

Lovko, V. J., W. K. Vogelbein, J. D. Shields, H. Kator, D. E. Zwerner and Y. Kiryu (2000). Development of a larval fish assay for testing *Pfiesteria* toxicity. CDC National Conference on Pfiesteria: from Biology to Public Health, Atlanta, Georgia.

Sub-optimal water quality, microbial enrichment, and prior fish-health issues have been major impediments in assigning cause of mortality in our routine 10-gallon toxicity bioassays for *Pfiesteria*. We therefore developed a rapid "fractionation assay" using larval mummichogs, *Fundulus heteroclitus*. The assay tests the different biotic constituents found in the complex microbial community of aquarium-sized bioassays. Seven-day-old lab-reared mummichogs and juvenile tilapia, *Oreochromis niloticus*, were exposed to "dinoflagellate," "bacterial," and "supernatant" (soluble *Pfiesteria* toxin) fractions and appropriate controls. The source of the fractionation medium was raw water from "toxic" cultures of *Pfiesteria* sp. B (20-50% daily tilapia mortality). Water was fractionated by centrifugation and filtration to obtain media enriched in dinoflagellates, bacterial contaminants, and toxin (supernatant fraction). Controls consisted of (1) artificial seawater (ASW), (2) ASW with NH₄ and pH adjusted to that of raw toxic culture water, and (3) raw toxic culture water. Mortality and water quality were assessed every 24 hrs for 96 hrs. Tilapia were exposed in jars with 500 ml medium (treatment: 3 replicates of 4 fish). Mummichogs were exposed in micro-titer well plates with 15 ml medium (treatment: 9 replicates of 5 fish/well). Results indicated that tilapia were marginally sensitive to *Pfiesteria* sp. "B" as extremely high cell densities were required to kill fish. However, larval mummichogs exhibited 100% mortality in raw water and "dinoflagellate" fractions, low mortality in "soluble" fractions, and negligible mortality in controls. Histopathology confirmed toxicity and mortality findings. The mummichog assay appears effective for rapid verification of dinoflagellate toxigenicity and provides a quantifiable approach to study *Pfiesteria* biology.