have practiced in class.

Background information on *Pfiesteria*

Each of the 5 figures focuses on a fascinating microscopic organism *Pfiesteria piscicada*. It is a dinoflagellate which is a common in marine environments. Dinoflagellates are single celled and are planktonic (drift in the water), although many forms have flagella and can therefore move. The are metabolically quite versatile; they can photosynthesize, absorb dissolved organic matter (like bacteria do), and also injest particles of organic matter. Dinoflagellates are brownish red in color and when they grow in large numbers (called blooms) the water can look red. Some dinoflagellates cause "red tides", which you may have heard of.

The *Pfiesteria* story is fascinating from many points of view - ecological, political, personal. Its life history is so bizarre that it sounds like a science fiction story. The politics center around JoAnn Burkholder, a microbial ecologist from North Carolina State University, who is either admired or chided, depending on who you are talking to. The politics are also those of hog industry in North Carolina.

Burkholder's first paper on *Pfiesteria piscicada* was published in the journal Nature in 1992. Since then researchers have learned a great deal about its highly complex life cycle (more than 20 different stages), its mode of attack on fish and other animals, the action and structure of the *Pfiesteria* toxin, factors that stimulate the toxic forms to grow in nature, and its presence in other estuaries in the U.S. and globally. There are many websites about *Pfiesteria*, some listed in the Resources section of this Issue.

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* In step one you first figure out how the figure or table is set up (e.g. what the labels on the axes mean). You also need to have a pretty good idea of the experimental design - how the researchers set up their study - and the hypotheses the study address. In step two you can go on to interpreting the data. For both steps write down any questions you have.

FIGURES

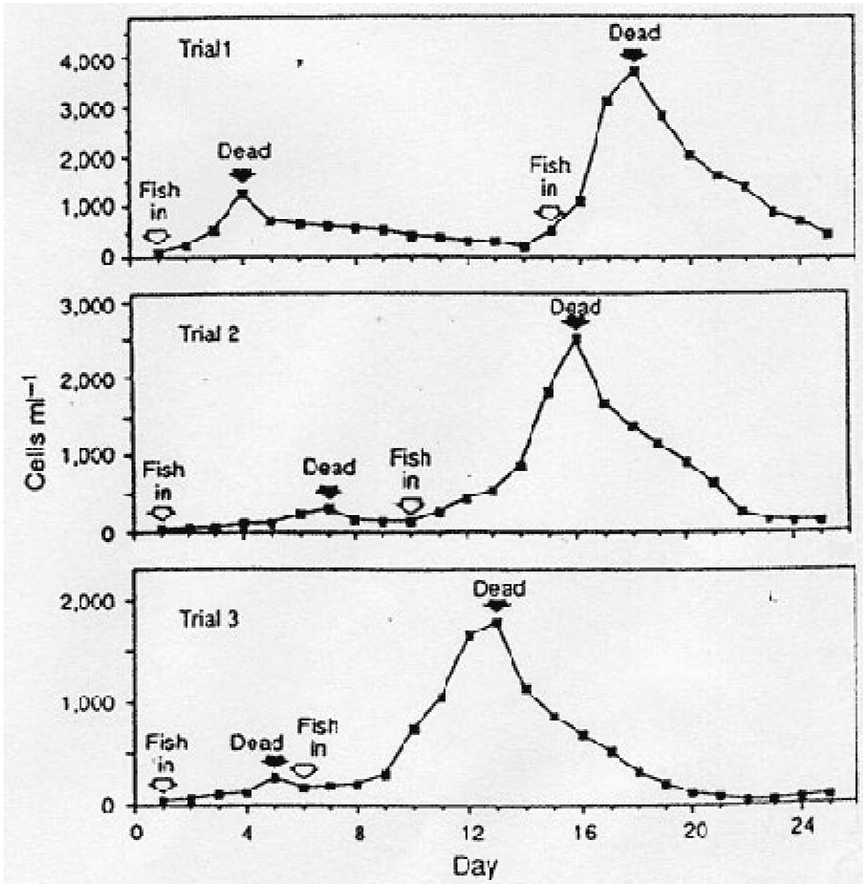

Figure 4A. Response of the dinoflagellate *Pfiesteria piscicada* (the toxic flagellated stage) to the fish tilapia (*Oreochromis aureus*) in repeat-trial experiments. The experiments were done in aquaria and the time between death of the first fish and addition of a second live fish was varied (cells ml-1 = cells/ml). From J. M. Burkholder, E. J. Noga, C. H. Hobbs, and H. B. Glasgow, Jr. 1992. New 'phantom' dinoflagellate is the causative agent of major estuarine fish kills. Nature 358: 407-410.

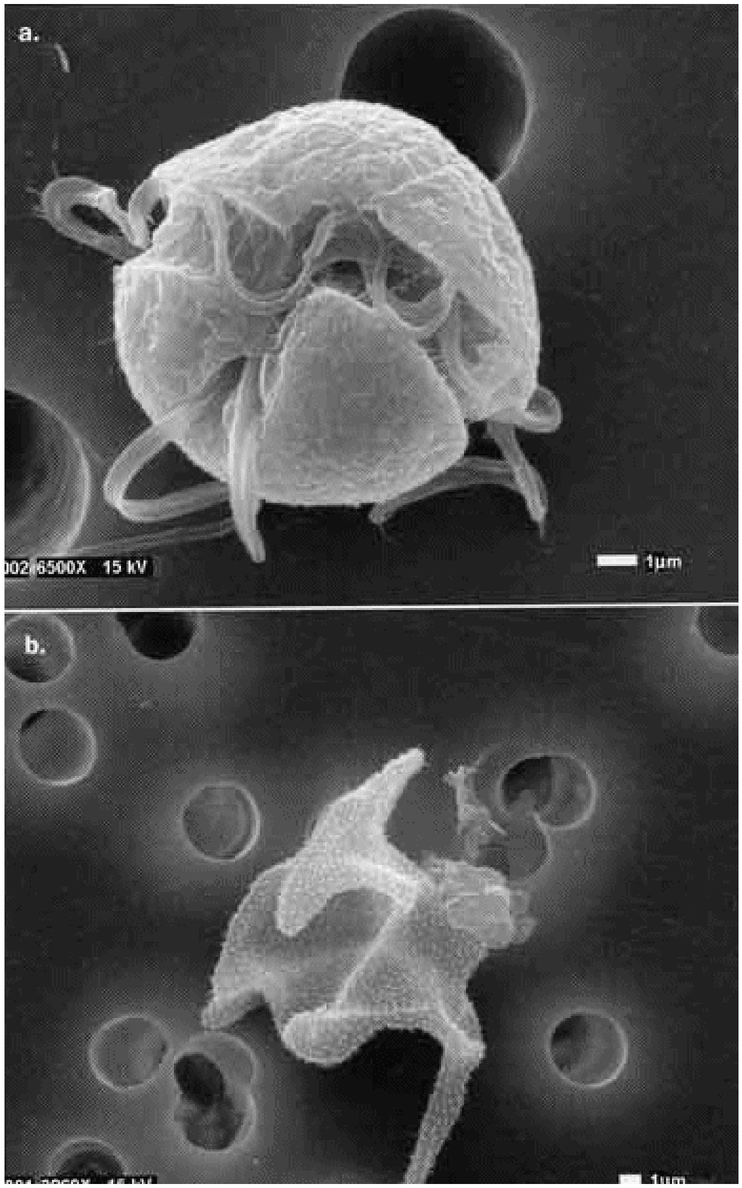

Figure 4B. Scanning electron micrographs of two common stages of *Pfiesteria piscicada*. (a) is toxic zoospore (diameter = 7-14 microns) and (b) amoeba that transformed from a toxic zoospore (cell length = 20-40 microns). Photos from NCSU Center for Applied Aquatic Ecology. From J. M. Burkholder and H. B. Glasgow, Jr. 2001. History of toxic *Pfiesteria* in North Carolina estuaries from 1991 to the present. BioScience 51: 827-841.

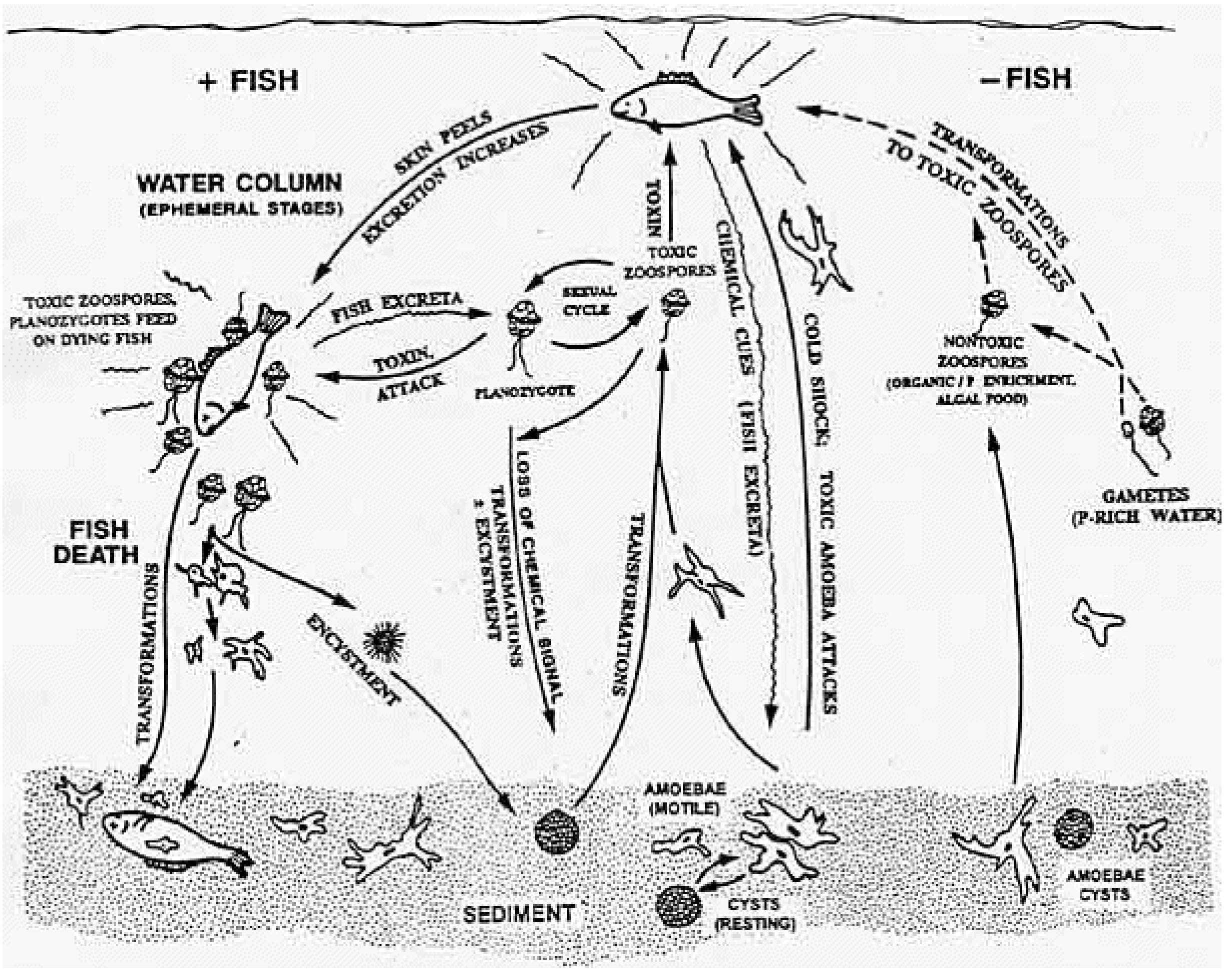

Figure 4C. Relationship between life stages of *Pfiesteria piscicada* and presence or absence of fish. See legend on figure for more details. From J. M. Burkholder and H. B. Glasgow, Jr. 1997. *Pfiesteria piscicada* and other *Pfiesteria*-like dinoflagellates: Behavior, impacts, and environmental controls. Limnology and Oceanography 42: 1052-1075.

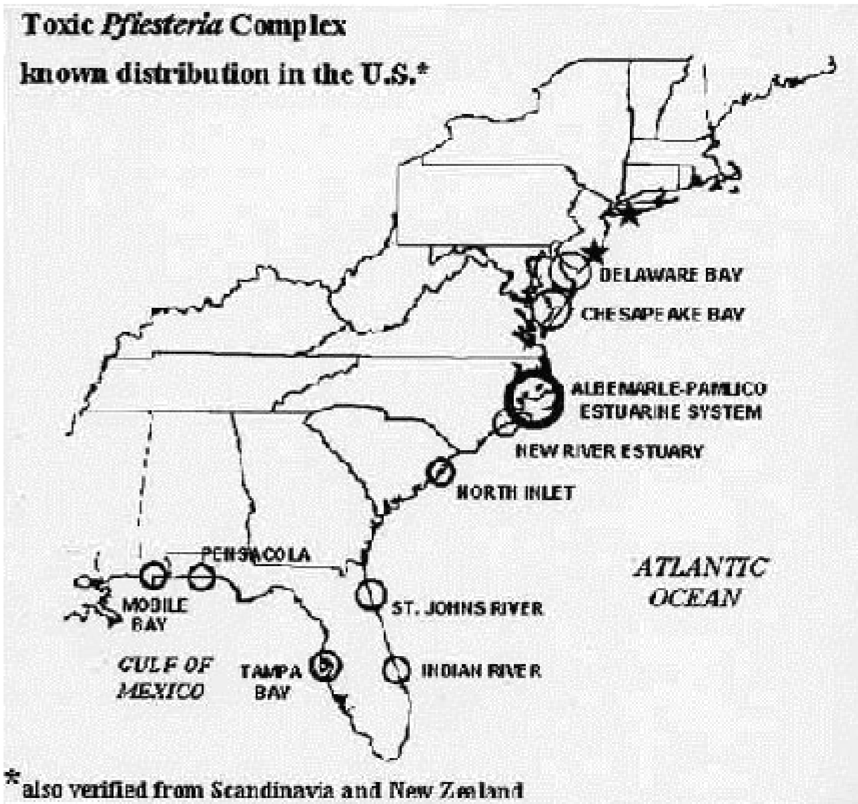

Figure 4D. U.S. distribution of toxic strains of *Pfiesteria* (TOX-B cells are nontoxic to grown fish but sometimes have residual toxicity to sensitive larval stages of fish). From J. M. Burkholder and H. B. Glasgow, Jr. 2001. History of toxic *Pfiesteria* in North Carolina estuaries from 1991 to the present. BioScience 51: 827-841.

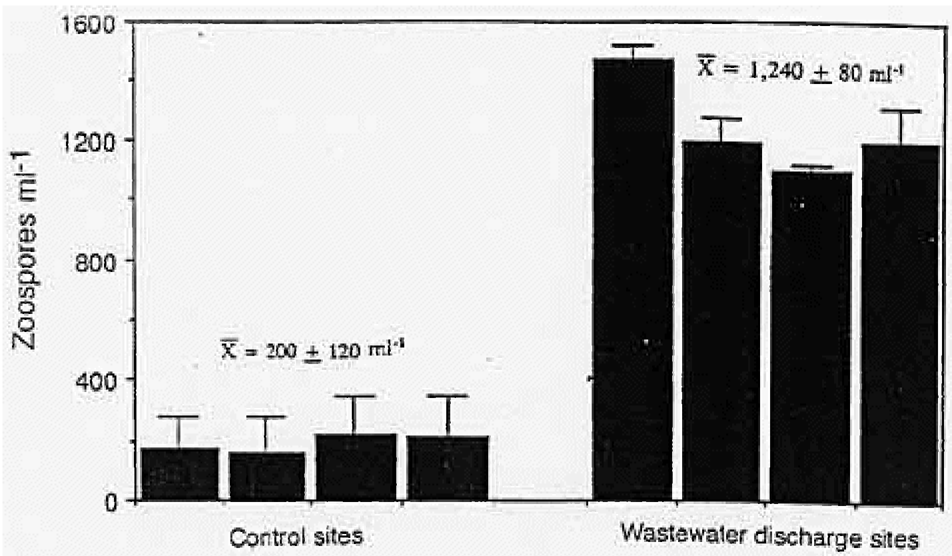

Figure 4E. Cells (NTZs - nontoxic zoospores) of *Pfiesteria*-like dinoflagellate (look like cells grown in the laboratory) from a depth of 0.25m at 4 control sites and 4 wastewater discharge sites (within about 100m of discharge point) in the New River estuary, North Carolinea. Histograms are averages + 1 standard deviation of 3 replicate samples taken at each site. From J. M. Burkholder and H. B. Glasgow, Jr. 1997. *Pfiesteria piscicada* and other *Pfiesteria*-like dinoflagellates: Behavior, impacts, and environmental controls. *Limnology and Oceanography* 42: 1052-1075.

FACULTY NOTES

For suggestions on doing a jigsaw see the Jigsaw tutorial in the Teaching section.

In my experience students are truly amazed by the *Pfiesteria* — the ecology, history, and politics — and are quite interested to learn more about the relevant ecological concepts. Therefore via *Pfiesteria* you can engage students in topics such as N and P cycling, autotrophy vs. heterotrophy, marine plankton, estuarine ecology, and plant nutrition. More applied issues are nutrient loading and eutrophication, management of human sewage and animal waste, environmental problems in the Chesapeake Bay, and the politics of "big" agriculture and environmental contamination.

For this jigsaw you can divide up the figures for the 3 groups as you wish. One possibility is Figure 4A & Figure 4B for Group A, Figure 4C for Group B, and Figure 4D and Figure 4E for Group C.

You can use class time for both groupings of the jigsaw or ask students to meet with their first group outside of class as a homework assignment. In either case, allow the students enough time (5 minutes per group) for the mixed group component (A, B, & C students explain the figures to each other). After this you can project the figures to the class and ask for interpretations and questions.

Discussion question for the cognitive skill "evaluation": Lead a discussion about how the ecology and biology of *Pfiesteria* contributes to the controversy over management of hog waste. There are several ecological/biological (as opposed to political) reasons why the *Pfiesteria* debates have been so heated. The *Pfiesteria* smoking gun is especially difficult to find during the massive fish kills that Burkholder and others have studied. Burkholder named *Pfiesteria* a 'phantom' in the Nature paper for good reason. The dinoflagellate has many forms that are quite different in appearance. Therefore, while the toxic zoospore may have been the lethal agent on day one, by day two when researchers take water samples *Pfiesteria* has likely morphed into an amoeba (or other) shape. In addition, swine waste can enter a river far upstream from the fish kill site, and hog farmers can safely then claim that someone else (e.g. a sewage treatment plant) is responsible. Finally, the very complicated life cycle and versatile nutritional metabolism makes *Pfiesteria* a challenge to study, even in the lab; this has allowed people opposed to regulation of animal wastes into NC/MD estuaries to claim that "we don't know enough yet".

Students can easily get background information about *Pfiesteria* using the websites in the Resources section of this Issue. If you ask them to do the first grouping on their own outside of class time, they can also learn more about *Pfiesteria* on the web and bring that information to class.

Student Assessment: Newspaper article.

Write an approximately 300 word article for a newspaper audience about *Pfiesteria*. In the article, describe/explain these points: what *Pfiesteria* is, hypotheses concerning factors leading to *Pfiesteria* blooms, why people are so concerned about *Pfiesteria*, and what is known and not known about *Pfiesteria* blooms. Your audience is the general public, but for people who do not live in the Chesapeake Bay region.

Evaluating an Issue: How do you know whether it is working?

On-going (also called formative) evaluation of the approaches your are using is critical to the success of student-active teaching. Why try out new ideas if you don't know whether or not they are working? This is a brief overview of formative evaluation. For more information, go to the Formative Evaluation essay in the Teaching Section.

Course Goals:

Formative evaluation only works if you have clearly described your course goals - because the purpose of the evaluation is to assess whether a particular technique is helping students reach these goals. For instance, most of us have "learn important ecological concepts and information" as a course goal. If I reviewed the nitrogen cycle in a class, for evaluation I might ask students to sketch out a nitrogen cycle for a particular habitat or system. Each student could work alone in class. Alternatively, I might ask students to work in groups of 3 and give each group a different situation (e.g. a pond receiving nitrate from septic systems, an organic agricultural field, an agricultural field receiving synthetic fertilizer). The students could draw their flows on a large sheet of paper (or an overhead transparency) and present this to the rest of the class.

The Minute Paper:

Minute papers are very useful evaluative tools. If done well they give you good feedback quickly. Minute papers are done at the end of a class. The students are asked to respond anonymously to a short question that you ask. They take a minute or so to write their response in a 3x5 card or a piece of paper. You collect these and learn from common themes. In the next class it is important that you refer to one or two of these points so that students recognize that their input matters to you. The UW - FLAG site (www.wcer.wisc.edu/nise/cl1/flag/) gives a good deal of information about using minute papers including their limitations, how to phrase your question, step-by-step instructions, modifications, and the theory and research behind their use.