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Characterization of Pfiesteria Ichthyocidal Activity

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Letter to the Editor

Characterization of *Pfiesteria* Ichthyocidal Activity

Drgon et al. (4) concluded that the "aquarium bioassay format is unsuitable to accurately assess the ichthyocidal activity of *Pfiesteria* spp." and "ichthyocidal activity of *Pfiesteria* spp. is mostly due to direct interactions of the zoospores with fish skin and gill epithelia rather than to soluble factors." These conclusions are not justified, because microbial community analyses of control aquariums were not included and previous studies (5, 8) that utilized similar experimental approaches and found significant (100%) fish death attributable to soluble factors were overlooked.

It is known a priori that aquariums containing fish or inoculated with whole sediment will develop a complex microbial community. This has been previously noted for the aquarium bioassay for *Pfiesteria* ichthyotoxicity (2). Given this complexity, a key factor in attributing fish death to *Pfiesteria* spp. is the difference between *Pfiesteria*- or sediment-inoculated aquariums and corresponding controls.

Drgon et al. focused on the presence of potentially pathogenic *Vibrio* spp. in experimental aquariums as a problem with attributing fish death to *Pfiesteria* spp. A recently published study of which the authors would not have been aware (1) showed that *Vibrio* spp. can be more abundant in control aquariums where fish remain healthy, and a previous publication showed that total bacterial numbers do not differ significantly between experimental aquariums with *Pfiesteria* spp. and control aquariums (7). Denaturing gradient gel electrophoresis of bacterial assemblages from control and experimental aquariums has shown that there is no consistent difference in community composition between experimental and control aquariums (1). These studies illustrate the importance of comparison between microbial communities in aquariums inoculated with clonal *Pfiesteria* cultures or sediment and corresponding controls. The organisms that toxic aquariums consistently have in common and that are absent from controls are *Pfiesteria* spp., implicating *Pfiesteria* spp. in fish death.

Drgon et al. concluded that most of the fish death in aquariums containing *Pfiesteria* spp. requires direct contact between fish and *Pfiesteria* spp. While this was true for their experiment and is consistent with another recent study that utilized similar methods (6), the authors failed to cite earlier publications that showed up to 100% death of finfish in cell-free filtrates from toxic *Pfiesteria* cultures (5) and 100% death of bivalve larvae in containers where toxic *Pfiesteria* spp. were held in dialysis tubing (molecular mass cutoff, 12 to 14 kDa) (5). Their results should have been discussed in the context of these previous studies using different *Pfiesteria* strains, where mortality was mostly attributable to soluble factors.

In summary, the article by Drgon et al. is flawed, since data on microbial communities in controls corresponding to sediment- or *Pfiesteria*-inoculated aquariums were not included. Thus, no conclusion regarding the suitability of the aquarium bioassay format can be made from their study. The authors also failed to acknowledge previous publications that showed high mortality in cell-free filtrates from toxic *Pfiesteria* sp. cultures. When the available data are objectively considered, the conclusion that emerges is that toxicity by *Pfiesteria* spp. is mediated by both direct contact and soluble toxic factors. The relative contribution of each depends on the *Pfiesteria* strains studied, culture history, and methods utilized to detect soluble toxins (1, 3).

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Authors' Reply

Gordon et al. criticize the conclusions of Drgon et al. (6) for concluding that the aquarium bioassays are unsuitable for *Pfiesteria* ichthyocidal activity determination.

Gordon et al. should recognize the work by Quesenberry et al. (10), which demonstrates (i) the diverse microbial compositions of control and experimental flask bioassays, (ii) the distinct microflora contributed by experimental fish, and (iii) the dramatic fluctuation of the microbial assemblages during the course of the experiment.

In Drgon et al. (6), the microbial assemblage was assessed by amplicon length heterogeneity fingerprinting (see Fig. 5 in the cited article) and sequence analysis (see Tables 1 and 2 in the cited article), and the results confirmed previous findings (10). Furthermore, Burkholder et al. (4) also reported different microbial denaturing gradient gel electrophoresis patterns for each aquarium, whether toxic or nontoxic, that are indicative of variable microbial compositions (curiously cited as supporting Gordon et al.). Thus, the proposal made by Gordon et al. to match control and experimental aquaria (1–4, 7–9, 11) is unrealistic. The idea that analysis of experimental results can be based on the abovementioned flawed controls, as proposed by Gordon et al., is very dangerous, as it leads to selective interpretation of the data.

Gordon et al. suggest that Drgon et al. are misguided in their consideration of microbial pathogens in experimental aquaria. However, the presence of *Vibrio* spp. was never concluded to be the source of the ichthyocidal activity, but it was rather emphasized that this could be equally attributed to other putative pathogens in the system (6).

Gordon et al. point out that Drgon et al. failed to cite earlier studies which demonstrated 100% death of finfish and bivalve larvae in a cell-free fraction of toxic *Pfiesteria* spp. (5, 8, 11). Drgon et al. made great efforts to cite all published relevant work. However, studies lacking any characterization of the microbial assemblage in the bioassay (8, 11) were not relevant for comparison. Even if there was a soluble ichthyocidal agent as they concluded, it cannot be rigorously attributed to *Pfiesteria* spp. without characterizing the composition of the microbial community. Gordon and Dyer reported substantial drops (70 to 90%, approximately) in fish mortality in assay aquaria when fish were separated from the environment by membranes (7), which is strikingly similar to the results reported by Drgon et al. Consequently, Drgon et al. concluded that most of the ichthyocidal activity was caused by direct contact of the experimental fish with *Pfiesteria* spp. and other established pathogens present in the assay and not by an organism(s)-associated toxin. In contrast, Gordon and Dyer attributed all the ichthyocidal activity to a *Pfiesteria*-associated exotoxin. Their conclusions are unjustified and emphasize the fact that these microbial communities are so complex that it would be surprising if orphan toxic compounds were not present in the bioassay.

In summary, it should be clearly understood that Drgon et al. do not rule out the potential contribution of *Pfiesteria* spp. to the observed fish deaths. However, there is no rigorous scientific justification for attributing soluble ichthyocidal activity to the presence of any specific organism in the bioassay, including *Pfiesteria* spp. The value of the controls proposed by Gordon et al. is limited at best. In the absence of a characterized bona fide *Pfiesteria* toxin and biochemical tools to assess its concentrations in the bioassay and of establishing the causal relationships of its proposed activity, it must be concluded that the fish tank bioassay is unsuitable for assessing *Pfiesteria*-caused fish deaths and any related phenomena.

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