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Pfiesteria piscicida and Ulcerative Mycosis of Atlantic Menhaden—Current Status of Understanding

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Abstract.—Ulcerative lesions in estuarine finfish are associated with a variety of parasitic, bacterial, and fungal infectious agents as well as water quality and other abiotic stress factors. Atlantic menhaden *Brevoortia tyrannus* are a severely affected species typically exhibiting solitary, perianal, focal, deep, granulomatous lesions containing oomycete hyphae. Intense recent emphasis in the scientific literature and in the popular press has been placed on *Pfiesteria*-like dinoflagellates and their toxins as the causative agents for ulcerative finfish lesions in east coast estuaries of the United States. Dramatic descriptive terms for *Pfiesteria,* including ''phantom,'' ''ambush-predator'' and ''the cell from hell,'' capable of affecting humans, have appeared in scientific journals and the news media. However, there is no scientific evidence to date that supports a single, causal relationship between the presence of toxic *Pfiesteria*-like dinoflagellates and fish kills or the associated ulcerative lesions. This is due, at least in part, to the difficulty of maintaining toxic *Pfiesteria*-like dinoflagellates in clonal culture and, in part, to the presence of other microorganisms in laboratory culture systems. Further, there is at present no toxin-specific probe to identify *Pfiesteria* toxins in estuarine environments during fish kills, nor has *Pfiesteria* been isolated from fish lesions. Yet, based on the limited published scientific evidence and the popular press coverage, there have been river closures, notable economic losses, and public alarm due to *Pfiesteria.* Future research into agents infectious to menhaden, such as *Aphanomyces* (Oomycetes) and toxin-producing estuarine inhabitants like *Pfiesteria,* is needed. These organisms and the disease processes in which they are implicated may also serve as markers for overall estuarine condition. Control of microorganisms, including *Pfiesteria,* to improve finfish health in estuaries is unlikely. However, using the presence of these microorganisms and their Atlantic menhaden hosts as sentinels to help monitor water quality may be a viable approach to assist future estuarine management.

Ulcerative mycosis in Atlantic menhaden *Brevoortia tyrannus* and concomitant fish kills along the eastern seaboard have recently attracted intense interest. This acute interest persists despite the fact that recurring epizootics of such ulcerative lesions have been well documented for more than 15 years in estuarine waters of the Chesapeake Bay, Albemarle Sound, Pamlico and Neuse rivers, and St. John's estuary in Florida (Noga and Dykstra 1986; Sindermann 1988). Similar lesions have been described in fish in Southeast Asia (Roberts et al. 1993), Japan (Hatai et al. 1977; Wada et al. 1994), and Australia (Callinan et al. 1995) since the 1970s and have recently been attributed to a newly described and characterized the Oomycete, *Aphanomyces invadans* (Lilley and Roberts 1997; Lilley et al. 1997).

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Taxonomically, members of the Oomycete class are no longer considered to be true fungi but have been placed in the phylum Oomycota in the Kingdom Stramenopila (Alexopoulos et al. 1996). However, *Aphanomyces* spp. are still commonly referred to as fungi and have been called such for the purpose of this review.

Much of the renewed interest in ulcerative lesions in menhaden appears due to extensive press coverage followed by scientific publications in the early and mid-1990s that implicated a newly described dinoflagellate, *Pfiesteria piscicida* (Steidinger et al. 1996), as the causative agent of epizootic fish kills in estuarine waters of the eastern United States from the mid-Atlantic to the Gulf of Mexico coast (Burkholder and Glasgow 1995, 1997; Burkholder et al. 1995; Noga et al. 1996). That *Pfiesteria* has been implicated recently as the primary cause of fish lesions and mortalities in estuaries of the eastern United States (Burkholder

et al. 1992; Noga et al. 1996) is notable given the abundant literature for more than 20 years that implicates pathogenic fungi and a variety of other biotic agents. Are *Pfiesteria*-like organisms or their toxins pathogenic? At present, it is difficult to say conclusively because there is no toxin-specific probe to identify a causal relationship between the presence of *Pfiesteria* and fish pathology. Unfortunately, *Pfiesteria* is viewed as an indicator of hazardous waterways when it is coincident with ulcerated fish despite the fact that *Pfiesteria* has been found in waters where there were no reports of harm to people or fish (Hathcock et al. 1999). Recent circumstantial evidence has been used to implicate *Pfiesteria* as the etiologic agent responsible for ulcerative lesions (Burkholder and Glasgow 1995; Burkholder et al. 1995; Glasgow et al. 1995). Whether this presumption is accurate is important because management regulations to protect public and environmental health are presently based on relatively limited, and at times anecdotal, scientific evidence. In this paper, we examine the current literature on epidemic ulcerative finfish lesions and estuarine fish kill events and the implication of toxic dinoflagellates (i.e., *Pfiesteria*-like organisms) in their etiology. We propose a hypothesis that ulcerative lesions in menhaden and other finfish are caused by a variety or a combination of predisposing factors, including suboptimal water quality.

Pathogenesis of Ulcerative Fish Lesions

A lesion is any alteration of a cell, tissue, or organ system, microscopic or otherwise, that deviates from normal. An ulcerative lesion may be defined as an open sore of the skin or of a mucus membrane, often penetrating through the underlying basement membrane of the affected epithelial cell layers. Ulcers may be caused by physical trauma, parasites, viruses, bacteria, or biotic or abiotic toxins. The word ''chronic'' refers to alterations that take place over many days to weeks. Ulcerative lesions may be characterized microscopically by chronic inflammatory responses evidenced by the presence of macrophages, giant cells, or granulomas. Granulomas are focal, organized lesions with a center of densely packed macrophages and a periphery of cells with deeply basophilic nuclei that sometimes appear epithelioid (Boorman et al. 1997). A granuloma may be a host response to wall off infectious agents such as parasites, bacteria, or fungi by surrounding them with host cells, as seen in the response of menhaden to the invading fungal hyphae found in ulcerative mycosis (Noga et al. 1988).

Chronic ulcers are the hallmark of ulcerative mycosis in Atlantic menhaden (Figure 1a, b) but may also be associated with bacterial infections (Figure 1c, d) and viral infections (Figure 1f) in other finfish species. Figure 1 illustrates that the presentation of ulcerations associated with different causative agents may be variable. For example, mycobacteriosis (Figure 1c) is a chronic, slowly progressing bacterial infection producing skin alterations including ulcers as well as granulomas. These granulomas, however, have an appearance distinct from those found in ulcers produced by ulcerative mycosis. A variety of protozoan (Figure 1e), metazoan, and even vertebrate parasites are also known to cause skin ulcers in fish (Noga 1988; Roberts 1989). Recent observations of ulcerated juvenile menhaden collected from Maryland waters of the Chesapeake Bay include myxosporidian parasites including *Kudoa* (R. Reimschuessel and others, University of Maryland, personal communications).

Chronic granulomatous lesions in fish take many days to weeks to develop (Timur et al. 1977). Finally, ulcers, as with other skin pathologies, may be primary or secondary lesions that can result from a variety of stressors. These stressors include suboptimal physical and chemical water quality, contaminants, spawning, and trauma. Trauma may result from predator–prey interactions, net stress, handling, and repeated capture.

Mycotic Ulcerations of Finfish from the Atlantic Coast of the USA, Asia, and Australia Associated with Oomycetes

Typical freshwater fish infections caused by Oomycetes such as *Saprolegnia* and *Achlya* produce a superficial, generalized infection with hyphae rarely penetrating past the dermis into the musculature (Pickering and Willoughby 1982). In contradistinction, infections with *Aphanomyces* in freshwater and estuarine fish from Asia, Australia, and the United States are characterized by deep, focal chronic ulcers that contain granulomas produced in response to hyphal elements (Noga and Dykstra 1986; Noga et al. 1988; Callinan et al. 1989; Wada et al. 1994). Penetration of the musculature is characteristic of *Aphanomyces* infections, and multiorgan involvement is not uncommon.

With ulcerative mycosis in Atlantic menhaden, oomycete hyphae have been associated with 90% or more of the lesions examined in short-term

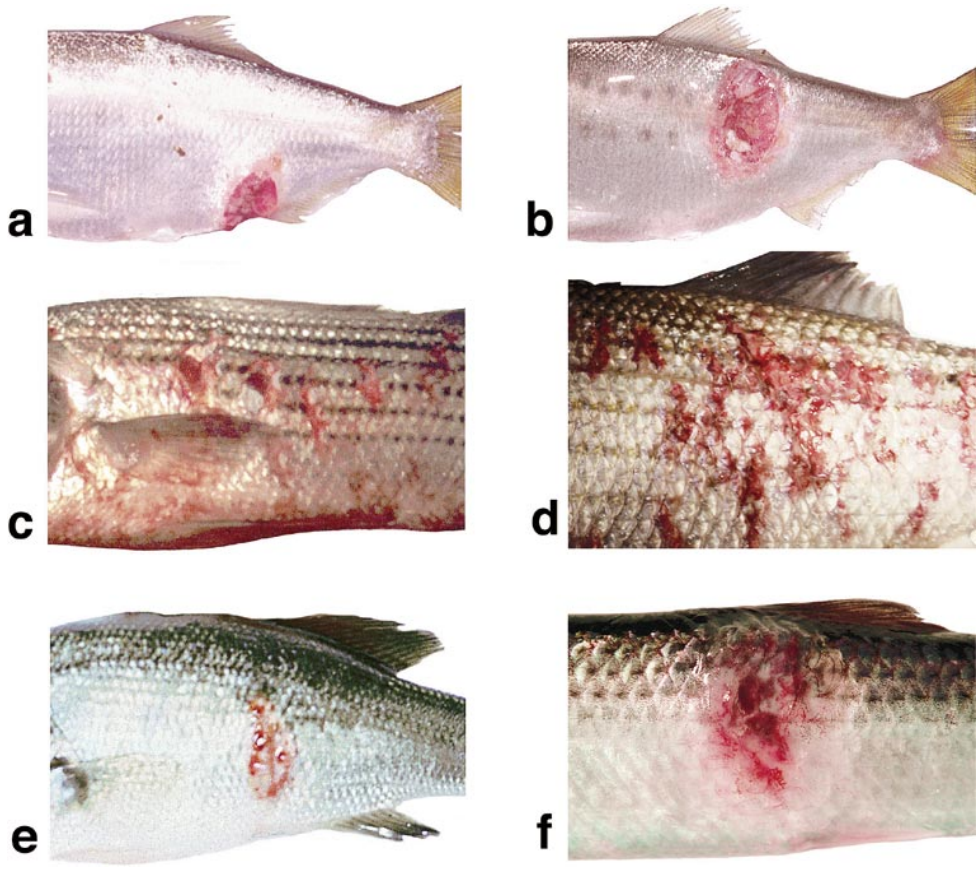

FIGURE 1.—Chronic ulcerative lesions in finfish associated with **(a, b)** fungus-like Oomycetes in Atlantic menhaden, **(c)** *Mycobacterium* in striped bass *Morone saxatilis,* **(d)** *Edwardsiella* bacteria in striped bass, **(e)** microsporidian parasites in largemouth bass *Microptera salmoides,* and **(f)** viral hemorrhagic septicemia virus in Pacific herring *Clupea pallasi.* Photographs (c), (d), and (e) are courtesy of A. Baya; photograph (f) is courtesy of G. Marty (reproduced with permission from Inter-Science Publishing).

(Kane et al. 1998) and long-term (Levine et al. 1990a) field studies of menhaden populations. These lesions are typically solitary, and a majority of them are perianal. However, what are believed to be very early lesions of ulcerative mycosis in menhaden seen in our studies (Kane et al. 1998) and in those of Wada et al. (1994) in other fish species do not appear to contain fungal hyphae.

Research on the salinity requirements of Oomycetes isolated from menhaden indicates a fairly broad salinity tolerance, particularly when sufficient nutrient levels are available (Padgett 1984; Hearth and Padgett 1990; Shafer et al. 1990). The *Aphanomyces* isolate 84-1240 appears to have optimal growth in 2–10‰ salinity, will grow vegetatively in up to 26‰, and will sporulate in salinities up to 20‰ (Dykstra et al. 1986). This information enabled fish disease survey teams searching for diseased menhaden (Levine et al. 1990a) to narrow their focus in North Carolina estuaries to zones within the 2–10‰ salinity range. These areas were found to contain the highest numbers of fish (juvenile menhaden) with lesions. Fish with lesions could be collected in the absence of any associated fish kills. Although some older menhaden exhibiting ulcerative mycosis lesions were isolated from higher salinity levels in East Coast estuaries, they represented a relatively small portion of the diseased menhaden population (Levine et al. 1990b). Two affected fish at least 5 years old were collected in open ocean waters (37‰ salinity) off South Carolina and Georgia (M. J. Dykstra, unpublished results). These fish would not be expected to enter estuarine waters at that age yet had gross lesions characteristic of ulcerative mycosis.

Physiological studies of North American, Asian,

and Australian cultures of *Aphanomyces* isolated from ulcerated fish (Lilley and Roberts 1997) indicated that the Asian and Australian isolates had different growth characteristics than one North American isolate (84-1240) from menhaden in regard to temperature and selected growth media. This becomes particularly relevant because Lilley et al. (1997) suggested that the *Aphanomyces* isolate 84-1240 characterized by Dykstra et al. (1986) was possibly not ''actually the invasive pathogen involved in the disease,'' based on differences in protein and carbohydrate profiles when compared with *A. invadans,* formerly *A. invaderis* (Lilley and Roberts 1997). However, it should not be overlooked that the majority of epizootic ulcerative syndrome (EUS) *Aphanomyces* isolates were made from freshwater fish (Willoughby et al. 1995; Lilley et al. 1997), whereas the various oomycete isolates taken from menhaden were from estuarine salinities of up to 18–20‰. Because Dykstra et al. (1986) isolated several different cultural types of *Aphanomyces,* as well as *Saprolegnia* spp. from menhaden ulcers on fish collected in water with salinities of 0–20‰, it appears that there are at least two oomycete genera opportunistically infecting menhaden. This situation is unlike that described by Lilley and Roberts (1997), who suggested that a single pathogenic strain of *Aphanomyces, A. invaderis* (now named *A. invadans*), is responsible for Asian and Australian infections. The work by Dykstra et al. (1986) and Hearth and Padgett (1990) on isolate 84-1240 and by Shafer et al. (1990) on one of the *Saprolegnia* isolates from menhaden showed tolerance to salinity levels greater than 20‰, both for vegetative growth and for sporulation. These findings may be contrasted with those of Lilley et al. (1998), who stated that outbreak sites had salinity levels less than 2‰, allowing *A. invadans* to sporulate. Thus, it is possible that the Asian–Australian isolates are different from the North American isolates, but it is premature to say that the North American *Aphanomyces* isolate 84-1240 is not a pathogen.

Lilley and Roberts (1997) challenged striped snakehead *Channa striata* (a fish species susceptible to EUS in the wild) with zoospore suspensions from *Aphanomyces* cultures derived from fish ulcers. The zoospore suspensions were injected intramuscularly, fish were sacrificed 7 d after infection, and histological preparations were examined for evidence of pathogenesis. The isolates designated as *A. invaderis* (*A. invadans*) by the authors were seen to have hyphae penetrating muscle tissue and some mild granulomatous response. No mention was made of ulcerative lesions similar to those described for natural infections.

It is not surprising that ulcers characteristic of natural infections were not seen, because injecting zoospores does not reflect a natural infection process. If zoospores are the infective agent, as is assumed for most Oomycetes (Alexopoulos et al. 1996), they would attach to the epithelial tissues of the host, encyst, and then produce hyphae that would penetrate the host tissue by hyphal extension. Whether the site of entry was from the gastrointestinal tract (through ingestion of fungal zoospores or hyphae associated with food or debris) or from penetration of the skin or by attachment and growth into areas of trauma, zoospores would not be found deep within tissue as was the case with the Lilley and Roberts (1997) experiments. In addition, the relatively mild-to-nonexistent host response to invading fungal hyphae shown by Lilley and Roberts (1997) seems quite distinct from the pathology encountered in lesions on menhaden (Noga and Dykstra 1986; Noga et al. 1988) and other fish (Callinan et al. 1989; Wada et al. 1994).

The Reported Relationship between *Pfiesteria* and Fish Ulcers

Pfiesteria has been described as a ''phantom predator'' that emerges from estuarine sediments to stun fish with its toxin(s) so that it can consequently eat their flesh (Burkholder et al. 1992; Burkholder and Glasgow 1997). The term phantom derives from the observation that the dinoflagellate appears to encyst and return to the sediments after stunning and killing fish (Burkholder et al. 1992). Dramatic descriptions for *Pfiesteria,* including phantom, ''ambush-predator'' and ''the cell from hell'' have appeared in scientific journals and news media (Burkholder et al. 1992, 1995; Smayda 1992; Burkholder and Glasgow 1995; Lewitus et al. 1995; Broad 1997; Grant 1997).

Laboratory studies in which fish were exposed in aquaria with *Pfiesteria* have described behavioral anomalies, alteration to the mucous coat, skin color changes, epidermal sloughing, hemorrhage, the development of chronic ulcers, the destruction of myelinated nerve tissue, and death (Noga et al. 1993a; Burkholder et al. 1995; Lewitus et al. 1995; Noga et al. 1996). Although these reports suggest that *Pfiesteria* is responsible for ulcerative lesions in menhaden and fish kills, conclusive evidence does not exist. This is largely due to the current inability to isolate and identify bioactive compounds for the development of specific probes. Finally, there is an inherent difficulty in relating con-

trolled laboratory aquarium exposures with complex field events.

Laboratory studies wherein Nile tilapia *Oreochromis niloticus* and striped bass were exposed to *Pfiesteria* in aquaria demonstrated the development of skin ulcerations in 8–48 h (Noga et al. 1996). However, these lesions were most often superficial and distinct from an invasion of muscle tissue, a hallmark of ulcerative mycosis in menhaden (Noga and Dykstra 1986). The rapid development of these lesions also differentiated them from the chronic granulomatous lesions characteristic of ulcerative mycosis in menhaden (Noga et al. 1988). Noga et al. (1993b) observed that up to 43% of fish developed ulcerative lesions in the absence of *Pfiesteria* when exposed to circulating estuarine water. At the same time, the presence of lesions was strongly associated with salinities of 4–8‰, which are ideal for the growth of *Aphanomyces* (Dykstra et al. 1986). In a second experiment, fish were exposed to waters containing high levels of *Pfiesteria*, but the fish developed no lesions.

It also should be noted that other *Pfiesteria*-like dinoflagellate species (Landsburg et al. 1995; Steidinger et al. 1997) have been discovered in freshwater and estuarine field and laboratory studies associated with ulcerative fish lesions or fish mortality. The toxicity of these other organisms and their impact on fish health has not been determined.

Burkholder and Glasgow (1997) reported that *Pfiesteria* produces toxin(s) responsible for ulcerative finfish skin lesions and massive menhaden fish kills in North Carolina estuaries. However, there is evidence arguing against a singular, causal relationship between the presence of *Pfiesteria* and fish kills with ulcerated fish. First, juvenile menhaden (70–110 mm in fork length) netted in low-salinity (2–8‰) areas can be found with lesions in the absence of fish kills (Levine et al. 1990b). Second, menhaden kills occur without the presence of high levels of *Pfiesteria* (Noga et al. 1993b). Finally, waters with high *Pfiesteria* counts have been known to contain lesion-free menhaden (Noga et al. 1993b).

Potential Health Risks to Humans Exposed to Estuarine Waters Containing Diseased Atlantic Menhaden or *Pfiesteria*

Reported health impacts on humans from *Pfiesteria* toxin(s) are difficult to investigate because of the lack of an appropriate animal model. In addition, there have been no reports in the literature of morbidity or mortality in avian populations such as of gulls or herons observed feeding on menhaden floating in estuarine waters containing ulcerated fish after fish kills (Dykstra and Kane, unpublished observations). Toxin-affected or poisoned birds should be easily spotted on or over open waters. Further, there have been no published reports of dogs, cats, or other mammalian species exposed to estuarine waters with unusual health problems. Although novel occurrences of marine biotoxins are frequently signaled by effects on birds, other wildlife, and domesticated animals, the species specificity of toxin(s) from *Pfiesteria*-like organisms remains unknown at present. Further, there is no evidence that *Pfiesteria* produces bioactive compounds that can be carried up the food chain as is reported for other algal biotoxins (Coulson et al. 1968; Work et al. 1993).

Reports in the scientific literature (Burkholder et al. 1992, 1995; Lewitus et al. 1995; Burkholder and Glasgow 1997) and in the popular press (Barker 1997; Broad 1997; Grant 1997) that *Pfiesteria* is a toxic, fish-eating, phantom, ambush-predator, capable of affecting human health have captured the attention of the public and policy makers who are responsible for estuarine management and allaying public fear. Fish kill observations associated with the presence of *Pfiesteria*-like organisms and concerns catalyzed by extraordinarily extensive media coverage have prompted state officials to close several regional waterways in Maryland and North Carolina. These closures resulted in further public alarm. Concerns with the safety of Chesapeake Bay waters and seafood safety led to a notable loss of regional tourism and an estimated US$43 million decline in seafood sales and consumption in Maryland alone (Lipton 1998).

The current state of knowledge concerning the effects of *Pfiesteria* on humans exposed to estuarine water stems from a relatively small number of studies with small sample sizes and from anecdotes that have arisen since the popular press coverage of humans exposed to toxin(s) in a laboratory setting. Memory loss and motor difficulties in humans due to exposure to *Pfiesteria* were first reported in a laboratory setting in 1995 (Glasgow et al. 1995), followed by a study indicating diminished cognitive function in rats exposed by intraperitoneal injection to *Pfiesteria* culture water (Levin et al. 1997). More recent reports indicate that self-reporting fishermen working in estuarine waters presumed to have significant populations of *Pfiesteria* had reversible memory loss and nonspecific respiratory, gastrointestinal, and skin ir-

ritation complaints (Grattan et al. 1998). Although these effects appeared to be associated with occupational exposure and regional fish lesion–fish kill events that occurred at the same time, the association between the human endpoints examined and exposure to toxic *Pfiesteria*-like organisms remains poorly documented.

Epidemiological studies to assess health effects on bay workers chronically exposed to estuarine waters presumed to contain *Pfiesteria* compared with those exposed to ocean waters containing no *Pfiesteria* populations are underway at the University of Maryland School of Medicine, the University of North Carolina, and through other state studies supported by the Centers for Disease Control. Until this needed, detailed work is completed, enacting regulations to restrict human exposure to estuarine waters potentially infested with *Pfiesteria,* although perhaps appropriately conservative, may be premature.

Conclusions

Atlantic menhaden are the species of estuarine fish most severely affected by ulcerative lesions that are characterized by solitary, typically perianal, focal, deep, granulomatous lesions containing oomycete hyphae, primarily those of *Aphanomyces.* The young-of-year in the 70–110-mm fork length range is the most heavily affected group. An *Aphanomyces* isolate from an infected menhaden demonstrated maximal growth and sporulation at 2–10‰ salinity (Dykstra et al. 1986), the salinity range within which the preponderance of the juvenile menhaden grossly exhibiting the signs of ulcerative mycosis can be found. These salinity levels are below the 15‰ salinity reported as optimal for *Pfiesteria* growth (Noga et al. 1996). Aphanomyces-infected fish can be collected in the absence of fish kills, and fish kills typically contain many fish without ulcerative lesions.

A single laboratory study with Nile tilapia (Noga et al. 1996) demonstrated a relationship between chronic ulcerative, albeit nongranulomatous, fish skin lesion formation, and *Pfiesteria.* However, the ability to induce lesions or to cause fish mortality in the laboratory is difficult to reproduce. This is likely due to problems maintaining the toxicity of *Pfiesteria*-like dinoflagellates in clonal culture or to the presence of other toxic, pathogenic, or synergistic microorganisms in the system. Further, it is very difficult to link controlled laboratory studies to the complexities of the field environment. Regardless, *Pfiesteria*-like organisms have not been reported from menhaden skin or from within menhaden lesions. High concentrations of *Pfiesteria* have been reported to be negatively correlated with menhaden lesions in limited estuary studies (Noga et al. 1993b). In addition, large menhaden kills have occurred that cannot be attributed to *Pfiesteria* (Noga et al. 1993b).

To date, evidence for human health risks from exposure to *Pfiesteria* toxin(s) is largely based on limited cognitive testing. Further, there have been no reports of health effects on mammals exposed to estuarine waters or on avian populations that feed on menhaden from active fish kills containing diseased fish in *Pfiesteria*-infested waters. The etiology of ulcerative mycotic lesions in menhaden and other fish is unclear at present, though it is most likely multifactorial in nature (Lilley et al. 1998). The association of ulcerative fish lesions with viral, bacterial, fungal, and other parasitic agents (Noga and Dykstra 1986; Noga 1988; Sindermann 1988; Roberts 1989; Lilley et al. 1998; Marty et al. 1998) certainly does not preclude the involvement of biotic or abiotic toxins, including those from *Pfiesteria*-like dinoflagellates. These toxins could predispose fish to epithelial lesion-initiating events. In addition, other predisposing factors (stressors) may include suboptimal or rapidly changing chemical and physical water quality (Wedemeyer 1974; Noga et al. 1993b), sublethally depressed dissolved oxygen (Scott and Rogers 1980), trauma (Kane et al. 1998), and the presence of irritants (Murty 1986; Heath 1987; Anderson and Zeeman 1995).

Needed research includes discerning the factors that cause fish ulcer initiation and progression, as well as species-specific sensitivities. Further, we need to characterize the toxin(s) from *Pfiesteria* and other closely related dinoflagellate species, determine the mechanism(s) of biological activity of these toxins, and develop a widely utilitarian, toxin-specific probe to identify and quantitate the presence of toxic *Pfiesteria* activity.

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