Tissues such as skin and muscle have a limited repertoire of morphological response to injury. The two most important phenomena that determine the outcome of cell injury appear to be all critical cell membrane damage, with associated fluid and ionic imbalances; and b) inability of mitochondria, the powerhouse of the cell, to restart ATP synthesis. In fish, skin ulcers can have many different etiologies, including infectious agents, toxins, physical causes, immunologic causes, and nutritional and metabolic perturbations. This article is concerned primarily with the possible pathways of disease involved in ulcerative lesions of fish. In particular, the high prevalence of ulcerative lesions in Atlantic menhaden found along the mid-Atlantic coast, especially in North Carolina estuarine waters, has received much recent attention. These ulcerative lesions are likely to be initiated by a series of factors that lead ultimately to a breach of the normal barrier function of the skin. Bioassays that attempt to define the role of individual etiologic agents such as fungi (oomycetes) or putative *Pfiesteria* toxin(s) should recognize this multiplicity of factors and should include appropriate quality control measures for water quality parameters (temperature, dissolved oxygen, nitrogenous wastes, etc.) as well as bacterial and other contaminants that may confound bioassay results and their interpretation. Consideration of these factors along with the whole animal in the context of its environment can only advance the science, perhaps provide clues to the causative pathways of skin ulcers in fish, and give us keener insight into the health of the aquatic environment. 

**Key words:** Atlantic menhaden, *Brevoortia tyrannus*, epizootic ulcerative syndrome, fish, muscle necrosis, skin ulcers, ulcerative mycosis. — *Environ Health Perspect* 109(suppl 5):681–686 (2001). http://ehpnet1.niehs.nih.gov/docs/2001/suppl-5/681-686law/abstract.html

Recent massive fish kills in North Carolina estuarine waters, often associated with sores or ulcerative lesions in fish, along with possible human health effects, have brought the toxic alga *Pfiesteria piscicida* Steidinger & Burkholder and related organisms to the forefront of public attention. Although this dinoflagellate has been blamed for many of the kills and lesions (1–3), the actual cause(s) of most fish die-offs remains difficult to resolve. Compounding the problem, the pathogenesis of the ulcerative skin lesions in fish that are sometimes associated with these kills is poorly understood.

Since 1984, skin and muscle lesions termed “ulcerative mycosis” (UM) have been recognized in fish from western Atlantic estuarine waters (4–7). Interestingly, the predominant species affected has been the Atlantic menhaden (*Brevoortia tyrannus*). Although not usually consumed by people, menhaden are a commercially important source of fish meal and a critical prey species in the aquatic food web.

Ulcerative mycosis was first described as a deep, ulcerative fungal infection most often limited to the area around the anal pore of the fish and often complicated by other microbes such as bacteria and/or protozoa (8,9). However, several new etiologies for this disease have since been proposed. When the *Pfiesteria* organism was reported in 1992 (1), many of the fish lesions and fish deaths were attributed to it. In controlled laboratory experiments, Noga and co-workers (2) provided evidence that, when stimulated, *Pfiesteria* can cause epithelial erosions that appear to progress to necrosis of the epithelium and underlying muscle in striped bass and tilapia. However, the initiating cause(s) of UM in the menhaden that appear in schools in large numbers in western Atlantic estuaries remains a mystery. Several questions need to be answered regarding UM: a) Why is the Atlantic menhaden often the only fish species significantly affected in disease outbreaks? Our stratified random sampling efforts in the Neuse and Tar–Pamlico River systems of North Carolina during 1998 to 2000 showed periods of lesion prevalence in menhaden of 80–90% (10). No other species had anywhere near this level of disease prevalence. b) If *Pfiesteria* (and presumably its biotoxins) are involved in lesion pathogenesis, why are other animals not affected, such as scavenging seabirds, which undoubtedly have eaten flesh from dead and/or distressed fish? c) If the lesions are caused by a direct-acting toxin in the water, why are the gills not affected? The gills are essentially a cluster of blood vessels directly exposed to the water. In our examinations of over 2,000 lesioned and nonlesioned menhaden from these waters, few remarkable gill lesions have been detected thus far (10). d) Why are the lesions usually solitary in individual fish, typically centered on the anal pore? Direct action of a dermonecrotic toxin might be expected to affect multiple areas of the skin, and in random fashion. Perhaps certain areas of the menhaden’s skin are deficient in the barrier function inherent to other parts of the body.

To date, the answers to these questions are clearly insufficient. This article is not intended to be a comprehensive review of ulcerative lesions in fish [an excellent review on this subject was recently authored by Noga (11)]. Rather, I focus on possible pathways of the disease in Atlantic menhaden and on recent data from North Carolina and other mid-Atlantic states, with comparisons to ulcerative lesions in other species.

**Pathways of Cell Injury**

To fairly and properly determine the causes of skin ulcers in fish, we should take advantage of lessons learned from comparative pathology. Cells of the skin and muscles, and the corresponding tissues they make up, have a limited repertoire of morphological response to injury. Thus, it is unlikely that histopathologic examination alone will reveal the pathogenesis of these ulcerative syndromes (11,12). However, careful morphological examination by the use of light and/or electron microscopy can provide important clues. Several biochemical mechanisms serve as common denominators in determining the ultimate outcome of cell injury, thus accounting for the morphological appearance of cells within lesioned tissues. These responses depend, of course, on the nature of the injury, its duration and severity, and the adaptability of the injured...
cells. Skin cells in one area of a fish’s body, for example—perhaps due to differences in natural defenses or in vascular perfusion (and, thus, oxygen tension and nutrient supply)—may be more susceptible to injury than other cells. Four intracellular systems are the most vulnerable targets for injurious agents: a) cell membranes; b) aerobic respiration involving mitochondria, the powerhouses of the cell; c) protein synthesis; and d) the genetic apparatus of the cell (13). All of these systems are closely interrelated. Thus, attack at one point will almost certainly disrupt the other systems. Notably, irreversible injury to cells is most consistently characterized by two phenomena: a) loss of cell membrane integrity; and b) critical injury to mitochondria, resulting in the inability to maintain proton motive force sufficient to restart ATP synthesis (13). Damage to mitochondrial membranes allows leakage of cytochrome c into the cytosol, which also can trigger apoptotic death pathways (14). Loss of cell membrane integrity allows an increase in cytosolic calcium, which in turn activates phospholipases, proteases, ATPases, and endonucleases, resulting in widespread cellular damage. Loss of membrane phospholipids, cytoskeletal damage, lipid breakdown products, loss of intracellular amino acids, and reactive oxygen species all contribute to further loss of membrane integrity.

Irreversibly injured cells in time undergo the morphologic manifestations of cell death. These include breakdown of the nucleus, lysis of the endoplasmic reticulum, membrane defects, swollen mitochondria, and accumulation of large mitochondrial densities. At the macroscopic level, necrotic tissue takes on a different color and consistency than the surrounding, normal tissue, depending on the age of the lesion and the amount of blood within, at the time of examination. Focal necrosis of epithelial tissues becomes an ulcer when the necrotic material sloughs away. “Ulcer” implies breach of the epidermal basement membrane, whereas “erosion” refers to loss of epithelium over an intact basement membrane.

Irreversible injury to muscle tissue results in swelling and fragmentation of myocytes, with loss of cross-striations and pale staining intensity. There is intracellular vacuolation, alteration of structural proteins, and accumulation of intracytoplasmic deposits. Segmental necrosis is followed by infiltration of macrophages, which phagocytose the necrotic cell debris. Cessation of the injurious stimulus may in time result in regeneration, characterized by large, more internally located myocyte nuclei, increased basophilia, and fiber hypertrophy. If there is extensive damage, reaction may involve local proliferation of fibroblasts, resulting in regenerated fibers interspersed with segmental areas of scar tissue (15). In our field sampling efforts, we have seen occasional menhaden with depressed areas of skin and muscle that appeared grossly to be old, scarred lesions. Histologically, these areas contained shrunken muscle fibers and areas of replacement by broad sheets of fibroblasts (fibrosis) and small numbers of infiltrating lymphocytes and macrophages.

The shape of an ulcer that involves skin, plus or minus the underlying muscle tissue, may provide clues to solving the pathogenesis of the lesion (Figure 1). An ulcer that started from the outside (“outside-in”) tends to have a base-narrow shape, whereas an ulcer that began as a lesion below the surface and worked its way outward (“inside-out”) tends to be base-wide. Menhaden from North Carolina estuaries have been observed with ulcerative lesions of both types. The majority of visibly diseased menhaden have a focal ulcer with severe granulomatous dermatitis and underlying myositis. These are usually found at the advanced stage of lesion progression, which takes many days to several weeks to form (7,16). Much like a “cold” crime scene, in fish with these advanced lesions or in fish poorly preserved or found dead, it is practically impossible to determine much about how the crime, i.e., the lesion, was committed or who committed it. Unfortunately in field sampling, the confounding effects of postmortem degeneration (autolysis) are exacerbated by warm water, in which most of the menhaden UM problems occur. However, our recent stratified random sampling efforts have afforded sampling of large numbers of fish with minimal postmortem autolysis and many with no visible lesions (16). In our examinations of numerous menhaden without observable skin lesions, we have found many specimens with areas of deep muscle necrosis, but we can detect no apparent etiologic agents such as bacteria or parasites. The cause of these areas of deep necrosis, some of which are accompanied by focal infiltrates of inflammatory cells, is uncertain. Some possible causes are discussed below.

Many Agents, Few Pathways

The pathogenesis of ulcerative syndromes in fish undoubtedly involves multiple factors, but a number of etiologic agents may be considered primarily responsible for causing the disease. Recent reports by Noga (6,11), Sindermann (12), and others discuss these ulcerative syndromes in more detail. Possible causes of ulcerative lesions in menhaden in the context of general categories of disease agents are discussed in the present article. Generally, differential diagnoses for a given disease process should include infectious agents, toxins, physical causes, immunologic causes, and nutritional and metabolic perturbations. There is, of course, much overlap in these categories of causative agents, as illustrated in Figure 2.

Infectious Agents

Fish literally swim in a sea of pathogens. Thus, any breach in the normal barrier function of the skin can allow colonization of the skin by infectious organisms, or invasion by microorganisms that normally colonize the skin but are typically of low pathogenicity. Differential diagnoses for skin lesions in fish should include fungal, bacterial, parasitic, and viral organisms:

**Fungal.** Fungi are associated with a variety of skin diseases in fish as well as in other animals (6,17). UM as originally described, involves water mold–type fungi, or more accurately, Oomycetes of the genera *Aphanomyces* and possibly *Saprolegnia* (9,18). The lesions typically consist of mature epithelioid granulomas centered around variable numbers of fungal hyphae. In a recent report,
Blazer and colleagues examined Atlantic menhaden from Chesapeake Bay and its tributaries in Maryland and Virginia (19). Of 121 lesioned menhaden from the Chesapeake Bay collected in 1997 and another 31 collected from the Pocomoke and Wicomico Rivers in Maryland in 1998, all had deeply penetrating fungal hyphae surrounded by intense granulomatous inflammation. In fish with raised lesions (i.e., before the necrotic material had sloughed away), hyphae were observed penetrating through and around muscle bundles beneath areas of intact skin and muscle. The lesions appeared identical to UM as well as to epizootic ulcerative syndrome, an ulcerative skin disease of fish of the Indo-Pacific attributed to *Aphanomyces invadens* (20,21). Indeed, organisms with culture characteristics identical to *A. invadens* were isolated from the Chesapeake Bay menhaden. This would suggest that highly invasive and highly pathogenic strains of this organism have been introduced to naïve fish populations of the U.S. Atlantic coast and may be responsible for at least some of the lesion events that have occurred in the past two decades.

In our stratified random sampling efforts over the past several years targeting Atlantic menhaden in North Carolina, we have examined over 2,000 lesioned and nonlesioned fish. Of 192 menhaden with inflammatory skin and/or muscle lesions confirmed by histopathology, only 66 (34%) contained fungal hyphae. Instead, many lesions were characterized by loosely organized infiltrates of inflammatory cells (mixed macrophages, lymphocytes, fewer granulocytes) either with no discernable etiologic agents, or less often with bacteria or protozoa within the lesion (Figures 3–5). When present, fungal hyphae virtually always elicited the mature, epithelioid-type granulomas that surrounded the hyphal elements. When fungi were not present, there was usually a milder, more loosely organized inflammatory infiltrate (Figures 3, 4) or occasionally, areas of deep muscle necrosis without remarkable inflammation.

Even if we are dealing with a highly invasive strain of *A. invadens* as a cause of the major manifestations of the lesions of UM in some lesion events, these organisms are unlikely to be the initiating cause of the lesions (7,11). Lessons learned from some of the better characterized mycotic diseases, such as aspergillosis, in man and other animals suggest that fungal pathogens require some predisposing condition of the host, such as debilitation or breach of the normal mucosal barriers along with favorable growth conditions before they can become established (22). Such conditions would include compromise of the animal’s normal immune status (i.e., systemic and/or mucosal immunity). Oomycetes certainly appear to be more invasive than higher fungi, but the precise predisposing factors that allow oomycetes to reach that point of invasion remain to be discovered.

**Bacterial.** Bacteria are ubiquitous in the aquatic environment. Genera such as *Aeromonas* and *Vibrio* are considered primary pathogens and have been implicated in several ulcerative disease syndromes, such as ulcus syndrome of cod in Denmark and red sore disease of sheepshead and black drum in the Gulf of Mexico (12,23). Bacterial pathogens are undoubtedly involved in worsening the lesions of many of the menhaden specimens presented with typical UM. However, to date, no single species has been consistently isolated to suggest it as an initiating pathogen or major cause of UM. Of note is the recent isolation, from the Chesapeake Bay, of *Mycobacteria* spp. from striped bass with skin lesions (24,25), which provides further evidence for

**Figure 3.** Cross-section of skeletal muscle and overlying skin from an Atlantic menhaden (*B. tyrannus*) that had no visible skin lesions. The section has a large area of necrosis and loss of skeletal muscle fibers, with infiltration of mixed inflammatory cells (macrophages, lymphocytes, and granulocytes, seen as dark blue/purple or basophilic). No etiologic agents were found, and overlying muscle and skin are intact. The inflammatory infiltrates extend downward along muscle planes. H & E, original magnification ×25.

**Figure 4.** Cross-section of skeletal muscle and overlying skin from an Atlantic menhaden that had no visible skin lesions. This fish has an area of deep, relatively loosely organized inflammation (basophilic area) that appears to form a dissecting tract along muscle planes. The arrow points to an area of muscle necrosis and inflammation at a point where the lesion might be expected to break out and form a skin ulcer, given additional time. Hematoxylin and eosin (H & E), original magnification ×25. Inset: Skeletal muscle fiber surrounded by inflammation. This muscle fiber is undergoing irreversible injury, with swelling, pale staining intensity, loss of striations, and separation from the surrounding muscle tissue. H & E, original magnification ×66.
either immunocompromise in estuarine fishes or a shift in ecological balance in these regions, or both.

**Parasitic.** Parasites cause sporadic skin lesions in fish. A myxosporean protozoan, probably of the genus *Kudoa*, is considered a possible cause of lesion events in menhaden. In mid-summer in the Pocomoke River, a Chesapeake Bay tributary, large numbers of juvenile menhaden have been found with ulcers apparently caused by these *Kudoa*-like organisms (26). Numerous protozoal cyst forms are seen massed within muscle bundles and closely associated with severe ulcerative dermatitis and myositis. *Kudoa* is a recognized cause of rapid muscle softening in several fish species including net pen-reared salmon (27,28). The parasite is thought to elicit release of the proteolytic enzyme cathepsin, which hastens autolysis and, thus, spoilage of the flesh under certain environmental conditions or particular physiological conditions of the menhaden host. Sporadic cyst rupture could account for some of the deep muscle inflammatory lesions seen in menhaden with no overlying skin lesions (Figures 3–5). The ensuing inflammatory response may then mask the presence of any spores from the ruptured cyst. If the inflammatory reaction is severe enough, it could then lead to skin ulceration with an inside-out pathogenesis (Figures 1, 5). Species-specific host–parasite interactions could also partly explain why menhaden are the predominant species affected by UM. Toxins are unlikely to be species specific, unless menhaden may possess some unique metabolic machinery able to bioactivate such toxins. More research is needed on the potential role that stages of this parasite play in necrosis of skeletal muscle in menhaden.

Other parasites also may contribute to skin ulcers in fish. Occasionally menhaden are caught with lernaeid-type anchor worms embedded deeply into their skin and underlying musculature. These worms may initiate ulcerative lesions in a small proportion of the fish. Large mouth bass in the Chowan River, North Carolina, a tributary of the Albemarle Sound, were reported with a significant anchor worm problem thought to contribute to the initiation of skin lesions (31). However, anchor worms are unlikely to be a significant cause of ulcerative disease in menhaden except in severe infestations.

**Viral.** Viruses such as rhabdovirus have been implicated in several ulcerative syndromes of fish (1,2). However, no consistent associations have been made between viral infections and UM-like lesions in Atlantic menhaden or other western Atlantic fish species. Stephens et al. (32) reported infectious pancreatic necrosis-like virus as a cause of spring menhaden epizootics in the Chesapeake Bay. A virus isolated from menhaden with neurologic signs (“spinning disease”) was injected into normal menhaden, which subsequently developed signs of spinning disease along with hemorrhages at the base of fins, in the eyes, and along the body (32). Such lesions could certainly lead to secondary infections and subsequent overt skin ulcers. This virus deserves consideration on the list of possible differentials, especially since distressed menhaden reportedly affected by toxic *Pfiesteria* have been observed with neurotoxic signs that may be similar to spinning disease (1). Interestingly, I have found no other reports of infectious pancreatic necrosis-like virus associated with disease in menhaden.

**Toxins**

*P. piscicida* and its putative biotoxins have been implicated as possible causes of ulcerative lesions in menhaden. Tilapia (*Oreochromis niloticus*) and striped bass exposed in the laboratory to toxic *Pfiesteria* developed intra- and extracellular edema and necrosis of the epithelium, culminating in severe erosions within 8–48 hr (2). These lesions differed from those seen in menhaden with UM and formed more rapidly than the chronic granulomatous inflammation seen in UM. The superficial pattern of epidermal necrosis suggests a direct-acting toxin in the water. However, no remarkable gill lesions were seen. This finding is consistent with our recent sampling efforts in which no remarkable gill lesions have been detected in menhaden. Another possible mechanism of epidermal damage was posed in this study, namely, shutdown in peripheral vascular perfusion (2). However, primary epidermal lesions are not a feature of skin lesions that involve blood vessels, such as vasculitis (33). Such vascular lesions usually consist of dermal hemorrhage and edema, and necrosis (infarction) involving both the dermis and epidermis. Fibrin thrombi may also be seen within blood vessels.
Unfortunately, laboratory studies have been hampered by the failure to isolate biotoxins from *Pfiesteria* for development of specific probes. We recently examined tilapia from *Pfiesteria* bioassays and found a small percentage of fish with focal necrosis of basal epithelial cells of the skin, as well as an interesting multifocal necrosis and apoptosis of liver cells. Menhaden from our recent sampling efforts have also been seen with necrosis of basal epithelial cells, occasionally forming a blisterlike lesion with a raised epidermis (Figure 6). However, lesions are difficult to reproduce between laboratories and between *Pfiesteria* cultures. In addition, the stringent biosafety conditions required for *Pfiesteria* bioassays make tight control of water quality conditions problematic. Obviously, isolation of individual biotoxins from *Pfiesteria* will help to clarify these issues.

The question remains, however: How could a systemic toxin cause only single, focal, ulcerative lesions as is the case with UM? There are examples from comparative pathology. Ergotism is the oldest known mycotoxicosis (34). The ergot alkaloids produced by *Claviceps* spp. fungi stimulate adrenergic nerves supplying arteriolar smooth muscle in cattle. This produces peripheral vasoconstriction, capillary thrombosis, and ischemic necrosis, resulting in necrosis (dry gangrene) of the distal limbs and tips of the ears and tail. In menhaden, it is possible that toxins from *Pfiesteria* may act indirecly or in concert with other toxins or environmental conditions to predispose certain skin and muscle tissue sites to injury.

**Physical Causes, Immunologic Causes, and Nutritional and Metabolic Perturbations**

Nutrient enrichment of estuarine systems, primarily due to nitrogen loading, creates increases in algal biomass and zones of severe oxygen depletion, or hypoxia (35–37). These phenomena appear to be increasing in western Atlantic estuaries and creating hypoxic zones in which schools of menhaden and other fish may become trapped. Fish kills attributed to low dissolved oxygen are relatively common in North Carolina waters in the warm water temperatures of summer and early fall (38). However, it is the sublethal effects of chronic hypoxia that may have wider reaching consequences such as immunosuppression, elevated stress hormones, or the development of ulcerative skin lesions (11). Plumb and others observed that catfish exposed to chronic, sublethal hypoxia developed sterile skin and muscle necrosis (39,40). Bacteria could not be cultured from the lesions until several days later, most likely as secondary infections. If the effects of hypoxia were mediated through immunosuppression, the necrotic lesions would most likely have contained infectious organisms from their inception. This would suggest that at least part of the contribution of chronic hypoxia to skin ulcers in fish is a more direct effect due to loss of cellular oxidative phosphorylation. In addition, when normal oxygen levels are restored, reperfusion injury comes into play, mediated through reactive oxygen species such as superoxide, peroxynitrite, or the potent hydroxyl radical (13,41).

As discussed above, lipid peroxidation in critical cell membranes results in the cascade of damage to intracellular systems, culminating in irreversible cell injury.

Figure 6. Cross-section of skeletal muscle and overlying skin from an Atlantic menhaden. In this section there is necrosis of the basal epithelial layer of the skin, creating a lifting or blisterlike lesion of the epidermis. Rupture of this blister could lead to secondary infections and extensive ulceration. H & E, original magnification ×10. Inset: This photo shows one corner of the blisterlike lesion characterized by coagulative necrosis and perivascular infiltration of mixed inflammatory cells. H & E, original magnification ×80.

Figure 7. Cross-section of a blood vessel within skeletal muscle from an Atlantic menhaden that had no visible skin lesions. The vessel is infiltrated and cuffed by mixed inflammatory cells and has swollen endothelial cells. However, fibrinoid change (eosinophilic, fragmented material) in the vessel wall would have to be more obvious before it could be classified as vasculitis. Fibrin thrombi are occasionally seen within vessels with this degree of perivascular inflammation. The arrows point to individualized muscle fibers, which, given further time and development of this lesion, would probably undergo irreversible cell injury and necrosis. H & E, original magnification ×66.
Another indirect effect of hypoxic zones in estuarine systems is the crowding of large numbers of fish into much smaller areas than normal, creating a greater demand on food supplies, increased risk of physical injuries and predation, and more rapid spread of infectious diseases. Decreased nutritional status in individuals may in turn lead to immunosuppression as well as a deficiency of critical antioxidants, making the fish even less able to cope with oxidative stresses created by toxins or hypoxia. Furthermore, overcrowding may have more direct effects. Noga et al. (42) recently demonstrated the induction of skin ulceration on the fins of striped bass and hybrid bass due simply to 2 hr of confinement stress. Similar but less severe losses were produced by injecting fish with epinephrine, suggesting a possible role for catecholamines in the induction of skin lesions. Chronic hypoxia can also cause increased cortisol levels in fish (43).

Immune system activation or hyperfunction should not be discounted as a possible contributing cause of ulcerative lesions. Infectious organisms as well as sudden physiological stresses may inappropriately activate components of the immune system, such as the complement system or antigen–antibody complexes, which may lead to vasculitis (13,33). In our sampling efforts we have seen a number of menhaden that exhibited perivascular cuffing by leukocytes and/or vasculitis (Figure 7). Further research is needed to determine whether these lesions contain immune complexes caused by infectious agents or are perhaps due to some degree of immune system hyperfunction.

Similar pathways of cell injury may be induced by other changes in water quality, such as sudden shifts in water temperature or pH, or influx of anthropogenic pollutants. “Winter kill” in catfish is associated with focal ulcerative dermatitis and myositis, often invoked by fungi. Sudden temperature drops in the shallow ponds probably cause shunting of blood to vital organs and local immunosuppression (44). Additionally, sudden changes in water quality, physiological changes, and decreased nutritional status may all contribute to changes in the protective mucus layer on the skin, which is the fish’s primary line of defense against a hostile aquatic environment.

Quality Control in Bioassays

It is clear that many interrelated pathways may lead to cell injury and death (Figure 2), often culminating in ulcerative skin lesions in fish with a very stereotypical morphological appearance. Laboratories performing bioassays to define the role of individual etiologic agents, such as fungi (oomycetes) or Pfiesteria toxin(s), should be mindful of this multiplicity of factors. Attempts to impose quality assurance/quality control measures on such bioassays should include appropriate controls for water quality parameters (temperature, dissolved oxygen, nitrogenous wastes, etc.), as well as for bacterial and other contaminants that may confound bioassay results and their interpretation. Water sources should be well defined, and the use of split samples to measure the degree of interlaboratory variation should be instituted. Finally, we must move beyond the concept of “one etiology, one disease,” accept the notion that disease syndromes may have multiple causes, and view ecosystems as a whole. Estuarine ecosystems provide some of the best examples of integrated biological systems, in which perturbation of one component will almost certainly affect every other component to some degree.

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