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**Molecular Characterization of Potential Fish Pathogens in Waters Where Reported
Pfiesteria piscicida Outbreaks Have Occurred**

By

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Abstract

Fish kills occurring along Atlantic slope waters, including in the Chesapeake Bay have been attributed to toxins released by the dinoflagellate *Pfiesteria piscicida* and to a proposed amoeboid form of *P. piscicida*. However, recent studies have indicated that the organism's presence is unrelated to fish kills and that its proposed amoeboid form does not exist. The purpose of this research, therefore, was to characterize protists and bacteria in waters where reported *Pfiesteria* outbreaks have occurred to molecularly determine if the organism is related to fish kills. DNA was extracted, amplified by polymerase chain reaction using fluorescently labeled protist and bacterial primers, and fingerprinted by amplicon length heterogeneity (ALH) from soil sediments taken from five rivers that empty into the Chesapeake Bay, from eighteen samples taken from an experimental fish tank from the Center of Marine Biotechnology (COMB), and from twenty-eight samples purported to be pure amoeboid *Pfiesteria* cultures (Tester samples). The fingerprints from the Chesapeake rivers found that *P. piscicida* was present in small amounts only in three of the five rivers, preliminarily indicating that *P. piscicida* may not have had a role in the fish kills. The fingerprints of the COMB samples also showed no direct correlation between the presence of *P. piscicida* and the occurrence of fish death. The fingerprints from the Tester samples revealed that many samples were not pure because other organisms were present in them. From this data, it is reasonable to question whether *P. piscicida* is a cause of fish kills. The data instead supports the hypothesis that other factors may be involved with the kills. Additionally, the bacterial ALH fingerprints may help determine if fish kills are the result of endosymbiotic bacteria associated with *P. piscicida*. This study demonstrates the importance of accurately studying all the possible causative factors in an epizootic event before definitively naming a cause.