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An Epizootic of *Edwardsiella tarda* in Largemouth Bass (*Micropterus salmoides*)

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ABSTRACT: *Edwardsiella tarda*, an opportunistic bacterial pathogen, was isolated from dying largemouth bass (*Micropterus salmoides*) during an epizootic in a eutrophic lake system, Lochloosa Lake, Florida, USA. Approximately 1,500 adult fish died over a 6-wk period during the late summer and early fall of 1991. A mixed population of aerobic bacteria (*E. tarda*, *Aeromonas hydrophila*, and *Pseudomonas* sp.) was isolated from deep cutaneous ulcers and intestines of moribund bass. However, *E. tarda* in pure culture was the only bacterium isolated from several viscera of several fish; *E. tarda* may be the etiologic agent responsible for some episodes of seasonal mortality in largemouth bass.

Key words: *Edwardsiella tarda*, largemouth bass, *Micropterus salmoides*, microbiology, pathology.

Freshwater sportfishing represents a major component of Florida's (USA) tourism industry. Of the freshwater game fish species indigenous to Florida waters, the largemouth bass (*Micropterus salmoides*) is one of the most economically important, by itself producing an annual income of $600 million in recent years (Hardin et al., 1987). Concerns regarding the continued health of game fish populations are mounting as the use of public waterways increases.

Epizootic mortality, particularly affecting largemouth bass, is a sporadic but common occurrence affecting the game fish populations of eutrophic and mesotrophic Florida lakes during late summer and early fall. Such fish kills routinely have been attributed to low dissolved oxygen concentrations in warm water (Bennet, 1970). Because of this assumption, few studies have been performed to identify other potential etiologic agents. In this report we describe an epizootic of mortality in largemouth bass caused by fatal systemic infection with the opportunistic bacterial pathogen, *Edwardsiella tarda*.

During early September 1991, approximately 1,000 dead largemouth bass were found floating in Lochloosa Lake, a 2,300 ha, eutrophic lake in north-central Florida (29°30′N, 82°06′E). Most dead bass were adults (>1 kg). A few dead bluegill (*Lepomis macrochirus*), black crappie (*Pomoxis nigromaculatus*), and gar (*Lepisosteus platyrhincus*) also were observed. Mortality continued for 6 wk with a cumulative loss of approximately 1,500 largemouth bass. A second, smaller epizootic of largemouth bass mortality was reported simultaneously by fisherman in the nearby Oklawaha River.

For sampling, Lochloosa Lake was divided into four quadrants. During the second week of the epizootic, surface water was collected from each quadrant (8 samples total), placed on ice, and transported to the University of Florida Fish Disease Laboratory (FDL) for analysis with a standard water quality test kit (Model No. FF-1A, Hach Chemical Company, Loveland, Colorado, USA). Dissolved oxygen concentrations ranged from 5.2 to 9.0 mg/liter; water pH was 7.2; total alkalinity and total hardness were both 68.4 mg/liter; total ammonia nitrogen ranged from 0.7 to 1.0 mg/liter; and nitrites were not detected. All values were considered within normal seasonal limits. A slight elevation in total ammonia nitrogen was attributed to the large quantity of decaying vegetation.

After water samples, live largemouth

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bass were collected from each quadrant using electrofishing techniques described by Bennet (1970). Fifteen bass were captured at random, weighed, measured, and examined for external abnormalities. About half of the fish had deep cutaneous ulcers. Ten of the most severely affected fish, ranging in weight from 0.01 to 0.55 kg, as well as one floating, moribund individual (1.06 kg), were bled from the caudal vein (Campbell and Murru, 1990). Both bass and blood were transported on ice to the FDL; all fish survived the 30-min trip. An additional moribund female largemouth bass (2.8 kg) from the Oklawaha River was frozen before shipping.

Bass from Lochloosa Lake were removed from ice and euthanized by application of a tricaine methanesulfonate (Finquel, Argent Chemical, Redmond, Washington, USA) overdose to the gills. The Oklawaha River bass was thawed. Aerobic bacterial cultures were obtained from the cutaneous ulcers, posterior kidney, and liver. Material was cultured on tryptose agar (Difco Laboratories, Detroit Michigan, USA) containing 5% bovine blood, and Ordal's medium for isolation of *Myxobacteria* (Anacker and Ordal, 1959). These primary cultures were incubated at 25 C for 48 hr. The identity of the bacterial isolates was determined using the techniques of Austin and Austin (1987) and Shotts and Bullock (1975).

Edwardsiella tarda, *Aeromonas hydrophila*, and *Pseudomonas* sp. were isolated from cutaneous ulcers of all bass of Lochloosa Lake and from the intestine of the Oklawaha River fish. In contrast, *E. tarda* was obtained in pure culture ≤24 hr from the posterior kidney of two Lochloosa Lake fish, one of which was moribund and floating. Pure *E. tarda* also was isolated from the posterior kidney, swim bladder, and liver of the Oklawaha River fish. Other bacteria were not isolated from the viscera of any fish. The identity of the visceral isolate was confirmed as *E. tarda* both morphologically, by demonstration of 2-μm-long, gram-negative, motile bacilli in the small, transparent colonies, and biochemically through elaboration of indole and hydrogen sulfide.

Packed cell volume (PCV) was determined on the heparinized blood taken from the caudal vein by the microhematocrit method (Coles, 1986), and total plasma protein was determined with a clinical refractometer (Coles, 1986). Two fish with systemic *E. tarda* infections were anemic with a mean (±SE) PCV of 18.0 (±1.0%) compared to fish which only had infected ulcers (mean PCV = 29.6 (±0.9%)). Total plasma protein (TP) also was lower in the two fish with systemic infections (mean TP = 2.8 (±0.4 g/dl)) compared to fish with external infections (mean TP = 5.0 (±0.1 g/dl)).

Gross examination of the body surfaces of Lochloosa Lake bass revealed deep cutaneous ulcers (usually 1 to 3 per fish) filled with malodorous, necrotic material. The width of ulcers varied from 0.5 to several cm, but most were <1 cm in diameter. Most ulcers were located on the lateral or posterio-lateral body wall, but the lesion on one fish was located between the eyes. A 12-mm-diameter vesicle filled with clear fluid was located between the eyes of the single moribund fish. Based on the necropsies, we found pale mottled livers in two individuals and an absence of ingesta in the alimentary tract of all fish. Gross lesions from the Oklawaha River fish consisted of gill pallor and fibrinohemorrhagic foci (≤2.5 cm in diameter) in the serosae of the intestine and swim bladder.

We fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin samples of skin, skeletal muscle, gills, and several viscera from the Lochloosa Lake bass. We noted necrohemorrhagic myositis of the trunk muscles as the principle lesion. In each instance, myodegenerative changes were associated with an overlying dermal ulcer, chronic cutaneous ulcer and the muscular lesions were most severe in the superficial muscle bundles. A mild response was characterized by loss of myofibrillar striations and a large, round

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clear vacuole replacing the fiber core. More severely affected fibers were fragmented. A chronic, active inflammatory infiltrate consisting of heterophil, macrophages and lymphocytes was prominent in the dermis, epimysium and (in severely affected superficial muscle) perimysium. Bacilli (2 to 3 μm long) were present in the inflamed dermal connective tissue and epimysium of superficial muscle bundles of two fish. During the evaluation of the viscera, we observed multifocal granulomas and scattered melano-macrophage centers in the interstitial tissues of the posterior kidney, spleen, and periportal liver. The granulomas were fresh as indicated by the large core of necrotic but recognizable inflammatory cells and the thin capsule. The hepatocytes in the mottled livers were diffusely vacuolated.

The pathologic and bacteriologic findings observed in dying largemouth bass from these Florida waterways were generally consistent with the diagnosis of *Edwardsiella tarda* septicemia (Bullock and Herman, 1988). In addition, a more chronic manifestation was the presence of deep cutaneous ulcers. *Edwardsiella tarda* ulcers in channel catfish are associated with emphysema of the subadjacent skeletal muscle (Meyer and Bullock, 1973). In this epizootic, however, the ulcers in bass were not associated with putrefication and gas release in the deep muscle tissues as demonstrated by the absence of gas bubble ghosts during histologic examination.

Based on the isolation of *E. tarda* from two separate epizootics of mortality affecting largemouth bass, we propose that this opportunistic pathogen also may be associated with seasonal mortality in Florida game fish populations. *Edwardsiella tarda* has been isolated in surface waters and moribund largemouth bass from a lake in northern Florida during a mid-summer fish kill (White et al., 1973). From the date of the earlier episode (1971), it would appear that fatal infections induced by *E. tarda* are not a new problem. Further research is warranted both to define the incidence and pathogenesis of *E. tarda* infections in game fish populations and to assess the zoonotic potential associated with human exposure to fish and surface waters containing this opportunistic pathogen.

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