



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), Ambi-

phrya (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 tomtom (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 $5.9 \pm 1.7 \text{ mg/L}$, temperature was $22.6 \pm 1.3 \text{ }^\circ\text{C}$, pH was 7.0 ± 0.2 , ammonia was $0.3 \pm 0.1 \text{ mg/L}$ 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 anti-serum 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 $22\text{--}24 \text{ }^\circ\text{C}$ 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 ($8.3 \pm 0.8 \text{ cm}$ in length and $6.9 \pm 1.3 \text{ g}$ in weight) and 18 blue catfish ($8.9 \pm 0.5 \text{ cm}$ and $7.2 \pm 0.9 \text{ g}$) 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 ($11.6 \pm 1.2 \text{ cm}$ and $12 \pm 2.7 \text{ g}$) and 16 CB hybrids ($10.6 \pm 0.5 \text{ cm}$ and $10.8 \pm 3.5 \text{ g}$) 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 ($\text{C}_{30}\text{H}_{26}\text{N}_2\text{O}_{13}$, 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 $150 \text{ mm} \times 25 \text{ mm}$ 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 ($5 \times 5 \text{ mm}$) 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 $40\times$ magnification (optical $10\times$ and objective $4\times$), 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

in fish following challenge. The infection levels, fish mortalities and MDD were compared by Paired-T test between channel and blue catfish or between channel catfish and CB hybrid in the same treatment group. The Duncan's multiple range tests were used to compare the infection levels and fish mortalities for different treatment groups (SAS Institute, 1989). Probabilities of ≤ 0.05 were considered statistically significant.

3. Results

3.1. Infection level on body surface of channel and blue catfish

White spots were seen on skin and fins of channel catfish and blue catfish at 4 dPTC. The spots became larger and more easily seen at 5 dPTC. More than 85% of channel catfish showed heavy infection (more than 150 spots per fish) when exposed to 5000 or 10,000 theronts per fish (Table 1). Fish exposed to the same concentration of theronts, only 53% of blue catfish showed heavy infection when exposed to 5000 theronts/fish and 63% became heavily infected when exposed to 10,000 theronts/fish. The average infection scores in channel catfish (2.8 and 2.9) were slightly higher than blue catfish (2.5 and 2.7) when infected by theronts at 5000 theronts/fish and 10,000 theronts/fish, respectively. However, the difference of mean infection scores did not reach statistical significance between channel and blue catfish ($p > 0.05$). Visible spots disappeared on the body surface of fish 12 dPTC. Channel catfish and blue catfish had the same appearance of white spots for up to 8 days for fish exposed to 5000 or 10,000 theronts per fish.

3.2. Mortality in channel and blue catfish

Fish showed mortality 6 days dPTC in all groups infected by theronts. When gills and body surface of newly deceased fish were examined, some mature trophonts and numerous young trophonts could be seen in wet mount samples, indicating that fish suffered heavy infection (Fig. 1). The majority of the fish died in 6 dPTC and the cumulative mortality increased until day 10 (Fig. 2). All fish died by 8 dPTC when exposed to theronts at 10,000 theronts per fish. The mortalities were lower in fish exposed to 5000 theronts/fish than fish exposed to 10,000 theronts/fish. The cumulative mortalities were 86.3% and 80.6%, respectively for channel catfish and blue catfish when exposed to 5000 theronts per fish. The MDD ranged from 6.1 ± 0.2 days for channel catfish to 6.4 ± 0.5 days for blue catfish exposed to 10,000 theronts/fish. There were no statistical difference in the cumulative mortalities and MDD between channel catfish and blue catfish. None of channel catfish or blue catfish died in the non Ich-infected control tanks.

Table 1

The infection level of *Ichthyophthirius multifiliis* (Ich) trophonts on fish body surface after 5 day exposure to Ich theronts on channel catfish and blue catfish. The infection level was assessed by assigning scores of 0, 1, 2, and 3 to fish that showed no infection, <50, 50–150, and >150 trophonts/fish, respectively. The mean infection score is the average infection score of 15 sampled fish from each group except control group (5 fish). Within a column, means followed by the same lower case letter are not statistically different ($P > 0.05$).

Theronts per fish	Fish	Numbers of fish with infection level				Mean infection score
		None	<50	51–150	>150	
0	Channel catfish	5	0	0	0	0 ^a
	Blue catfish	5	0	0	0	0 ^a
5000	Channel catfish	0	1	0	14	2.8 ^b
	Blue catfish	0	0	7	8	2.5 ^b
10,000	Channel catfish	0	1	1	13	2.9 ^b
	Blue catfish	0	1	4	10	2.7 ^b

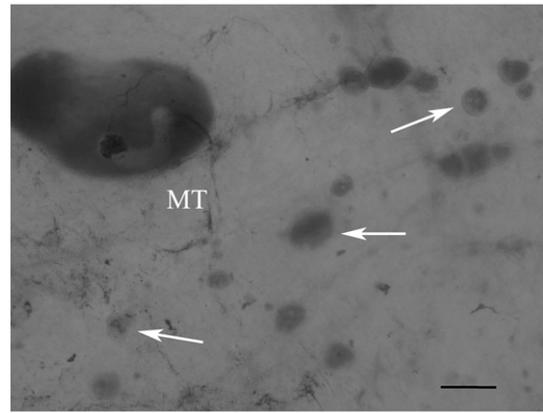


Fig. 1. A wet mount of skin mucus from a dead channel catfish shows a mature trophont of *Ichthyophthirius multifiliis* (Ich) with a typical “C” shaped macronucleus (MT) and many young trophonts (arrow). Catfish infected by exposure to Ich theronts and died 6 days post initial infection. The mature trophont was from the first infection cycle and young trophonts were from the second infection cycle. Scale bar = 140 μ m.

3.3. Infection level on body surface of channel catfish and hybrid catfish

On day 5 post cohabitation with Ich-infected fish, all channel catfish and CB hybrids became infected and showed visible white spots on the body surface, including head, skin and fins. Six channel catfish and 6 CB hybrids were sampled from each group and all fish showed an infection level of >150 trophonts/fish (Table 2). Visible spots disappeared on the body surface of channel catfish and CB hybrid 12 days post cohabitation with an Ich-infected fish and 13 days post cohabitation with 3 Ich-infected fish. Most of the fish showed visible spots for 7–8 days post cohabitation. Channel catfish and CB hybrid had similar spot appearance duration.

In fish exposed to theronts at the dose of 2500 theronts/fish, channel catfish and CB hybrid showed various infection levels from <50 trophonts/fish to >150 trophonts/fish (Table 2). All fish exposed to theronts at the dose of 10,000 theronts/fish had a heavy infection and showed an infection level of >150 trophonts/fish. There was no statistical difference on infection level on body surface in any group of channel catfish and CB hybrid. Visible spots disappeared on the body surface of fish 12 dPTC to 2500 theronts/fish or 13 dPTC to 10,000 theronts/fish. Channel catfish and CB hybrid had similar spot appearance duration from 8 to 9 days.

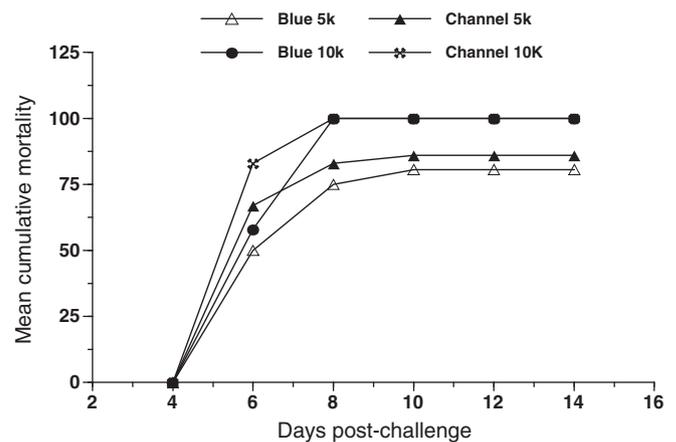


Fig. 2. Mean cumulative mortality of channel and blue catfish following exposure to *Ichthyophthirius multifiliis* at 5000 theronts/fish or 10,000 theronts/fish. Blue 5 k = blue catfish exposed to 5000 theronts/fish, Blue 10 k = blue catfish exposed to 10,000 theronts/fish, Channel 5 k = channel catfish exposed to 5000 theronts/fish, Channel 10 k = channel catfish exposed to 10,000 theronts/fish.

Table 2

The infection level of *Ichthyophthirius multifiliis* (Ich) on fish body surface after 5 day cohabitation with fish infected by Ich and showing visible spots or exposure to Ich theronts on channel catfish and CB hybrid (channel catfish × blue catfish). The infection level was assessed by assigning scores of 0, 1, 2, and 3 to fish that showed no infection, <50, 50–150, and >150 trophonts/ fish, respectively. The mean infection score is the average infection score of 6 fish sampled from each group. Within a column, means followed by the same lower case letter are not statistically different (P>0.05).

Infection methods	Doses	Fish	Numbers of fish with infection level				Mean infection score
			None	<50	51–150	>150	
Cohabitated	1 fish	Catfish	0	0	0	6	3 ^a
		Hybrid	0	0	0	6	3 ^a
	3 fish	Catfish	0	0	0	6	3 ^a
		Hybrid	0	0	0	6	3 ^a
Exposed to theronts	2500	Catfish	0	2	3	1	1.8 ^b
		Hybrid	0	1	4	1	2.0 ^b
	10,000	Catfish	0	0	0	6	3 ^a
		Hybrid	0	0	0	6	3 ^a
Non infected control	0	Catfish	6	0	0	0	0 ^c
		Hybrid	6	0	0	0	0 ^c

3.4. Parasite load in gills of channel catfish and hybrid catfish

Both channel catfish and CB hybrid catfish had higher number of trophonts in gills in fish cohabited with 3 Ich-infected fish than fish cohabited with an Ich-infected fish. An average of 50 ± 20 and 44 ± 23 trophonts per view were observed in channel catfish and CB hybrid cohabited with 3 Ich-infected fish, which were significantly higher than parasite loads of channel catfish (14 ± 8) and CB hybrid (17 ± 9) cohabited with an Ich-infected fish (Table 3). Fish had similar parasite counts in gills of channel and hybrid catfish exposed to theronts at 2500 theronts/fish or 10,000 theronts/fish. There was no statistical difference in parasite counts in gills between channel catfish and CB hybrid (p>0.05).

3.5. Mortality in channel catfish and CB hybrid

Both channel catfish and CB hybrid showed mortality on day 8 post cohabitation with an Ich-infected fish (Fig. 3). The cumulative mortalities continuously increased to day 14 post Ich cohabitation. The mortality was 80% in CB hybrid and 70% in channel catfish. The MDD was 9.2 and 11 days, respectively for CB hybrid and channel catfish when cohabited with an Ich-infected fish. When cohabiting with 3 Ich-infected fish, both channel catfish and CB hybrid began dying on day 6 post cohabitation. All of the CB hybrids died by day 10 and channel catfish by day 16 after cohabitation with 3 Ich-infected fish. The MDD was 7.7 days in CB hybrid and 9 days in channel catfish. There was no statistical difference in the mortalities and MDD for these two groups of fish.

Table 3

The infection level of *Ichthyophthirius multifiliis* (Ich) on gills after 5 day exposure to Ich on channel catfish and CB hybrid. The mean number of Ich per view is the average trophont numbers counted randomly from 24 viewing areas under microscope at 40× magnification, representing the mean from 12 gill samples collected from 6 fish in each group. Within a column, means followed by the same lower case letter are not significantly different. Within a row, means followed by the same upper case letter are not significantly different.

Infection methods	Doses	Numbers of view on gill samples	Mean numbers of Ich per view	
			Catfish	Hybrid
Cohabitated	1 fish	24	14 ± 8 ^{aA}	17 ± 9 ^{aA}
	3 fish	24	50 ± 20 ^{bA}	44 ± 23 ^{bA}
Exposed to theronts	2500	24	36 ± 16 ^{cA}	39 ± 12 ^{bA}
	10,000	24	37 ± 14 ^{cA}	44 ± 15 ^{bA}
Non infected control	0	24	0 ± 0 ^{dA}	0 ± 0 ^{cA}

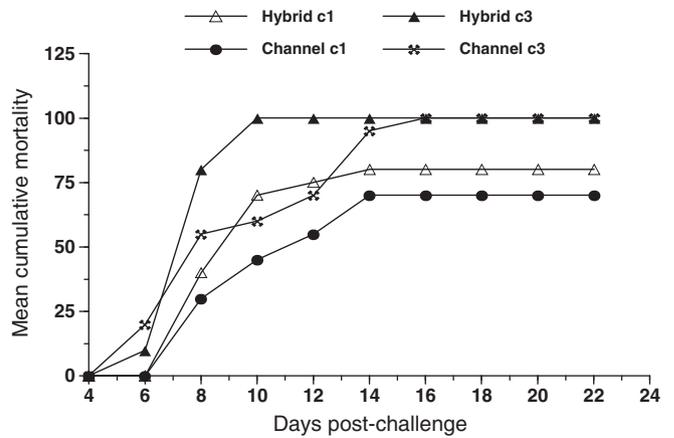


Fig. 3. Mean cumulative mortality of channel catfish and CB hybrid after cohabiting with fish infected by *Ichthyophthirius multifiliis* and showed visible spots (Ich-infected fish). Channel c1 = channel catfish cohabiting with an Ich-infected fish, Channel c3 = channel catfish cohabiting with 3 Ich-infected fish, Hybrid c1 = CB hybrid cohabiting with 1 Ich-infected fish, Hybrid c3 = CB hybrid cohabiting with 3 Ich-infected fish.

When fish were infected with theronts, mortalities increased greatly from day 10 to day 12 dPTC at the dose of 2500 theronts per fish (Fig. 4). The cumulative mortality reached 90% for channel catfish and 80% for CB hybrid. The MDD was 10.8 days in CB hybrid and 11 days in channel catfish. Fish death increased rapidly from day 6 to 12 when fish were exposed to 10,000 theronts per fish. The mortalities were 85% for channel catfish and 100% for CB hybrid. The MDD was 9.1 and 9.7 days, respectively in CB hybrid and channel catfish. There was no statistical difference in the mortalities and MDD for these two groups of fish. None of either the channel catfish or CB hybrid died in the control tank without theront exposure.

4. Discussion

In Trial I, channel catfish and blue catfish were exposed to *I. multifiliis* in the same tanks at the concentration of 5000 or 10,000 theronts per fish. Both channel catfish and blue catfish were susceptible for Ich infection. These two species showed no difference in parasite infection level, fish mortalities and median days to death. These results does not support the impression that blue catfish is more susceptible to Ich infestation than channel catfish. It is possible

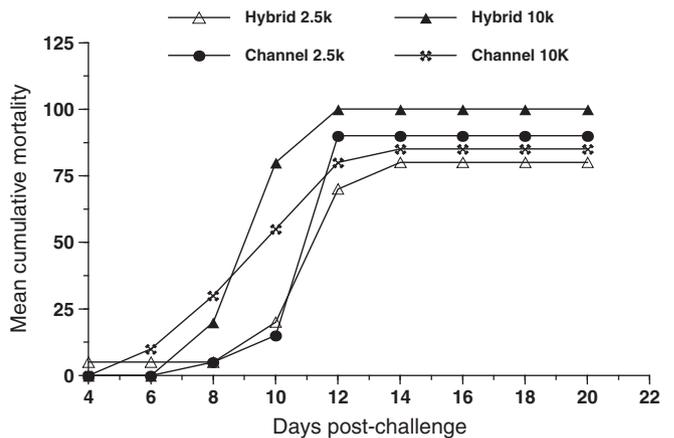


Fig. 4. Mean cumulative mortality of channel catfish and CB hybrid following exposure to *Ichthyophthirius multifiliis* at 2500 theronts/fish or 10,000 theronts/fish. Channel 2.5 k = channel catfish exposed to 2500 theronts/fish, Channel 10 k = channel catfish exposed to 10,000 theronts/fish, Hybrid 2.5 k = CB hybrid exposed to 2500 theronts/fish, Hybrid 10 k = CB hybrid exposed to 10,000 theronts/fish.

that “white spots” are more visible on blue catfish than on channel catfish due to a sharper color contrast that led to the impression. Channel catfish and CB hybrid were tested for their susceptibility to Ich by two infection methods with two concentrations in this trial. Channel catfish showed slightly higher mortality (90%) than CB hybrid (80%) when exposed to 2500 theronts/fish. CB hybrid showed slightly higher mortalities (80% and 100%) than channel catfish (70% and 85%) when cohoused with an Ich-infected fish or exposed to 10,000 theronts/fish. These differences on mortalities, however, were not significant. No statistical differences were noted in parasite infection level, fish mortalities, MDD and parasite infection duration between channel catfish and CB hybrid. Variation in susceptibility to Ich infection has been reported among fish species (Matthews, 2005; Dickerson, 2006). In a previous study, Nile tilapia were found more resistant to Ich infection than channel catfish and fewer tilapia were killed when these two species were subjected to heavy Ich infection (Xu and Klesius, 2006).

The majority of fish mortalities occurred at the second infection cycle of Ich in the trials. When fish were infected with theronts, they showed visible mature trophonts (white spots) 4–5 dPTC. The mature trophonts left fish and divided into infective theronts in water, indicating the start of second infection cycle. It usually took 5–6 days to complete an infection cycle at water temperature of 22–25 °C. A single Ich tomtom could divide and release several hundreds to thousands of infective theronts (Dickerson, 2006). This increment of theront numbers led to heavy infection. The infected fish then died due to the heavy infection of Ich. When moribund or newly deceased fish were examined, some mature trophonts from the first infection cycle and many young trophonts from the second infection cycle were observed to co-exist on the body surface.

The cohabitation challenge model was used in this study to evaluate fish infection by Ich between channel and blue catfish or between channel catfish and CB hybrid. Most studies that evaluated infections against fish pathogens have been conducted using different fish species challenged in separate units (Wolters and Johnson, 1994; Bosworth et al., 2003; Silverstein et al., 2008). Evaluation of infection within different fish species in separate units encounter difficulties in controlling variation between experimental units, such as the number of infective pathogens, exposure time, temperature, water quality, volume of flowing water, and amount of feed provided. In this study, channel catfish and blue catfish or channel catfish and CB hybrid were put in the same tanks, thereby decreasing the chance for variation between experimental units. The cohabitation models have been successfully used for evaluating protective immunity between immunized and non-immunized fish held in the same rearing unit (Nordmo, 1997; Klesius et al., 2006; Xu et al., 2007).

Channel catfish and CB hybrid can be differentiated easily when they are alive, but not after death. To avoid the misidentification of channel catfish and CB hybrid, channel catfish were marked by calcein before the trial. In a previous study, the calcein marking was demonstrated to have no effect on the susceptibility of channel catfish to Ich theronts (Xu et al., 2007). No difference was noted in fish infection level, mortality, and MDD caused by Ich between unmarked fish and fish marked with calcein regardless of concentration. In the trial to compare fish infection by Ich between channel catfish and CB hybrid, two infection methods were used. One method consisted of exposure of fish to known amounts of infective theronts for a defined length of time. This method enabled easy management of the number of theronts used and exposure duration, thereby providing acceptable challenge results. This challenge method is commonly used in immunization studies of Ich (Dickerson et al., 1981; Lin et al., 1996; Dalgaard et al., 2002; Xu et al., 2004). The second infection method places 1 or 3 Ich-infected fish in tanks with the channel catfish and the CB hybrids. After 5–7 days at 22–25 °C, the infected fish showed visible white spots on their body surface. Because there was a great variation in the number of trophonts on the infected fish and the

number of infective theronts released into the water, the theront concentration per fish is less easily to control. In this study, this factor was not a major concern since both channel catfish and CB hybrid were put in the same tanks and exposed to the same amount of infective theronts. Cohabitation of channel catfish and CB hybrid in the same tank minimized the effect of variation in trophont numbers from Ich-infected fish. This infection method is similar to pond or wild conditions since channel catfish and CB hybrid directly contact the infected fish carrying trophonts.

There are many difficulties in comparing two fish species for susceptibility to Ich under pond conditions. Some environmental factors are hard to control, such as parasite concentration, infection methods, water quality and water supply. Infection progress in fish is also hard to monitor, including infection level, fish pathological changes, infection duration and fish mortality. It becomes a problematic to eliminate effects of other pathogens if they co-existed in the same ponds. These pathogens could be primary pathogens which directly cause fish mortality or secondary pathogens which enhance Ich infection and then lead to higher mortality of fish. Laboratory trials using the cohabitation model overcome some of these disadvantages. Many important events associated with the two species can be monitored continuously and easily, such as infection level, fish pathological changes, infection duration and fish mortality.

In summary, channel catfish, blue catfish and CB hybrid were all susceptible to Ich infestation. No difference was noted in fish infection level and mortality between channel catfish and blue catfish following exposure to different concentrations of infective theronts. Similarly, there was no observed difference in the infection level and mortality between channel catfish and CB hybrid following exposure by cohabitation with fish infected by Ich or contacting infective theronts. Further studies are needed to verify species susceptibility to Ich infestation among channel catfish, blue catfish and CB hybrids under pond conditions.

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