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Experimental *Edwardsiella ictaluri* Infection Causes Mortality in White Perch (*Morone americana*)

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Abstract: *Edwardsiella ictaluri*, the causative agent of Enteric Septicemia of Catfish (ESC) has been isolated from other fish species from ten families, but not white perch (*Morone americana*). In order to determine whether white perch could be experimentally-infected with *E. ictaluri*, white perch were captured from the Corsica River in Centreville, Maryland, USA, using a castnet. Four perch and 4 cultured channel catfish were experimentally challenged by intraperitoneal injection with 1.0×10^7 colony-forming units of *E. ictaluri*/fish. While channel catfish exhibited clinical signs characteristic of ESC, the white perch showed limited and non-specific clinical signs. All challenged fish died within 48 h and 97.5% of nare, brain, head kidney, intestine and posterior kidney samples from the catfish and perch produced growth on 5% de-fibrinated sheep blood agar. The colonies were formed by oxidase-negative, Gram-negative rods and biolog analysis identified the isolates as *E. ictaluri* (Probability = 99%; SI = 0.91). Despite some differences in clinical presentation, both the channel catfish and white perch were susceptible to *E. ictaluri* and died due to acute systemic infections. The results of this study demonstrated that white perch are experimentally susceptible to *E. ictaluri* and this study provides the first description of *E. ictaluri* infection in white perch.

Key words: Channel catfish, *Edwardsiella ictaluri*, *Ictalurus punctatus*, *Morone americana*, white perch

INTRODUCTION

Edwardsiella ictaluri, the causative agent of Enteric Septicemia of Catfish (ESC), receives considerable attention due to its negative impact on the production and harvest of channel catfish (*Ictaluri punctatus*). This bacterium was initially considered a specific pathogen of channel catfish and other fish in the Ictaluridae family (Hawke, 1979; Hawke *et al*., 1981; Plumb, 2000) (Table 1). This conclusion has been supported by several studies reporting fish species from non-Ictaluridae families as resistant to infection after experimental *E. ictaluri* challenge or exposure: golden shiner (*Notemigonus crysoleucas*), largemouth bass, (*Micropterus salmoides*) and bighead carp, (*Hypophthalmichthys nobilis*) (Plumb and Sanchez, 1983) and white sturgeon, (*Acipenser transmontanus*) (Baxa *et al*., 1990). Nonetheless, Wolters and Johnson (1994) suggested that the blue catfish (*I. furcatus*) is also resistant to *E. ictaluri* challenge. They demonstrated that channel catfish strains can vary in their susceptibility depending on families and body weights.

Some of these same authors and others have also documented disease susceptibility in natural and experimental *E. ictaluri* investigations of other unrelated fish species from seven families, including green knife fish (*Eigemannia virescens*) (Kent and Lyons, 1982) blue tilapia (*Oreochromis aureus*) (Plumb and Sanchez, 1983) Bengal danio (*Devario devario*) (Waltman *et al*., 1985; Blazer *et al*., 1985) Chinook salmon (*Oncorhynchus tshawytscha*), striped bass (*Morone saxatilis*) (Baxa *et al*., 1990) and iridescent shark (*Pangasius hypophthalmus*) (Crumlish *et al*., 2002) and in related fish species including walking catfish (*Clarias batrachus*) (Kasornchandra *et al*., 1987) European catfish (*Silurus glanis*) (Plumb and Hilge, 1987) and tadpole madtom (*Noturus gyrinus*) (Klesius *et al*., 2003). At least one species, rudd (*Scardinius erythrophthalmus*) (Popovic *et al*., 2001) has been found to harbor *E. ictaluri*.

White perch (*Morone americana*) is a popular North American food and game fish (Kohler, 2000) with a habitat extending from Nova Scotia, Canada to South Carolina, USA. While the white perch is native to areas like the

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Table 1: Species of fish previously reported Susceptible (S) or Non-Susceptible (NS) to *Edwardsiella ictaluri* according to family, common name, species and associated reference

Family/Common Name/Species	S	NS	Reference
Ictaluridae			
Channel catfish, *Ictalurus punctatus*	X[a]		Hawke, 1979*; Hawke *et al.*, 1981*; Wolters and Johnson, 1994
Blue catfish, *Ictalurus furcatus*		X[b]	Wolters and Johnson, 1994
White catfish, *Ameiurus catus*	X		Hawke *et al.*, 1981*
Brown bullhead, *Ameiurus nebulosus*	X		Hawke *et al.*, 1981*
Tadpole madtom, *Noturus gyrinus*	X		Klesius *et al.*, 2003*
Clariidae			
Walking catfish, *Clarias batrachus*	X		Kasornchandra *et al.*, 1987*
Siluridae			
European catfish, *Silurus glanis*	X		Plumb and Hilge, 1987
Pangasiidae			
Iridescent shark, *Pangasius hypophthalmus*	X		Crumlish *et al.*, 2002*
Cichlidae			
Blue tilapia, *Oreochromis aureus*	X		Plumb and Sanchez, 1983
Cyprinidae			
Bengal danio, *Devario devario*	X		Blazer *et al.*, 1985*; Waltman *et al.*, 1985*
Rosy barb, *Puntius conchonius*	X		Humphrey *et al.*, 1986*
Golden shiner, *Notemigonus crysoleucas*		X	Plumb and Sanchez, 1983
Bighead carp, *Hypophthalmichthys nobilis*		X	Plumb and Sanchez, 1983
Rudd, *Scardinius erythrophthalmus*	?[c]		Popovic *et al.*, 2001*
Centrarchidae			
Largemouth bass, *Micropterus salmoides*		X	Plumb and Sanchez, 1983
Salmonidae			
Rainbow trout, *Oncorhynchus mykiss*	X		Baxa *et al.*, 1990*
Chinook salmon, *Oncorhynchus tshawytscha*	X		Baxa *et al.*, 1990*
Sternopygidae			
Green knife fish, *Eigemannia virescens*	X		Kent and Lyons, 1982*
Moronidae			
Striped bass, *Morone saxatilis*	X		Baxa *et al.*, 1990*
White perch, *Morone americana*	X		This study
Acipeseridae			
White sturgeon, *Acipenser transmontanus*		X	Baxa *et al.*, 1990*
Serranidae			
European sea bass, *Dicentrarchus labrax*	X		Blanch *et al.*, 1990*

[a]Wolters and Johnson (1994) indicated significant variability in channel catfish strain susceptibility to experimental *E. ictaluri* challenge. [b]Wolters and Johnson (1994) considered blue catfish resistant to *E. ictaluri* after observing 0.7% mortalities among experimentally-challenged blue catfish. [c]Popovic *et al.* (2001) cultured *E. ictaluri* from rudd but did not comment on its susceptibility. [d]An asterisk next to a reference indicates that the paper included study of a natural *E. ictaluri* infection

Chesapeake Bay, they are a non-native, invasive species in the Midwestern USA. Though another popular food and game fish from the Moronidae family, striped bass (*Morone saxatilis*) (Baxa *et al*., 1990) has been reported as susceptible to *E. ictaluri*, the white perch has not been reported with *E. ictaluri* infection. The study presented here provides the first description of experimental *E. ictaluri* infection in white perch.

MATERIALS AND METHODS

Fish: White perch (*M. americana*; mean weight = 15.6 g) were captured from the Corsica River in Centreville, Maryland, USA, using a castnet and the fish were transported in a cooler with ambient river water. Approximate mean water quality parameters at the sampling sites were as follows: Temperature, 20.22°C; dissolved oxygen, 8.26 mg L^{-1}; salinity, 0.76% and pH, 8.36. The perch were housed at the Aquatic Animal Health Research Laboratory in Chestertown, Maryland, USA and they were held for 8 weeks before challenge to acclimate the fish to the laboratory water quality conditions. The fish were kept in 57 L aquaria supplied with flow-through de-chlorinated tap water, salt was added to maintain approximately 1% salinity and the fish were maintained on a 12 h : 12 h light: Dark period. Daily water temperature averaged 25.03±1.12°C, mean daily dissolved oxygen was 3.26±0.83 mg L^{-1}, mean daily total ammonia concentration was 0.59±0.38 mg L^{-1} and mean daily pH was 7.13±0.16. A two-week effort to induce perch to eat fish pellets was unsuccessful; therefore, the perch were subsequently fed every three days with live Nile tilapia (*O. niloticus*) fry hatched at the facility. National Warmwater Aquaculture Center (NWAC)-103 channel catfish (mean weight = 14.8 g) were also used in the challenge study; these fish had been held at approximately the same environmental conditions as the perch for 6 months prior to challenge.

Bacterial challenge: For the challenge experiment, *E. ictaluri* (ARS ID: EILO Thailand), originating from walking catfish (*C. batrachus*) was grown at 30°C for 24 h on 5% de-fibrinated Sheep Blood Agar (SBA; Remel, Lexena, Kansas, USA). The isolate had been frozen in a -78°C freezer and subsequently passed through channel catfish twice before it was grown for the challenge experiment. Four perch and four catfish were challenged by intraperitoneal injection with 1.0×10^7 colony-forming units of *E. ictaluri*/fish, the same or approximately the same dose used in *E. ictaluri* experimental infectivity studies conducted by Hawke (1979) Plumb and Sanchez (1983) Waltman *et al.* (1985) and Klesius *et al.* (2003). Fish were monitored daily for clinical signs of disease and mortality and moribund and dead fish were promptly removed. Samples for microbiologic examination were aseptically obtained from the nares, brain, head kidney, intestine and posterior kidney of all morbid and dead fish to confirm the presence of *E. ictaluri*; samples were cultured at 30°C for 24 h on 5% de-fibrinated sheep blood agar. Isolate identity was confirmed as *E. ictaluri* using the BIOLOG MicroLog3™ Microbial Identification System (BIOLOG, Hayward, California, USA) according to a modification of the manufacturer instructions (Arias *et al.*, 2003; Klesius *et al.*, 2003). BIOLOG results were obtained with the Gram-Negative (GN) Micrролog and User databases and a Similarity Index (SI) of >0.50 was considered a positive identification.

RESULTS AND DISCUSSION

Within 24 h after experimental challenge, all challenged channel catfish began to show clinical signs commonly associated with *E. ictaluri* infection: going off feed, lying on the bottom of the tank, severe lethargy, occasional spiral swimming throughout the water column, exophthalmia, petechial hemorrhages around the mouth, fins and abdomen and death (Hawke, 1979; Plumb and Sanchez, 1983). No fish exhibited cranial lesions or ascites accumulation. Two of the channel catfish died one day after challenge and the other two died 2 days after challenge. Three of the perch died one day after challenge and the one remaining fish died 2 days after challenge; the perch only exhibited three clinical signs: Mild lethargy, refusal to eat offered live prey and death. No gross external or internal lesions were observed in the perch. Nare, brain, head kidney, intestine and posterior kidney samples from all of the catfish and perch produced pure and significant growth on SBA, except one posterior kidney of a perch. The colonies were formed by oxidase-negative, Gram-negative rods and biolog analysis after 20 h incubation at 30°C identified the isolates as *E. ictaluri* (Probability = 99%; SI = 0.91).

Clinical signs of infected catfish were similar to those observed by other authors (Hawke, 1979; Areechon and Plumb, 1983). However, clinical signs in the perch were different than the catfish, mirroring limited or atypical clinical signs of *E. ictaluri* infection in other susceptible non-*I. punctatus* fish (Blazer *et al.*, 1985). Some authors have indicated that other susceptible species did not exhibit clinical signs during infection (Plumb and Hilge, 1987; Baxa *et al.,* 1990). Despite some differences in clinical presentation, both the channel catfish and white perch were susceptible to *E. ictaluri*. Based on the recovery of *E. ictaluri* from 97.5% of organs cultured, the catfish and perch died due to acute systemic infections.

A small number of bacterial species have been isolated from wild white perch, including *Pasteurella piscicida* now *Photobacterium damselae* subsp. *piscicida* from a fishkill in the Chesapeake Bay (Snieszko *et al.*, 1964; Janssen and Surgalla, 1968) and *Serratia marcescens* cultured during a survey of the Back River in Maryland, USA (Baya *et al.*, 1992). This is the first report of experimental *E. ictaluri* infection in *M. americana*, although it has been reported from a related family member *M. saxatilis* (Baxa *et al.*, 1990). Baxa *et al.* (1990) considered striped bass susceptible to *E. ictaluri* after observing one mortality among 20 experimentally-challenged striped bass, increased mean agglutination titers and histopathologic changes in the single striped bass mortality. It is possible that white perch may harbor and be susceptible to many of the infectious diseases reported from a closely related family member, striped bass. The results of this study demonstrated that white perch are experimentally susceptible to *E. ictaluri* and the apparent susceptibility may have been due to direct pathogenic mechanisms. However, since stress is highly correlated with *E. ictaluri* disease susceptibility (Sink *et al.*, 2006) laboratory water quality parameters and husbandry conditions may have played a role in infection despite the eight-week acclimation period.

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