Virginia Journal of Science
Volume 50, Number 4
Winter 1999

Pfiesteria piscicida and Dinoflagellates Similar to *Pfiesteria*

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Pfiesteria pisiccida is a microscopic, unicellular organism that is classified as both a mixotrophic and heterotrophic dinoflagellate, which has been associated with both fish deaths and a cause of human illness (Burkholder et al., 1992; Glasgow et al., 1995; Burkholder and Glasgow, 1997). This species possesses a complex life cycle that includes motile forms (e.g. zoospores, gametes, amoebae) and a cyst stage that may remain dormant in the sediment (Burkholder et al., 1995b). *Pfiesteria piscicida* is known to have toxin and non-toxin producing populations, where cyst transformation into the toxic motile zoospores may be initiated by the presence of certain unknown fish excretions, or secretions (Burkholder et al., 1995a; 1999). These zoospores can attack fish and cause their death, then pass through several vegetative and reproductive stages, including the formation of amoebae and cysts that may leave the water column and pass to the sediment (Burkholder et al., 1995b; Glasgow et al., 1998). These transitions occur over a relatively short time period, so their concentrations in the water after a fish kill are often greatly diminished. This rather rapid departure into the sediment resulted in this species to be initially labeled the "Phantom dinoflagellate" (Burkholder et al., 1992).

If a toxin producing population of *Pfiesteria piscicida* is exposed to live fish (e.g. Tilapia, *Oreochromis massambica*), the toxin producing stage (motile zoospores) ensues, killing the fish (Burkholder and Glasgow, 1995; 1997). By removing the dead fish, and replacing them with live fish, *P. piscicida* will continue to increase in abundance and produce toxin(s). It is during these periods of high toxin production in the laboratory that in the absence of adequate ventilation controls, the toxin may be transmitted as an aerosol from the water containing *P. piscicida* and come in contact with, or inhaled by, humans. A variety of severe and potential debilitating illnesses have been associated with this type of exposure (Glasgow et al., 1995; Franklin, 1995). As a result of this danger, culturing these toxin producing cells requires their growth in a facility that has Biohazard Level III standards. Individuals entering this unit would wear the appropriate respirators, and protective gloves and clothing, to prevent contact with these organisms and their toxins.

Although the life cycle of *Pfiesteria piscicida* is most frequently associated with the events described above, under natural conditions it may also feed on microscopic algae and other micro-organisms (Burkholder and Glasgow, 1995; 1997). This is made possible by the special organelle it possesses, the peduncle, which is tube shaped and can be extended between its sulcal plates on the ventral side of the cell to capture and convey prey into the cell's interior (Glasgow et al., 1996; 1998). Algae that may be used as a natural food source would include *Cryptomonas* spp. and *Rhodomonas* spp., which are often common constituents in estuarine waters. In this manner of prey capture, whole chloroplasts from the algal cells may enter the *Pfiesteria* cell (klepto-

plastidy) and continue to conduct photosynthesis (Steidinger et al., 1995; Lewitus et al., 1999; Burkholder, 1999). This alternative ability to function as an autotrophic cell also results in non-toxin producing *P. piscicida* cells. In a similar fashion, *P. piscicida* cultures may be maintained by feeding them these algae (Burkholder and Glasgow, 1995). In the laboratory, Glasgow et al. (1998) have shown the non-toxic zoospores are capable of ingesting a variety of algae (diatoms, cyanobacteria) as well as fish erythrocytes. The toxicity of these cells may be re-established over time in the presence of live fish (Burkholder, 1999).

The environmental conditions that have been associated with *Pfiesteria* development and fish kills are discussed by Burkholder et al. (1995a) and Burkholder et al. (1997). They indicate these cells can tolerate broad salinity and temperature ranges, but conditions most favorable for *P. piscicida* development are those found in shallow eutrophic estuaries, with a salinity around 15 ppt, and water temperatures 26°C. Factors favoring *Pfiesteria* growth were discussed at several Maryland Workshops on *Pfiesteria* and these included nutrient enrichment of the waters and attributes of a river channel that would have shallow regions with a reduced flushing rate (Magnien et al., 1999), Burkholder and Glasgow (1997) report higher abundance of the non-toxic zoospores near waste water discharge sites, in comparison to locations without this discharge. In addition, they report from laboratory and field observations that *Pfiesteria* growth may be enhanced by inorganic nutrients. These findings include direct and indirect relationships regarding the densities of *Pfiesteria* to nitrate and phosphate concentrations (Burkholder et al., 1992; Fensin and Burkholder, 1996). For instance, Burkholder et al. (1996) indicate higher nutrient levels enhances the development of algal prey that would be available for *Pfiesteria* intake and development.

The challenging activities associated with the study of *Pfiesteria piscicida* includes its identification and verification that the cells identified are toxin producers. *P. piscicida* belongs to a dinoflagellate category where the individuals are identified on the basis of a unique assemblage of plates possessed by each species. These plates which cover the cell will vary among different species in their shape, size, number, and position. The specific tabulation of these plates on the motile zoospores of *P. piscicida* are used as the basis to identify this species (Steidinger et al., 1996b). For many of the larger dinoflagellates, these plates can be seen using light microscopy. However, due to the small size of *Pfiesteria* zoospores (18 microns), scanning electron microscopy (SEM) is necessary to examine and identify these plates. SEM protocols for examining *Pfiesteria* and other similar dinoflagellates have been described by Burkholder and Glasgow (1995), Lewitus et al. (1995), Steidinger et al. (1996a), and Truby (1997). A factor that often complicates these procedures is that these plates are covered by several membranes. This condition is resolved during the SEM protocols by either stripping away these membranes, or swelling the sutures between the plates for them to become more visible. If the configuration of the plates match that given for *P. piscicida*, this species identity can then be verified. However, these cells may not be toxin producers. To confirm this species is capable of killing fish, a toxic fish bioassay using these cells and live fish needs to be conducted (Burkholder et al., 1995a). To conduct toxic culture studies on *Pfiesteria piscicida* investigators need to have a known and actively producing toxic population of *Pfiesteria*. However, a culture maintained on algae will not necessarily revert back to a toxin producing form. For instance, the two procedures (SEM analysis and fish bioassay) are presently necessary to link the *P. piscicida* at a

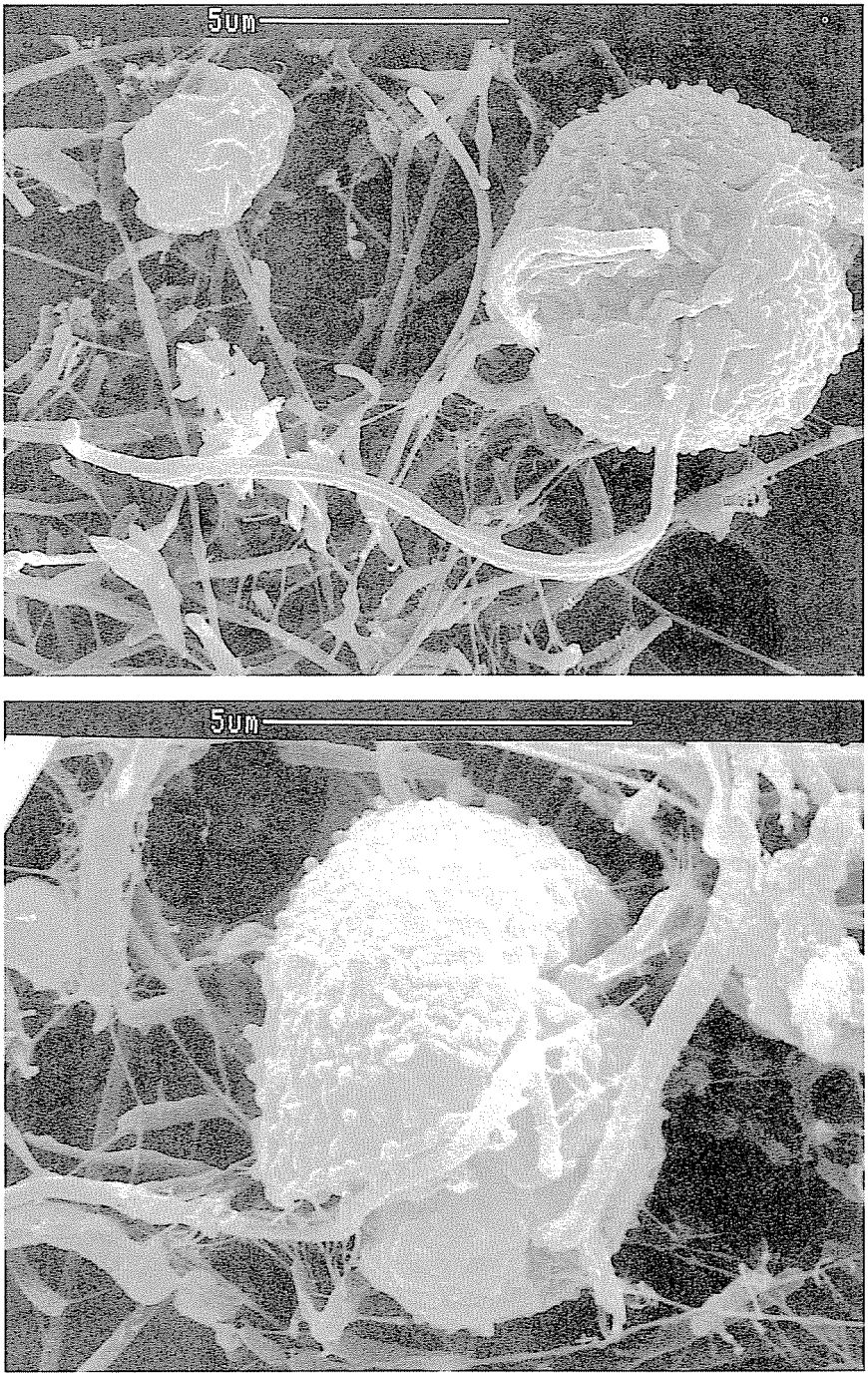

FIGURE 1. *Pfiesteria piscicida* (above) and *Cryptoperidiniopsis* sp. (below), a *Pfiesteria*-like organism (PLO)

fish kill site to these deaths. More recently, species specific genetic probes have been developed to identify *P. piscicida* and can initially be substituted for the SEM analysis, thus reducing the time necessary to identify the presence of this species (Oldach et al., 1998; Rublee, 1999). However, to date these probes look for genetic markers, not toxin production capability, so the fish bioassay is presently necessary for confirmation of toxicity. Also, SEM analysis is still used as the back-up for species identification.

Considering the large number of phytoplankton species in estuaries, it is not uncommon to find species that appear similar in size and morphologic characteristics to each other. *Pfiesteria piscicida* also has other dinoflagellates with a similar appearance that may co-exist with each other in these estuaries (Fig. 1). These may include other *Pfiesteria* species and representation from other generic categories that are not necessarily toxin producers. These *Pfiesteria* "look alike" species were originally described in 1997 by JoAnn Burkholder and Karen Steidinger during their numerous presentations in 1997 (Burkholder, 1997; Steidinger et al., 1997). These different species were originally placed in a group called the *Pfiesteria* complex organisms (PCO). More recently the term *Pfiesteria*-like organisms (PLO) has been adopted, in addition to the classification of the toxic *Pfiesteria* complex (TPC), which refers to those *Pfiesteria* species that are known to produce ichthyotoxins (Burkholder and Glasgow, 1997). We have noticed in culture that several of these look-alike species also have stages in their life cycles that mimic *Pfiesteria*, including prey capture mechanisms (Seaborn and Marshall, 1998). These include *Cryptoperidiniopsis* sp., plus *Gymnodinium galatheanum*, which are both common in Virginia estuaries. During routine water analysis for *Pfiesteria piscicida*, the total number of these PLO cells are initially counted, and referred to as "presumptive cell counts of PLO". These PLO cells are considered common in many estuaries and have been found to have wide spread distribution in Virginia estuaries (Marshall et al., 1998; 1999). So the presence of PLO cells does not mean *Pfiesteria* cells are included, only that they may be present. The verification whether any of these PLO cells are *Pfiesteria* and toxin producers would require further examination using the SEM (or genetic probe) and the fish bioassay analysis as indicated above. Presently, the routine scanning of water samples for the PLO provides the initial step to determine the likelihood of *Pfiesteria* cells being present (Marshall and Seaborn, 1998; Marshall et al., 1999).

A rewarding aftermath in the search for *Pfiesteria piscicida* in regional estuaries is that it has revealed a group of similar organisms (PLO) whose presence and life cycles are now receiving increased attention from the scientific community. Several of these species appear to be ubiquitous inhabitants of the Virginia estuaries and are actively feeding heterotrophs that prey upon cryptophycean species and other microplankton within these estuaries (Seaborn and Marshall, 1998; Seaborn et al., 1999). With further investigation, some of these PLO may also prove to be toxin producers, with these and other PLO being recognized as having a specific (unique) contribution in the utilization and transfer of organic matter within these ecosystems.

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