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EDWARDSIELLOSIS IN WILD STRIPED BASS FROM THE CHESAPEAKE BAY

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ABSTRACT: The first epizootic of edwardsiellosis, caused by *Edwardsiella tarda*, is described. The epizootic occurred in the Chesapeake Bay, Maryland (USA) during the summer and autumn of 1994, and affected wild adult striped bass (*Morone saxatilis*). Clinical signs included numerous irregular coalescing hemorrhagic ulcers on the body and fins that were distinctly malodorous. Internally, the body cavity was filled with abundant yellowish or sanguinous mucoid fluid, and the visceral organs had multiple tiny white foci. The intestines contained thick white opaque mucus. Histopathological lesions included ulcerative dermatitis, cardiac endothelial hyperplasia, and necrotic foci and granulomata in multiple organs. A bacterium isolated in pure culture was characterized taxonomically and serologically as the wild-type or classical biotype of *E. tarda*. In infectivity trials, it was pathogenic for striped bass, gilthead seabream (*Sparus aurata*), and turbot (*Scophthalmus maximus*) with an LD_{50} of about 10^5 cells; however, the isolate was non-virulent for mice ($LD_{50} > 10^8$ cells). The isolate also was resistant to the bacteriolytic activity of normal fish skin mucus.

Key words: *Edwardsiella tarda*, wild striped bass, Chesapeake Bay, microbiology, fish skin mucus test, fatty acid methyl esters (FAME), histopathology.

INTRODUCTION

Edwardsiellosis, caused by *Edwardsiella tarda*, is a subacute to chronic disease which affects a variety of fish taxa and has worldwide distribution in fresh and marine waters (Austin and Austin, 1993). The most serious epizootics have been reported in channel catfish (*Ictalurus punctatus*) in the USA and in eels (*Anguilla anguilla*) in Japan and Taiwan (Plumb, 1993, 1994; Kusuda and Salati, 1993). Although *E. tarda* generally is considered a problem in warmwater fishes, the bacterium was responsible for mortalities of economically important coldwater fishes, such as chinook salmon (*Oncorhynchus tshawytscha*) in the USA (Amandi et al., 1982), Atlantic salmon (*Salmo salar*) in Canada, and, more recently, in turbot (*Scophthalmus maximus*) in Spain (Nougayrede et al., 1994).

Edwardsiella tarda is considered a common constituent of the normal intestinal flora of normal-appearing aquatic animals (White, 1984), but this bacterium may cause intestinal and extra-intestinal disease and wound infections in reptiles, amphibians, marine mammals, and terrestrial endotherms, including humans (Bockemuhl et al., 1971; Sakazaki and Tamura, 1992; Janda and Abbott, 1993).

Edwardsiellosis in fish most often occurs secondary to handling, high content of organic material in water, other forms of poor water quality, crowding, and high or rapidly fluctuating water temperatures (Plumb, 1993). The only previous case of edwardsiellosis in striped bass (*Morone saxatilis*) was described in West Virginia (USA) in hatchery-reared fish (4 to 5 cm long) after stress related to handling and transportation (Herman and Bullock, 1986). In this report we describe the first record of mortality from edwardsiellosis in wild adult striped bass, characterize phenotypically and biochemically the strain of *E. tarda*, describe the gross and histological findings in affected fish, and assess the pathogenic potential of the bacterium in three fish species and laboratory mice.

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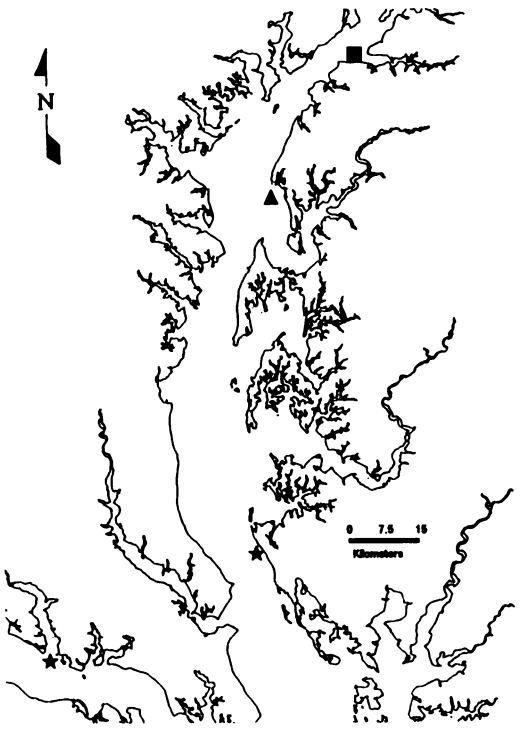


FIGURE 1. Map of northern and central region of Chesapeake Bay, Maryland (USA), with sites where wild striped bass were collected in September and October 1994. Mouth of Sassafras River (■) and Chester River (▲) where sick striped bass were collected by anglers. The star (★) on the right is the Taylors Island site and the star on the left is the Wicomico River site where sick striped bass were taken from pound nets.

MATERIALS AND METHODS

Affected striped bass were collected from pound nets at the mouth of the Wicomico River (38°15'N, 76°50'W) (a tributary of the Potomac River) and adjacent to the mouth of Punch Island Creek, Taylors Island (38°25'N, 76°20'W) in the Chesapeake Bay of the northeastern USA in September and early October 1994 (Fig. 1). Monitoring of pound net populations of striped bass from Taylors Island and the upper Chesapeake Bay at the mouth of the Sassafras River (39°23'N, 76°03'W) continued through October to assess temporal and spatial distributions of affected fish. Eleven affected striped bass (approximate mean weight: 4 kg) from the Wicomoco River and 14 affected bass from Taylors Island pound nets were taken alive and immediately placed on ice. Striped bass were transported to the Maryland Animal Health Diagnostic Laboratory in College Park, Maryland (USA), for necropsy and bacteriological, virological, and histological examinations. Specimens were collected within 0.5 to 3 hr of arrival. Additionally, five fish without skin lesions were taken as controls from the Taylors Island site.

Samples for bacterial cultures were collected from skin ulcers by wiping the skin surface with sterile gauze to remove mucus, then spraying with a steady stream of saline solution for 90 seconds, incising the margin of an ulcer, undermining the ulcer, and swabbing the undermined dermal-subcutaneous tissue; bacterial cultures also were collected by stabbing in situ the pronephros (head kidney), liver, spleen, and body cavity with a plastic disposable sterile inoculating needle (Fisherbrand Bac-Loops, Fisher Scientific, Pittsburgh, Pennsylvania, USA). Swabs of each organ were immediately inoculated onto tryptic soy agar (Difco Company, Detroit, Michigan, USA) supplemented with 2% NaCl (TSA-2) and thiosulphate-citrate-bile-sucrose agar (TCBS) (Oxoid, Unipath Ltd., Basingstoke, England) and incubated at 28 C for 48 hr. Pure cultures of the isolated colonies were identified using the morphological, biochemical and physiological plate and tube tests described by Smibert and Krieg (1994) and the API 20E system (bioMerieux, Marcy-l'Etoile, France). Additional characterizations of bacterial isolates were performed following the schema of Sakazaki and Tamura (1992) and Holt et al. (1994). Briefly, these tests included detection of hemolysins on sheep blood agar plates, growth of bacteria on TSA-2 agar plates at various temperatures (4, 10, 28, 37, 42, and 45 C), and detection of growth at various salinity levels (1.0, 3.0, 4.0, and 6.0% NaCl) at 28 C. Drug sensitivity tests were done by the disc diffusion method on Mueller-Hinton agar (Oxoid, Unipath Ltd.). The bacteria isolated in this study were compared to the reference strain, *E. tarda* 9.8 (U.S. Fish and Wildlife Service, National Fish Health Research Laboratory, Kearneysville, West Virginia, USA), first isolated from cultured striped bass (Herman and Bullock, 1986).

More detailed characterization of bacterial isolates (named strain FL4-53) from diseased striped bass were done by gas chromatography of fatty acid methyl esters (FAME) (Toranzo et al., 1994).

Serological studies of isolated bacteria were conducted by the slide agglutination test (Toranzo et al., 1987) using antisera raised against *E. tarda* 9.8 and *Edwardsiella ictaluri* ATCC 33202 (American Type Culture Collection, Rockville, Maryland). Tests were performed using the thermostable "O" antigens which were obtained by heating bacterial suspensions in

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10% volume/volume in phosphate-buffered saline (PBS) at 100 C for 1 hr.

Virus cultures followed the procedures of Thoesen (1994) and involved homogenizing pooled pronephros, spleen, and brain from each fish in Leibovitz's L-15 medium with 2% fetal calf serum (20% weight/volume) containing 500 μg/ml gentamycin (Sigma Chemical Company, Saint Louis, Missouri, USA), 800 μg/ml streptomycin, 10 μg/ml fungizone, and 800 units/ml penicillin (JRH Biosciences, Lexena, Kansas, USA). After 2 hr incubation at room temperature (22 to 24 C), the suspension was centrifuged at 3,000 × G for 20 min. Supernatant fluids were harvested, diluted 1:10, 1:50, and 1:500, and then 0.1 ml of each dilution was inoculated onto cell lines grown in 48-well microtiter plates. One row of wells was used per dilution, and one row of wells on each plate served as a control and received maintenance medium only. Cell lines were epithelioma papulosum cyprini (EPC) cells, (Fijan et al., 1983), chinook salmon embryo (CHSE) cells, (Lannan et al., 1984), rainbow trout gonad (RTG-2) cells (Wolf and Quimby, 1962), fathead minnow (FHM) cells (Gravel and Malsberger, 1965), and brown bullhead (BB) cells (Hay et al., 1992) and were incubated at 15 or 20 C. If no cytopathic effect (CPE) was observed within 10 days, fluids were removed from the wells and used to inoculate fresh monolayers (one blind passage). If no CPE developed after an additional 10 days, the sample was considered negative for virus.

For histological examinations, 1-cm slices of skin ulcers, spleen, liver, pronephros, heart, stomach, intestine, gill, and whole brain and eye were fixed by immersion in 10% buffered neutral formalin. Bony tissues were decalcified after formalin fixation in saturated EDTA (ethylene diamine tetraacetic acid) disodium solution (Fisher Scientific) for 48 hr prior to histologic processing. Tissues were embedded in paraffin, sectioned at 7 μm, and stained with hematoxylin and eosin (H&E) and the Brown and Brenn Gram stain.

For pathogenicity studies, the gilthead seabream (*Sparus aurata*) and turbot (10 to 15 g approximate weight) were selected as representative of economically important warm and cold water species, respectively. Striped bass (30 g approximate weight) also were used. Fish were divided into lots of six, and injected intramuscularly with 0.1 ml aliquots containing 10^3 to 10^7 bacteria suspended in sterile PBS. Controls were injected with sterile PBS. All turbot and seabream were maintained in aerated seawater at 22 C for 2 wk; striped bass were maintained in recirculating fresh water at 22 C. Fish found dead in the tanks were discarded while moribund fish and those surviving to the end of the study (2 wk) were euthanized by immersion in water containing 500 mg/L of tricaine methanesulfonate (MS-222) (Argent Chemical Laboratory, Redmond, Washington, USA), necropsied and examined bacteriologically and histologically, as described. The LD_{50} was calculated by the method of Reed and Muench (1938). Experimental striped bass, but not experimental turbot and seabream, were examined histologically, as described.

To determine if fish skin can be colonized by the isolate of *E. tarda* (FL4-53) from diseased wild striped bass, the bacteriolytic activity of cell-free skin mucus of fish was analyzed by the disc diffusion method (Magariños et al., 1995). Briefly, mucus was scraped from the skin of healthy turbot and seabream with a glass slide; the mucus was dissolved in 0.85% saline solution to produce a 1:2 mucous : saline ratio. Sterile 6-mm diameter discs were impregnated with 20 μl of the mucus solution. Bacteria were suspended in PBS to a concentration of 10^5 cells per ml, streaked over the entire surface of Mueller-Hinton agar plates, and the mucus-impregnated discs were applied to the agar plates. After incubation at 22 C for 24 hr, antibacterial activity was evident as a zone of inhibited growth around the discs.

To assess virulence of *E. tarda* in endothermic animals, a mouse pathogenicity test was performed using two strains of *E. tarda*: 9.8 and FL4-53. Groups of five BALB/c mice were inoculated intraperitoneally with bacterial concentrations ranging from 10^4 to 10^8 cells.

RESULTS

Morbidity among wild striped bass was first reported and investigated in September 1994. No moribund or dead bass were found by anglers or watermen using pound nets. Affected striped bass from pound nets had numerous red coalescing foci in the skin of the body and fins, some of which were distinctly ulcerated and malodorous (Fig. 2). The cloaca was occasionally prolapsed and hemorrhagic. Some bass had cloudy and opaque corneas. Internally, the body wall was pale, and the body cavity had prominent amounts of yellowish or sanguinous opaque mucoid fluid. The intestines, stomachs, and hearts were flaccid. The intestines were empty of food but filled with a thick white opaque mucus. Gallbladders were distended and

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FIGURE 2. Adult wild striped bass from the Chesapeake Bay affected by *Edwardsiella tarda* show extensive hemorrhagic areas and skin ulcers on the body and fins.

filled with bile. The spleens, livers, and pronephroi had miliary white foci.

We isolated a single colony type from all internal organs and coelomic fluids on TSA-2 agar. No bacteria were isolated on TCBS agar nor from five healthy-appearing wild striped bass taken from pound nets at Taylors Island during the epizootic. The wild striped bass isolate, hereafter referred to as strain FL4-53, possessed phenotypic traits consistent with *Edwardsiella tarda* and a biochemical profile identical to reference strain 9.8 (Table 1). Both *E. tarda* strains lacked proteolytic, lipolytic, and amylolytic activities but produced β-hemolysins against sheep erythocytes. Both strains grew in a temperature range of 10 to 42 C and tolerated salinity up to 4% NaCl. Both strains had similar drug sensitivity patterns, and were resistant only to streptomycin and erythromycin. On the basis of hydrogen sulfide production and inability to ferment the majority of sugars, the two strains resembled wild-type or classical biotype *E. tarda*.

The FL4-53 isolates had a FAME profile typical of *E. tarda*; the major peak being hexadecanoic acid (16:0) followed in descending amounts by saturated and unsaturated 14:0, 3OH 14:0, 16:1, 17:0Δ, and 18:1 fatty acids. Trace amounts of pentadecanoate and octadecanoate were detected.

The "O" antigen of strain FL4-53 agglutinated with the *E. tarda* antiserum but not with antiserum against *E. ictaluri*. Virological assays of affected wild striped bass were negative in all cell lines.

Striking histologic abnormalities were evident in the skin of wild striped bass, and to a much lesser extent in experimental striped bass. The epidermis had extensive coalescing foci of acute and subacute necrotizing dermatitis in which moderate numbers of Gram-negative bacilli were present. There was a loss of scales, edema of the dermis, and necrosis of dermal and subcutaneous fibrocytes; dermal vessels were proliferating, markedly dilated, congested, and lined by markedly hypertrophied, basophilic endothelial cells; some venules contained fibrin thrombi. Inflammatory cells consisted primarily of macrophages and lymphocytes surrounding vessels and infiltrating towards the ulcerated surface where they were degenerate and necrotic. Intense hyperplasia and hypertrophy of cardiac ventricular endothelium was evident. The spleens and pronephroi had hypertrophy of reticulo-en-

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TABLE 1. Phenotypic characteristics of the *Edwardsiella tarda* strain FL4-53 isolated from diseased striped bass in Chesapeake Bay.

Character	*E. tarda* strains FL4-53	9.8[a]
Gram	–	–
Motility (at 25 and 37 C)	+	+
Oxidase	–	–
Catalase	+	+
Oxidation (O)-Fermentation (F) (Glucose)	F	F
Methyl red	+	+
Voges-Proskauer	–	–
Indole production	+	+
Nitrate reduction	+	+
H_2S production	+	+
Citrate utilization	–	–
β-galactosidase (ONPG)	–	–
Reaction on Triple Sugar Iron agar	K/AG[b]	K/AG
Arginine dihydrolase	–	–
Lysine decarboxylase	+	+
Ornithine decarboxylase	+	+
Temperature range (C)	10–42	10–42
Salinity tolerance (% NaCl)	0–4	0–4
Acid from:		
Glucose	+	+
Arabinose	–	–
Cellobiose	–	–
Galactose	+	+
Mannose	+	+
Sucrose	–	–
Lactose	–	–
Rhamnose	–	–
Mellibiose	–	–
Trehalose	–	–
Inositol	–	–
D-mannitol	–	–
D-sorbitol	–	–
Esculin	–	–
Salicin	–	–
Amygdalin	–	–
Degradation of:		
Gelatin	–	–
Casein	–	–
Elastin	–	–
Tween 20, 40 & 80	–	–
Lecithin	–	–
DNA	–	–
Urea	–	–
Starch	–	–
Collagen	–	–
Hyaluronic acid	–	–
Hemolysis (sheep erythrocytes)	+β	+β

TABLE 1. Continued.

Character	*E. tarda* strains FL4-53	9.8[a]
Resistance/Sensitivity to (μg/disc):		
Ampicillin (10)	S (20)[c]	S (18)
Tetracycline (30)	S (28)	S (24)
Oxytetracycline (30)	S (30)	S (30)
Chloramphenicol (30)	S (28)	S (30)
Streptomycin (10)	R	R
Erythromycin (15)	R	R
Oxolinic acid (2)	S (30)	S (30)
Norfloxacine	S (40)	S (36)
Trimethoprim (23.75) sulphametoxazole (1.25)	S (22)	S (28)
Nitrofurantoin (300)	S (26)	S (24)
Furazolidone (100)	S (21)	S (20)

[a] Reference strain isolated from an epizootic in cultured striped bass.

[b] K, alkaline or no reaction; A, acid production; G, gas production.

[c] S, sensitive; R, resistant. In parentheses are indicated the inhibition halos in mm.

dothelial cells. Spleens, pronephroi, and livers had scattered foci of acute granulomatous necrosis. Stomachs had marked edema of the submucosa, and the intestinal mucosa had necrosis of the villous tips. Incidental lesions, including protozoan and metazoan parasites were few; encapsulated dead nematodes were present in the mucosa of the stomach and intestines of some fish. No internal or external trematodes, cestodes, protozoa, microsporidia or myxozoa were detected.

The *E. tarda* FL4-53 strain was highly pathogenic for striped bass, gilthead seabream and turbot with LD_{50} of 4×10^5, 7×10^5, and 3×10^5 cells, respectively. The inoculated strain was recovered in cultures from all the internal organs of dead and moribund experimental fish, as well as from survivors, evidence that the bacterium may have a capacity to establish a carrier state in experimental fish. The FL4-53 strain was not pathogenic for mice; the LD_{50} was $>10^8$ cells. Mucus-soaked discs failed to inhibit the growth of strain FL4-53 on TSA-2 agar.

At necropsy, moribund experimentally

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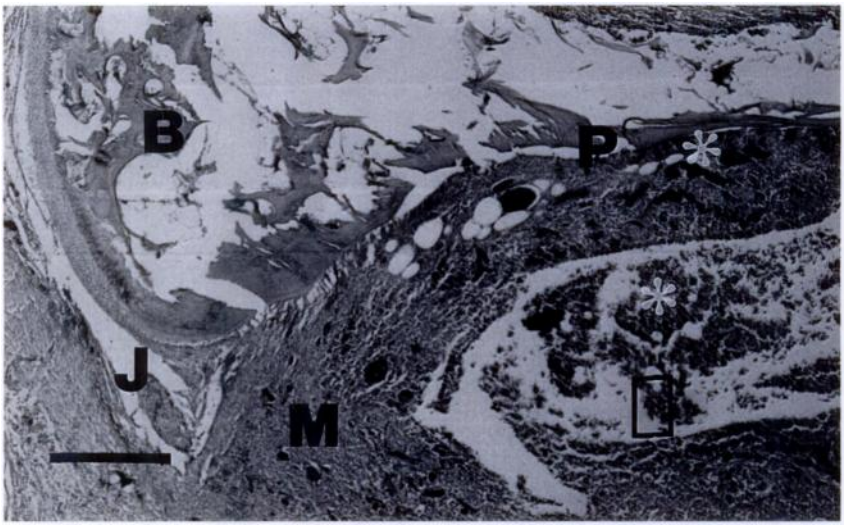


FIGURE 3. Bone (B), joint (J) and muscle of pectoral fin of a striped bass inoculated intramuscularly in the peduncle with *Edwardsiella tarda*. Massive cavitating necrosis and suppuration (asteriks) in the periosteum (P) and adjacent skeletal muscle (M) was diagnosed as periosteomyositis. Myocytic degeneration, necrosis, and suppuration projected superficially to the skin (not shown) around the pectoral fin. H&E, decalcification in saturated EDTA solution. Box is region in Fig. 4. Bar = 400 μm.

infected bass did not have all gross lesions of the natural disease. At the injection site, experimental striped bass had mild circular reddening and swelling of the skin surface; the underlying muscle was friable, reddish-brown, and malodorous. The spleens and pronephroi were moderately swollen. Meninges and coelomic membranes were diffusely hyperemic. White necrotic foci were not detected in internal organs. However, some experimental striped bass developed distinct foci of ulcerative and hemorrhagic dermatolysis at the base of the pectoral fins (Figs. 3 and 4). Histologically, longitudinal sections through affected pectoral bones had cavitating necrosis of muscles with extensive infiltrates of degenerate granulocytes and macrophages; suppurative and necrotic arthritis and periosteitis were prominent. Epidermal ulceration was marked. Many Gram-negative bacilli were present in inflammatory cells adjacent to the bone and joints, and extracellularly in regions of necrotic muscles. Bacilli were not detected in visceral organs, except for a very few in the ventricle of the brain adjacent to foci of acute minimal malacia.

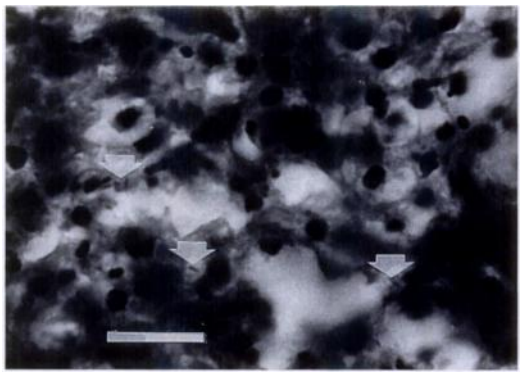


FIGURE 4. Degenerate inflammatory cells in core of cavitating suppurative periosteomyositis lesion show in box in Figure 3. Numerous intra- and extracellular Gram-negative bacilli (arrows) are present. Brown and Hopps Gram stain. Bar = 12 μm.

DISCUSSION

We report here the first description of an epizootic of edwardsiellosis affecting wild adult striped bass in the Chesapeake Bay. The bacterium, *E. tarda*, previously had not been associated with disease in wild fish in this region.

Based on taxonomic and serological

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characterization of the isolated bacterial strain from diseased wild striped bass, it belonged to the wild-type or classical biotype of *E. tarda*. Wild-type strains commonly are implicated in infections of animals and humans, while biogroup 1 has been recovered only from snakes and water (Sakazaki and Tamura, 1992; Holt et al., 1994).

The striped bass isolate (strain FL4-53) was pathogenic for three fish species; it had LD_{50} values in the range of those reported for channel catfish (*Ictalurus punctatus*) which are considered one of the most sensitive species to *E. tarda* infections (Amandi et al., 1982). We found a susceptibility to *E. tarda* infection in economically important estuarine fishes. At doses of 10^8, strain FL4-53 was not pathogenic to laboratory mice; bacteria are considered pathogenic in mice if the LD_{50} is $<10^7$ cells (Lupiani et al., 1993).

The resistance of this bacterium to the antimicrobial activity of skin mucus is evidence that the skin surface may not repel *E. tarda* strain FL4-53, and the skin may be an important route of infection and horizontal transmission in fish.

On histologic examinations of naturally infected striped bass, we observed lesions typical of bacterial septicemia. Although the lesions produced by *E. tarda* infections may vary according to the host species, age, and route of infection, multiple necrotic foci in internal organs have been reported in epizootics of edwardsiellosis in cultured striped bass (Herman and Bullock, 1986) and wild largemouth bass (*Micropterus salmoides*) (Francis-Floyd et al., 1993).

In our experimentally infected striped bass, skin, muscle, and joint lesions occurred far from the site of injection. Histological lesions in pectoral joints, periosteum and adjacent deep muscles were more extensive, longer in duration, and had more bacteria than the overlying skin and superficial muscles. This was evidence that the lesions of the joints and adjacent musculature preceeded development of skin ulcers; therefore, the inoculated *E. tarda* bacteria must have become bacteremic and were transported to pectoral joints via the circulatory system. In humans and domestic animals, the joints are a favorable site for localization and persistance of bacteria and other infectious microorganisms after clearance from the blood (Jubb et al., 1985). The skin ulcers adjacent to pectoral fins of experimentally inoculated bass were interpreted as extensions from the severe joint infections. Bacteria carried hematogenously to other internal organs, such as the liver, spleen, and pronephros, probably were effectively phagocytosed and destroyed. Wild fish, however, may be environmentally stressed, partially immunosuppressed by other infectious diseases, or may suffer continuous invasion of bacteria through wounds, and are thus less able to destroy hematogenous bacteria, resulting in widespread foci of necrosis and inflammation. Pathogenesis of *E. tarda* infections differs between species of host fish; in eels (*Anguilla anguilla*) the bacterium spreads from lesions in visceral organs to the musculature and then to the skin (with resulting ulcer formation), whereas in channel catfish, the infection originates in the skin and underlying muscles and then progresses to septicemia (Plumb, 1993).

Virulence factors of *E. tarda* in fish have been postulated to be dermatotoxins and cell-associated hemolysins (Janda and Abbott, 1993). Our results and previous reports (Waltman et al., 1986; Plumb, 1993) are evidence that this pathogen does not produce *in vitro* any other extracellular enzyme or toxin commonly detected in bacterial pathogens of fish (Thune et al., 1993; Toranzo and Barja, 1993).

While pound netted striped bass in two locations were confirmed culturally and histologically to be involved in this edwardsiellosis outbreak, it is not clear whether the pound nets contributed to the infections. The first reports of hemorrhagic ulcers on the body surface of 10 to 15% of wild striped bass were received from

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commercial pound netters in the Potomac River. Fisheries biologists of the Maryland Department of Natural Resources interviewed watermen and sport fisherpersons and determined that striped bass with red skin and ulcers had appeared as early as mid-August. Prevalence rose through September and involved bass in pound nets in both the Potomac River and near Taylors Island where infections were estimated at 25% and 40% respectively.

Recreational fisherpersons reported similar skin lesions in hook-and-line caught striped bass in the central Chesapeake Bay from August through November (Fig. 1). Although affected striped bass taken by hook-and-line, were not examined culturally, such angled fish could have been released or escaped from pound nets. Abrasions and overcrowding in pound nets may have contributed to this epizootic, but it is probable that other factors such as high water temperatures, salinity, high organic loads, and other unrecognized co-factors were involved.

Water temperature during the epizootic at three locations in the Chesapeake Bay ranged from 17 to 23 C. It is not known what portion of infected bass died, recovered, or became carriers after recovery. Sakai et al. (1994) recently found that *E. tarda* may remain in fresh and marine waters for extended periods in a viable but non-culturable form (dormant state). Favorable environmental conditions such as warm water or increased organic matter may contribute to the resuscitation of the bacterium and its potential to cause fish epizootics. Additional studies to track the spatial and temporal distribution of *E. tarda*, and to determine the presence of this bacterium in other resident species in the Chesapeake Bay are ongoing.

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