

Frequently Asked Questions about *Pfiesteria*

What is Pfiesteria?

Pfiesteria is a genus of heterotrophic dinoflagellates whose members inhabit the sediment and water column of shallow, quiescent estuaries and bays along the mid-Atlantic coast of the U.S. *Pfiesteria* occurs primarily in North Carolina, but has been reported in the Chesapeake Bay and its tributaries. The *Pfiesteria* genus includes two species, *Pfiesteria piscicida* and *Pfiesteria shumwayae*, that reportedly share a similar feeding strategy and life history. These two species, described as members of the "Toxic *Pfiesteria* Complex" (TPC), reportedly cause ulcerative lesions in fish, fish-kill events, and human health impacts in East Coast estuaries.

What is Aphanomyces?

Aphanomyces invadans is an invasive mold that is known to infect wild and farmed fish, particularly schooling species in low-salinity or fresh water. It is held responsible for ulcerative fish diseases around the world, including red spot disease (RSD) in Australia, epizootic ulcerative syndrome (EUS) in Asia, and mycotic granulomatosis (MG) in Japan. Recent research by scientists at VIMS, the National Fish Health Research Laboratory, and the University of Stirling shows that *A. invadans* is also the primary cause of the characteristic, deeply penetrating lesions that commonly afflict Atlantic menhaden.

What is the focus of Pfiesteria research at VIMS?

Pfiesteria research at VIMS is directed toward understanding the biology and toxicity of *Pfiesteria* and *Pfiesteria*-like organisms (PLOs). VIMS researchers study the relationships among PLOs, menhaden, *Aphanomyces* (a water mold), and the environmental conditions that modulate or contribute to fish-lesion and fish-kill events in East Coast estuaries.

What is the relationship between Pfiesteria shumwayae and Pfiesteria piscicida? What do results from experiments with P. shumwayae say about the behavior of P. piscicida?

Pfiesteria shumwayae and *Pfiesteria piscicida* are two species of heterotrophic dinoflagellates that have been described as members of the "Toxic *Pfiesteria* Complex" (TPC). Both species are reported to produce potent toxin(s) that "stun" fish and cause the skin of the fish to slough off, resulting in fish death. The presumed toxins are also accused of causing adverse effects in humans. Both species are reported to have a complex life-history that comprises various amoeboid, cyst, and dinospore stages, with toxicity allegedly occurring in several of these stages.

VIMS research with *P. shumwayae* indicates a different mechanism of fish death than secretion of a toxin. VIMS scientists have demonstrated that cells of *P. shumwayae* swarm toward the fish, where they attach to and rapidly denude the fish epidermis, resulting in fish death. Experiments with permeable membranes clearly demonstrate that no toxin is involved with fish death in these cultures. Because these same cultures are also able to kill juvenile tilapia in a system very similar to that which has been used to demonstrate toxicity of *P. piscicida*, it is likely that the presence of a

toxin in *P. piscicida* was incorrectly assumed based on gross observations of fish death. Thus, it is feasible that the mechanism of fish death that VIMS researchers describe for *P. shumwayae* is the mechanism involved with all pathogenic cultures of the *Pfiesteria* species. None of the many cultures tested by VIMS researchers show signs of toxicity, yet many of these cultures kill fish in a manner similar to what has been reported previously for "toxic" *Pfiesteria*.

What are the controversial areas surrounding Pfiesteria?

There are four controversial aspects of *Pfiesteria* biology:

1) The role of *P. piscicida* and *P. shumwayae* in causing fish-kill events

VIMS research shows that *P. shumwayae* kills fish by feeding on their skin rather than by releasing a toxin, as has been widely reported. This discovery casts doubt on the prevailing view that a toxin released by *Pfiesteria* species is the primary cause of fish-kill events in East Coast estuaries. A number of factors besides *Pfiesteria* can lead to fish-kill events. These include insufficient oxygen, chemical contamination, harmful algal blooms (e.g., red-tide algae), and the dumping of by-catch in estuaries. These other factors must be ruled out before ascribing *Pfiesteria* as an agent of mortality.

2) The role of *P. piscicida* and *P. shumwayae* in causing fish lesions

A number of agents can cause fish to develop skin lesions. These include disease agents (fungi, parasites, bacteria, and viruses), direct trauma, low dissolved oxygen levels (hypoxia and anoxia), and other physical stressors. However, the popular press and scientific literature have previously emphasized *Pfiesteria piscicida* and *P. shumwayae* (and their reported toxins) as the primary cause of finfish lesions, particularly the type of deeply penetrating lesions seen in Atlantic menhaden. Recent studies by VIMS researchers and colleagues contradict this view, showing that the characteristic, deeply penetrating skin lesions often attributed to the action of a *Pfiesteria* toxin are instead caused by the common water mold *Aphanomyces invadans*. In these studies, fish exposed to *A. invadans* develop skin ulcers without exposure to *Pfiesteria* species. The disease afflicting Atlantic estuarine menhaden is therefore best called ulcerative mycosis (UM), a disease that has been observed along the East Coast of the United States since at least the early 1980s. *Pfiesteria* may still play a role in field-observed lesions, possibly by inducing skin damage through epidermal feeding that predisposes fish to later infection by *A. invadans*. Further research is needed to determine *Pfiesteria*'s role relative to environmental stressors.

3) The nature of *Pfiesteria*'s life cycle

The team of researchers that identified and named *Pfiesteria piscicida* and *P. shumwayae* claims that the organism has a complex life cycle with at least 24 flagellated, amoeboid, and encysted stages or forms (Steidinger et al., 1996, Burkholder and Glasgow 1997, Glasgow et al. 2001). A recent paper by Litaker et al. (2002) disputes this assertion, arguing instead that *Pfiesteria piscicida* has a much simpler life cycle more similar to that of other free-living marine dinoflagellates. The Litaker paper contends that the previously reported amoeboid stages are actually separate, free-living contaminants introduced with the fish used in aquarium trials with *Pfiesteria*. VIMS researchers can corroborate Litaker et al.'s findings of a simpler life cycle for *Pfiesteria*.

At VIMS, amoebae occur not only in tanks where fish are exposed to *Pfiesteria*, but in control tanks that contain fish but are *Pfiesteria*-free. In addition, they have never seen amoebae attacking live fish in cultures with *Pfiesteria*.

4) Human health concerns

Anecdotal and laboratory evidence suggests that *Pfiesteria* secretes a toxin that can cause nausea, memory loss, and learning difficulties in people (Glasgow et al. 1995). Laboratory experiments show that rats injected with *Pfiesteria* cells from aquarium water samples experience difficulties in short-term memory [Levin, 2001; Levin, 1997; Levin, 1999; Rezvani, 2001]. These findings have fueled significant public concern about *Pfiesteria*'s possible effects on beach-goers, swimmers, recreational anglers, and commercial watermen [Greenberg, 1998]. VIMS scientists are skeptical of claims of human health impacts of exposure to *Pfiesteria*, as their research has produced no evidence for a *Pfiesteria* toxin or a toxic *Pfiesteria* strain (see Vogelbein et al. 2002). However, because *P. shumwayae* feeds on the epidermis of fish, and at high levels may kill fish through the loss of skin, VIMS researchers are developing a mammalian model to investigate the potential role of *Pfiesteria* in causing skin rashes in humans.

What implications do VIMS research have for regulatory policies concerning Pfiesteria?

Maryland, Virginia, and North Carolina currently use menhaden lesions as one of several indicators of local *Pfiesteria* activity. However, VIMS research suggests that use of lesions for this purpose is not valid, as the lesions are actually caused by a highly pathogenic water mold called *Aphanomyces invadans*. Examination of wild menhaden by VIMS researchers indicates that their lesions are a chronic (typically older than 5-7 days) response to infection by *A. invadans*, and not a direct response to a potential *Pfiesteria* toxin(s). Based on these findings, Virginia has taken steps to move away from using lesion-bearing menhaden as an indicator of local *Pfiesteria* activity. The VIMS Molecular Genetics group has developed alternative methods for identifying both *Pfiesteria* and *Aphanomyces*, based on new DNA fingerprinting methodology. This approach provides a quicker, more accurate means to determine if *Pfiesteria*-like organisms are present in local waters.