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Susceptibility of channel catfish, blue catfish and channel × blue catfish hybrid to *Ichthyophthirius multifiliis*

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ABSTRACT

Information on the infectivity of *Ichthyophthirius multifiliis* (Ich), a severe fish parasite that causes high mortality, is limited for blue catfish (*Ictalurus furcatus*) and catfish hybrids (CB hybrid) resulting from female channel catfish (*Ictalurus punctatus*) × male blue catfish crosses. The objective of this study was to compare channel catfish, blue catfish and CB hybrids on the infection level and fish mortality caused by Ich using a cohabitation model. To compare the susceptibility to Ich between channel and blue catfish, fish were exposed to 5000 or 10000 theronts per fish, respectively. There were no statistical differences in the infection levels and mortalities between channel catfish and blue catfish. Channel catfish showed an infection score of 2.9 and blue catfish of 2.7 when infected by theronts at 10,000 theronts per fish. The cumulative mortalities were 86.3% and 80.6 %, respectively for channel catfish and blue catfish when exposed to theronts at 5000 theronts per fish. To compare the susceptibility to Ich between channel catfish and CB hybrid, fish were infected by cohabiting with 1 or 3 Ich-infected fish or exposure to Ich theronts at 2500–10,000 theronts per fish. Channel catfish and CB hybrid showed similar infection levels of >150 trophonts/fish and infection duration of 7–8 days when cohabited with 1 or 3 Ich-infected fish. All channel catfish and CB hybrid exposed to theronts at the dose of 10,000 theronts/fish showed heavy infection of >150 trophonts/fish. The cumulative mortalities were 90% and 80% for channel catfish and CB hybrid after exposure to 2500 theronts per fish. No statistical difference was found in the infection levels and mortalities using two infection methods between channel catfish and CB hybrid. Overall results indicated that CB hybrid were as susceptible to Ich as channel catfish or blue catfish.

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1. Introduction

Channel catfish (*Ictalurus punctatus* Rafinesque 1818) have been cultured for several decades as the dominant aquaculture species in the USA (Wolters and Johnson, 1994). Recently, an increasing number of producers are growing the hybrid catfish resulting from mating of female channel catfish × male blue catfish (*Ictalurus furcatus* Valenciennes 1840) (CB hybrid) instead of channel catfish (Small, 2006). Several researchers have reported that CB hybrids exhibit several commercially desirable characteristics, including faster growth, better feed conversion, tolerance of low oxygen, increased resistance to some diseases, and tolerance to crowded growth conditions in ponds (Giudice, 1966; Yant et al., 1976; Tave et al., 1981; Dunham and Smitherman, 1987).

Blue catfish are more resistant than channel catfish to some diseases, such as enteric septicemia of catfish (ESC) (Wolters et al., 1996), channel catfish virus (CCV) (Silverstein et al., 2008), Ambiphrya (Tidwell and Mims, 1990) and proliferative gill disease (PGD) (Bosworth et al., 2003), but are less resistant than channel catfish to *Flavobacterium columnare* (Dunham et al., 2008). CB hybrid exhibited intermediate resistance between blue catfish and channel catfish to ESC (Wolters et al., 1996), but showed no difference in resistance to CCV and PGD when compared to channel catfish (Bosworth et al., 2003; Silverstein et al., 2008).

Ichthyophthirius multifiliis Fouquet, 1876, referred to as "Ich", is one of the most severe fish parasites which infects most freshwater fish at every growth stage, from fry, juveniles to brood fish. The disease leads to high fish mortality and causes heavy economic losses for aquaculture (Paperna, 1972; Jessop, 1995; Traxler et al., 1998). The life stages of the parasite include an infective theront, a parasitic trophont and a reproductive tomont (MacLennan, 1935; Hines and Spira, 1974).

Studies have been conducted to evaluate the susceptibility of channel catfish to Ich, immune protection against Ich and treatment of the parasite (Goven et al., 1980; Straus, 1993; Dickerson, 2006). However, there is limited information on the infectivity of Ich for blue catfish and CB hybrid. Blue catfish were observed to be more vulnerable to Ich infestation when compared to channel catfish

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(Dunham et al., 1994), but no experimental data has been reported on the survival of blue catfish infected by Ich. In this study, we compared Ich infection level and mortality between channel catfish and blue catfish and between channel catfish and CB hybrid using a cohabitation model. Channel catfish and blue catfish or channel catfish and CB hybrid were put in the same tanks and subjected to the same treatment, including infection methods, parasite concentration, parasite infection duration, water quality, food and feeding time.

2. Materials and methods

2.1. Fish, water quality and parasite

Channel catfish (Industry Pool Strain) and hybrid catfish (Norris strain *I. punctatus* × D&B strain *I. furcatus*) were obtained from disease-free stock from the USDA-ARS Catfish Genetic Research Unit, Stoneville, MS. Blue catfish (*I. furcatus)* of the D&B strain were obtained from the Fish Genetics Research Unit of Auburn University, Alabama. These fish were reared to the experimental size at the USDA Aquatic Animal Health Research Laboratory, Auburn, Alabama. No parasites were detected in fish skin and gills under microscopic examination prior to each trial.

Fish were acclimated in 57-L aquaria supplied with flowing dechlorinated water at approximately 0.5 L min^{-1} for 1 week prior to trials. A light:dark period of 12:12 h was maintained, and aeration was supplied by air stones. The dissolved oxygen (DO) and temperature were measured using an YSI 85 oxygen meter (Yellow Spring Instrument, Yellow Springs, OH). The pH, hardness, and ammonia were determined using the CEL/890 Advanced Portable Laboratory (Hach, Loveland, Colorado). During the trials, the mean ± standard deviation of DO was 5.9 ± 1.7 mg/L, temperature was 22.6 ± 1.3 °C, pH was 7.0 ± 0.2, ammonia was 0.3 ± 0.1 mg/L and nitrate was below detectable level.

I. multifiliis was isolated from pet fish obtained from a local pet shop and maintained by continuous serial passages through channel catfish as previously described (Xu et al., 2004). This isolate was a serotype-D culture determined with immobilization assay using antiserum from fish Immunized to serotype-D Ich (Xu et al., 2004).

2.2. Cultured parasite theronts for infection trials

Four fish infected heavily by Ich were killed and put into a tank with 10 L water. The dead fish were removed from the tank 6 h after fish death. Most of Ich trophonts left the dead fish and attached to the walls or bottom of the tank. The tomonts were incubated at water temperature 22–24 °C for 18–20 h. Theront numbers were enumerated in five 1-mL samples of theront solution with the aid of a Sedgewick–Rafter cell by adding 1 drop of 1% formalin solution. Theront concentration was calculated as numbers of theronts per mL and theront solution was added to each tank to have precise numbers of theronts per fish for the infection trials.

2.3. Trial I—susceptibility of channel catfish and blue catfish to I. multifiliis

Fish were stocked into seven 57-L aquaria, with 18 channel catfish (8.3 ± 0.8 cm in length and 6.9 ± 1.3 g in weight) and 18 blue catfish (8.9 ± 0.5 cm and 7.2 ± 0.9 g) in each tank. There were two treatment groups with 3 tanks per group and one control group with one tank of fish. Fish in the two treatment groups were exposed to 5000 or 10,000 theronts per fish, respectively and the control group received no theronts. Two theront concentrations were used to infect fish based on the result from a primary trial in which fish were exposed to 5000, 10,000 and 25,000 theronts per fish. The water level was lowered to approximately 10 L in each tank. Theronts were added to each tank at the required concentration and fish were exposed to Ich theronts for 1 h. Flowing water was resumed to 0.5 L/min in each tank after one hour exposure to theronts. The fish infection and mortality were monitored daily for 2 weeks.

2.4. Trial II—susceptibility of channel catfish and CB hybrid to I. multifiliis

Ten tanks were used and fish were divided into 5 groups for this trial. Each group had two tanks with 16 channel catfish (11.6 ± 1.2 cm and 12 ± 2.7 g) and 16 CB hybrids (10.6 ± 0.5 cm and 10.8 ± 3.5 g) per tank. The fish were exposed to Ich by 2 infection methods, cohabitation with fish infected by Ich (Ich-infected fish) and showing visible spots or exposure to infective theronts. Fish in each group received one of following treatments: 1) cohabitation with an Ich-infected fish, 2) cohabitation with 3 Ich-infected fish, 3) exposure to Ich theronts at 2500 theronts per fish, 4) exposure to Ich theronts at 10,000 theronts per fish and 5) exposure to no parasite as control.

To avoid the misidentification of channel catfish with CB hybrid, calcein (a green-fluorescent dye) was used to mark channel catfish. One week prior to Trial II, 160 channel catfish were marked with calcein in two buckets. Calcein ($C_{30}H_{26}N_2O_{13}$, Sigma Chemical Co., St Louis, MO) was dissolved in 10-L water to make 400 mg/L calcein solution in 2 buckets. Eighty channel catfish were immersed in the calcein solution in each bucket with aeration for 4 h. Then, fish were washed with fresh tank water several times to remove excess calcein and moved to tanks with flowing water at 0.5 L/min to continue washing for 2 days. The marked fish were then distributed to each of ten aquaria with 16 fish per tank. CB hybrids were kept in the bucket with 10 L water while channel catfish were marked with calcein. To inspect calcein fluorescent marks on fish, anaesthetized fish or dead fish were placed in 150 mm × 25 mm Petri dishes (Corning Incorporated, Corning, NY) and viewed under an Olympus fluorescence inverted microscope (Olympus American Inc., New York, USA). Calcified skeletal structures, such as fins of calcein-marked catfish showed intense fluorescence (Klesius et al., 2006).

2.5. Evaluated parasite infection level and fish mortality

The infection levels by Ich were determined by the numbers of visible trophonts on each fish as described previously (Xu and Klesius, 2004). When fish showed visible white spots 5 days post theront challenge (dPTC) in Trial I, 5 channel catfish and 5 blue catfish in each aquarium were sampled to determine infection by the number of spots on the body surface of each fish, including head, skin and fins. The number of spots on the body surface of each fish was counted, and the infection level was assessed by assigning scores of 0, 1, 2, and 3 to fish that showed 0 trophonts/fish (no infection), <50 trophonts/fish, 50–150 trophonts/fish, and >150 trophonts/fish, respectively.

In Trial II, 3 catfish and 3 CB hybrids in each aquarium were sampled to determine infection by the number of spots on the body surface of each fish. Gill filament samples (5 × 5 mm) were cut from the opercula cavity on both sides of each fish (2 samples per fish). Gill samples were observed under a microscope, and the numbers of trophonts per sample were randomly counted for 2 viewing areas at 40× magnification (optical 10× and objective 4×), approximately 19.6 mm^2 per viewing area. Trophont loads in fish gills were expressed as the number of parasites per viewing area. Cumulative mortality of fish in each aquarium was recorded daily for 14 dPTC in Trial I and 21 dPTC in Trial II. The body surface and gills of newly deceased fish were examined for parasite infection using wet mount samples.

All experimental procedures involving fish were approved by the Institutional Animal Care and Use Committee of USDA Aquatic Animal Health Research Unit, Auburn, Alabama.

2.6. Statistical analysis

Median days to death (MDD) were calculated by SAS Lifetest procedure (Kaplan–Meier method) to express the survival time-span

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