2012

Susceptibility of three strains of blue catfish, *Ictalurus furcatus* (Valenciennes), to *Ichthyophthirius multifiliis*

D-H Xu
USDA-ARS, dehai.xu@ars.usda.gov

P.H. Klesius
USDA-ARS, phillip.klesius@ars.usda.gov

Brian G. Bosworth
USDA–ARS, bbosworth@ars.usda.gov

N Chatakondi
USDA-ARS, nagaraj.chatakondi@ars.usda.gov

Journal of Fish Diseases 2012, **35**, 887–895 doi:10.1111/j.1365-2761.2012.01436.x

Susceptibility of three strains of blue catfish, *Ictalurus furcatus* (Valenciennes), to *Ichthyophthirius multifiliis*

D-H Xu[1], P H Klesius[1], B G Bosworth[2] and N Chatakondi[2]

1 USDA-ARS, Aquatic Animal Health Research Laboratory, Auburn, AL, USA
2 USDA-ARS, Catfish Genetics Research Unit, Thad Cochran National Warmwater Aquaculture Center, Stoneville, MS, USA

Abstract

This study compared the susceptibility of three blue catfish strains (D&B, USDA 101 and USDA 102) to the parasite *Ichthyophthirius multifiliis* (Ich). In Trial I, a cohabitation study (all strains stocked communally) was conducted and fish were exposed to theronts at 0, 200, 1000, 5000 or 25 000 theronts fish^{-1}, respectively. All fish died when exposed to theronts at 5000 or 25 000 theronts fish^{-1}. When exposed to 1000 theronts fish^{-1}, USDA 102 strain of blue catfish showed significantly lower mortality (78.5%) compared to USDA 101 and D&B strains (92.7% and 100%). In Trial II, the same three strains of blue fish were evaluated for their susceptibility to Ich with strains challenged in separate tanks by adding Ich theronts at 0, 200 and 1000 theronts fish^{-1}, respectively. All D&B and USDA 101 blue catfish died; however, 42.3% of USDA 102 strain survived the infection when exposed to 1000 theronts per fish. The results indicate that there are differences among strains of blue catfish for susceptibility to Ich, and these differences will be useful in the development of improved catfish germplasm for commercial aquaculture.

Keywords: blue catfish, cohabitation, *Ichthyophthirius multifiliis*, infection, mortality, susceptibility.

Introduction

The channel catfish, *Ictalurus punctatus* (Rafinesque), is the principal warm water species grown in the United States. Channel catfish posses a number of desirable qualities for aquaculture, including rapid growth, ease of spawning, tolerance to a wide range of temperatures and water quality, good product quality and high consumer acceptance of the product (Li *et al.* 2009). However, channel catfish are susceptible to certain species-specific diseases that cause millions of dollars in losses annually (Dunham *et al.* 1993; Hawke & Khoo 2004). Recently, an increasing number of producers have shown an interest in growing the hybrid catfish resulting from the mating of channel catfish × blue catfish, *I. furcatus* (Valenciennes) (Small 2006; Li *et al.* 2009). Large-scale field trials have confirmed the outstanding performance of the hybrids in commercial settings and in processing plants, resulting in high demand and premium prices for fingerling hybrids (Chatakondi, Yant & Dunham 2005). Hybrid catfish have the potential to replace the channel catfish that is predominantly used in the catfish industry (Dunham & Argue 2000). To meet the rapid expansion of hybrid production, it is necessary to evaluate disease resistance of blue catfish because the parental strain of blue catfish may affect the performance of the hybrid.

Ichthyophthirius multifiliis (Ich) is one of the most prevalent protozoan parasites of freshwater fish. The parasite causes high mortality in many fish when reared under intensive conditions and leads to heavy economic losses in aquaculture (Paperna 1972; Traxler, Richard & McDonald 1998). The parasite has a life cycle consisting of an infective theront, a parasitic trophont and a reproductive tomont (MacLennan 1935; Nigrelli, Pokorny & Ruggieri 1976). Infective theronts swim actively in water in search of hosts (MacLennan 1935). After burrowing into fish epithelium, theronts become trophonts and feed on host

Correspondence *D-H Xu, USDA-ARS, Aquatic Animal Health Research Laboratory, 990 Wire Road, Auburn, AL 36832, USA (e-mail: dehai.xu@ars.usda.gov)*

Journal of Fish Diseases

tissue until they reach maturity (McCartney, Fortner & Hansen 1985). The mature tomont drops off the host, attaches to substrates and undergoes multiple divisions to produce theronts (MacLennan 1935). The parasite spreads rapidly from fish to fish as a single Ich tomont can produce hundreds to thousands of infective theronts in less than a day (MacLennan 1935; Matthews 2005). The classic sign of an Ich infection is the presence of small white spots on the skin and gills (Dickerson 2006). Heavy infection by Ich damages fish skin and gills, causes loss of the respiratory, excretory and osmoregulatory functions, and eventually leads to fish death (Hines & Spira 1973; Dickerson 2006).

Many treatments for Ich have been evaluated, including external chemical treatments (Tieman & Goodwin 2001), oral therapeutants (Shinn *et al.* 2003) and non-chemical treatments (Farley & Heckmann 1980). These treatments aim at killing of infective theronts and the reproductive tomonts in water, blocking theronts from infecting hosts or eliminating parasitic trophonts from fish tissues. Control of Ich by using chemical treatments and oral therapeutants is difficult, costly or not effective after the Ich penetrates fish skin and gills (Tieman & Goodwin 2001; Shinn *et al.* 2003). Non-chemical treatments for Ich included water ozonation, radiation with ultraviolet light, submicron filtration, electrotherapy, increased water flow and temperature extremes (Farley & Heckmann 1980; Tieman & Goodwin 2001). None of the physical treatments are effective or economical for treating Ich, especially trophonts in fish tissues. Vaccination against Ich has been evaluated, and many studies demonstrated that fish developed immune protection against the parasite following immunization with Ich antigens (Hines & Spira 1973; Houghton & Matthews 1990; Dickerson & Clark 1998; Sigh & Buchmann 2001; Xu, Klesius & Shelby 2004). However, most of these studies are laboratory trials, and no commercial vaccine for Ich has yet been developed. Genetic improvement of disease resistance through selective breeding could be an alternative way to prevent fish diseases (Fjalestad, Gjedrem & Gjerde 1993).

Studies have been conducted to compare disease resistance between channel and blue catfish for viruses (Silverstein, Bosworth & Gaunt 2008), bacteria (Wolters, Wise & Klesius 1996; Dunham *et al.* 2008) and parasites (Tidwell & Mims 1990; Bosworth *et al.* 2003; Xu *et al.* 2011). However, there is no information available for the susceptibility of different strains of blue catfish to the parasite Ich. This study compared the susceptibility of three strains of blue catfish (D&B, USDA 101 and USDA 102) to Ich infection using both cohabitation and separate-tank methods. In the cohabitation method (Trial I), three strains of blue catfish were put in the same tank and subjected to the same treatment, including exposure to the same amount of Ich theronts, same water quality, same food and feeding time during the trial. In the separate-tank method (Trial II), three strains of blue fish were evaluated for susceptibility to Ich in separate tanks. Infection level, survival time-span and mortality were compared among three strains of blue catfish in both trials.

Materials and methods

Fish and water quality

Three strains of blue catfish, D&B, USDA 101 and USDA 102, were shipped from disease-free stock from the USDA-ARS Catfish Genetic Research Unit (CGRU), Stoneville, MS. The D&B strain of catfish is a strain commonly used in commercial aquaculture. D&B strain catfish at the USDA catfish genetics research unit were originally obtained from Dycus Farms, Arkansas. The USDA 101 and 102 blue catfish strains are currently being evaluated at the CGRU and were developed from fish obtained from the Missouri River Basin and Mississippi River Basins, respectively. Fingerlings from each strain used in challenges represent a pool of approximately equal numbers of fish from 10 to 15 full-sib families. Fish were acclimatised in six 400-L tanks supplied with flowing dechlorinated water at approximate 0.8 L min^{-1} (two tanks per strain of blue catfish) at the USDA-ARS, Aquatic Animal Health Research Laboratory, Auburn, Alabama for 2 week before the trials. During the acclimatisation period, fish were fed AquaMax Fingerling Starter 300 (PMI Nutrition International, LLC) daily until satiation.

During trials, dissolved oxygen (DO) and temperature in tanks were measured daily using a YSI 85 oxygen meter (Yellow Spring Instruments). The pH, hardness, ammonia and nitrite were determined using a Hach CEL/890 Advanced Portable Laboratory. The mean ± MSE of DO was 6.3 ± 0.4 mg L^{-1}, temperature was 23.2 ± 0.8 °C, pH was 6.9 ± 0.2, ammonia was 0.24 ± 0.2 mg L^{-1}

Published 2012.

and hardness was 89.3 ± 4.2 mg L^{-1}. Nitrite concentrations were below the detection limit.

Parasite and theront for infection trials

Ichthyophthirius multifiliis was originally isolated from infected pet fish obtained from a local pet shop and maintained by serial passages on channel catfish held in 50-L glass aquaria as previously described (Xu *et al.* 2004).

Trophonts were collected, and theronts were cultured as previously described (Xu *et al.* 2011). Briefly, fish heavily infected with maturing trophonts were anaesthetized with 150 mg L^{-1} tricaine methanesulphonate (MS-222), rinsed in tank water and the skin was gently scraped to dislodge the parasites. For evaluation effect of Alizarin red S (ArS) on Ich infection, isolated trophonts were placed in Petri dishes with fresh tank water (Xu *et al.* 2004). For infection trials, trophonts were placed in a tank with 20-L water and incubated at 22–24 °C for 18 h. Theronts were enumerated with a Sedgewick –Rafter cell after adding one drop of 1% formalin solution. Theront concentration was calculated as numbers of theronts mL^{-1}, and theront solution was added to each tank to have precise numbers of theronts per fish for the infection trials.

Marked blue catfish in Trial I

One week prior to Trial I, 140 blue catfish from the USDA 102 strain were marked with calcein in two buckets. Calcein ($C_{30}H_{26}N_2O_{13}$; Sigma Chemical Co.) was dissolved in 10 L water to make 400 mg L^{-1} calcein solution in each bucket (Xu *et al.* 2011). Seventy blue catfish were immersed in the calcein solution in each bucket with aeration for 4 h. Similarly, 140 D&B strain of blue catfish were marked with alizarin red S (ArS) as reported by Bashey (2004). Alizarin red S ($C_{14}H_7NaO_7S$; Sigma) was dissolved in 10 L water to make 400 mg L^{-1} ArS solution in each of two buckets. Seventy fish from D&B strain were immersed in 10-L ArS solution with aeration for 4 h. The USDA 101 strain of blue catfish was not marked, and 140 fish were kept in two buckets with 10 L water each as controls, while other blue catfish were marked with calcein or ArS. After marking, the fish were washed several times with tank water to remove excess fluorescent dyes (calcein or alizarin red S) and moved to tanks with flowing water at 0.5 L min^{-1} to continue washing for 2 days.

To check fluorescent marking on fish, anaesthetized fish or dead fish were placed in 150 × 25 mm Petri dishes (Corning Incorporated) and viewed under an Olympus 1X70 inverted fluorescence microscope equipped with a 10× objective lens and fitted with an Olympus DP11 digital camera (Olympus American Inc.). Calcified skeletal structures, such as fins of blue catfish, showed intense green fluorescence when marked with calcein or pink fluorescence when marked with ArS (Fig. 1).

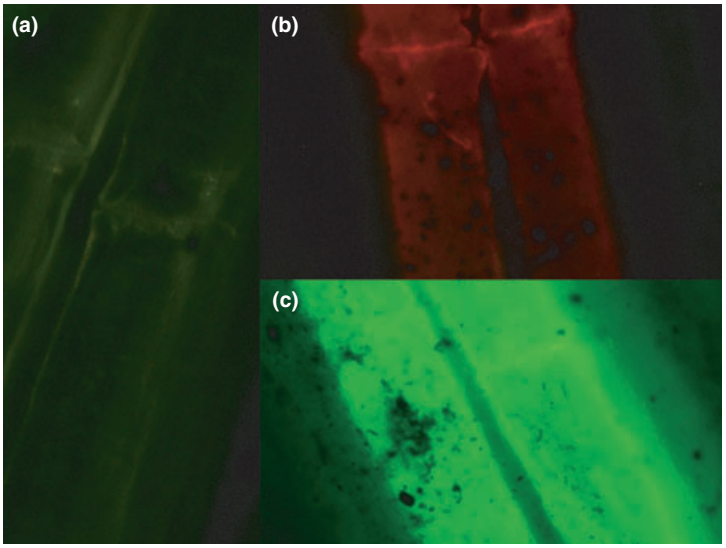

Figure 1 Photomicrographs of the caudal fins of blue catfish: (a) unmarked USDA 101 strain; (b) alizarin-marked D & B strain and (c) calcein-marked USDA 102 strain. These images were taken with the inverted fluorescence microscope equipped with an Olympus DP11 digital camera.

Effect of Alizarin red S on Ich theronts

In a previous study, marking fish with calcein was demonstrated to have no effect on the susceptibility of channel catfish to Ich theronts. No difference was noted in fish infection level, mortality and median days to death (MDD) caused by Ich, between unmarked fish and fish marked with calcein regardless of concentration (Xu, Klesius & Shoemaker 2007). A toxicity test was conducted to evaluate the effect of ArS on Ich theronts before marking fish with ArS. The ArS solutions were made at the concentration of 0, 25, 50, 100, 200 and 400 mg L^{-1} in a 24-well plate with 0.5 mL per well (Corning Costar). A 0.5 mL theront solution with approximately 2000 theronts was added to each well to yield 0, 12.5, 25, 50, 100 and 200 mg L^{-1} of ArS, respectively. Theronts were exposed to each concentration in triplicate wells, and 10 μL of sample was collected from each well at 0, 1, 2 and 4 h post-ArS treatment. Ten microlitres of sample was added to a Sedgewick–Rafter cell marked with a grid of 1000 × 1 mm squares, and a cover glass was placed over the counting chamber. All live theronts were enumerated under an Olympus BH-2 microscope at low magnification (4× object and 10× eye) and theront concentration was calculated as numbers of theronts mL^{-1} in each well. Non-moving theronts with non-beating cilia were considered dead.

Trial I evaluating susceptibility of blue catfish to *I. multifiliis* using cohabitation method

A cohabitation method was used to evaluate susceptibility of blue catfish strains to Ich in Trial I. A total of 378 fish were distributed to eighteen 57-L flow-through aquaria supplied with flowing dechlorinated water at approximately 0.4 L min^{-1} with seven fish from each strain of blue catfish (D&B, USDA 101 and USDA 102), a total of 21 fish per tank. Calcein and ArS were used to mark USDA 102 and D&B, respectively, as described above. Ten fish from each strain of blue catfish were inspected for parasites, and fish length and weight were measured prior to the trial (Table 1). Fish were acclimatised in the aquaria to laboratory conditions for 1 week before the trial.

Theronts were added to tanks, and blue catfish were exposed to theronts at 0, 200, 1000, 5000 or 25 000 theronts per fish for 1 h. There were four replicate tanks for each parasite concentration and two tanks for non-infected controls, accounting for a total of 18 tanks. The parasite infection levels were evaluated, and mortality of fish in each tank was recorded daily for 28 days post-theront challenge as indicated below.

Table 1 Average length and body weight of blue catfish used in trials

Blue catfish strains	Trial I		Trial II	
	Length (cm)	Weight (g)	Length (cm)	Weight (g)
D&B	12.3 ± 0.2	14.2 ± 0.5	14.4 ± 0.4	26.2 ± 1.5
USDA 101	12.5 ± 0.2	16.8 ± 0.9	14.6 ± 0.2	25.6 ± 1.2
USDA 102	12.7 ± 0.3	16.0 ± 1.4	15.5 ± 0.3	34.0 ± 2.9

Values are mean length and weight ± standard error from 10 fish.

Trial II evaluating susceptibility of blue catfish to *I. multifiliis* using the separate-tank method

In Trial II, three strains of blue catfish were put in different tanks. The infection level and mortality in different strains of blue catfish were evaluated and compared in separated tanks. A total of 390 blue catfish were distributed to 57-L aquaria with 15 fish per tank. There were 18 tanks supplied with flowing dechlorinated water at approximate 0.4 L min^{-1} for three strains of blue catfish and two parasite concentrations with triplicate tanks per treatment. Another six tanks were used as non-infected controls, two tanks for each strain of blue catfish. Ten fish from each strain of blue catfish were inspected for parasites, and fish length and weight was measured prior to the trial (Table 1). The blue catfish were challenged by exposing fish to Ich theronts at 0, 200 or 1000 theronts $fish^{-1}$ for 1 h to evaluate susceptibility to Ich. Infection level and mortality of fish in each tank were determined as follows.

Evaluated parasite infection level and fish mortality

Fish were examined for infection when trophonts were visible at 5 days post-theront challenge. In Trial I, two tanks of fish were evaluated for infection level in relative to each theront concentration. Fish were anaesthetized in water with 100 mg L^{-1} MS-222, placed on Petri dishes and the strain identified by viewing the marked caudal fin under an inverted fluorescence microscope. Then, numbers of trophonts on the fish skin and fins were evaluated

using a semi-quantitative scale. The infection level was assessed by assigning scores of 0, 1, 2, 3 and 4 to fish that showed no infection, <50, 50–150, 151–300 and >300 trophonts fish^{-1}, respectively. In Trial II, all fish were removed from each tank and parasitic infection was evaluated in a 2-L beaker individually. The fish were then returned to the tank to recover, and survival was evaluated. The mortality of fish in each tank was recorded daily after theront challenge. For newly dead fish, the body surface and gills were examined for parasitic infection using wet mount samples (Xu *et al.* 2011). A small sample of mucus was scraped from the body surface of each fish. Gill filament samples were cut from the opercular cavity on both sides of each fish. Skin and gill samples were observed under a microscope for Ich infection.

Statistical analysis

All data were analysed with SAS software (SAS Institute 1989). Median days to death were used to express the survival time-span in fish following Ich infection and were calculated by the Lifetest procedure (Kaplan–Meier method). The Kolmogorov–Smirnov test was used to test normality for experimental data. Fish size and theront survivals after exposing to then alizarin *in vitro* test were analysed with analysis of variance. The infection scores and MDD were analysed by maximum likelihood using SAS Genmod procedure. The fish mortality data were not normally distributed so mortality data was transferred to the natural logarithm number and analysed with Poisson regression (Stokes, Davis & Koch 2000). P value of 0.05 or less was considered statistically significant.

Results

Fish marking and effect of Alizarin red S on Ich theronts

In Trial I, USDA 102 and D&B strain were marked to distinguish strains of blue catfish cohabited in the same tank. The marked fish were easily identified under UV light. Alizarin-marked D&B strain showed pink florescence and calcein-marked USDA 102 strain showed strong green florescence in fins compared to unmarked USDA 101 strain (Fig. 1).

An *in vitro* test was conducted to evaluate the effect of Alizarin red S on Ich theront survival. No toxic effect of alizarin was noted on theronts.

Table 2 Number of *Ichthyophthirius multifiliis* (Ich) theronts (mL^{-1}) surviving in each well of 24-well plate post-exposure to alizarin red S at different concentration and exposure time

Alizarin red concentration (mg L^{-1})	Mean number of surviving Ich theronts mL^{-1}			
	0 h	1 h	2 h	4 h
0	1900 ± 458	1833 ± 267	2233 ± 338	1967 ± 233
25	1933 ± 425	2266 ± 785	2366 ± 317	2300 ± 513
50	1767 ± 348	1333 ± 202	1900 ± 305	2167 ± 333
100	1400 ± 153	2500 ± 501	2230 ± 845	2333 ± 504
200	1167 ± 267	1300 ± 305	2067 ± 536	2133 ± 883

Theronts showed high survival when exposed to alizarin at 200 mg L^{-1} for 4 h. There was no statistical difference ($P > 0.05$) between theronts treated with alizarin at 200 mg L^{-1} for 4 h and non-treated theronts (Table 2).

Susceptibility of blue catfish to *I. multifiliis* in Trial I using cohabitation method

There was no statistical difference ($P > 0.05$) on the length and weight among three strains of blue catfish in Trial I. Most fish were infected when exposed to theronts at 200 or 1000 theronts per fish and showed <50 visible spots per fish (infection scores ranged from 0.79 to 1.14). USDA 102 strain of blue catfish showed lower infection level than D&B strain (Table 3). Fish showed light (<50 spots fish^{-1})-to-moderate infection (50–150 spots fish^{-1}) when exposed to 5000 theronts fish^{-1}. Fish had heavy infection when exposed to 25 000 theronts fish^{-1}. All fish showed 151–300 visible trophonts. No difference was noted on infection level among three strains of blue catfish in the same tank when exposed to 5000 or 25 000 theronts fish^{-1}.

All fish died when exposed to theronts at 5000 or 25 000 theronts fish^{-1} (Table 4). These fish died with a MDD 7–9 days when exposed to 25 000 theronts fish^{-1} or 11–13 days when exposed to 5000 theronts fish^{-1}. No difference was noted on mortalities and MDD for the three strains of blue catfish when fish were exposed to 5000 or 25 000 theronts fish^{-1}. When exposed to 200 theronts fish^{-1}, USDA 101 and 102 strains of blue catfish showed significantly lower mortality (18.0% and 17.8%) compared to the D&B strain (35.5%). When exposed to 1000 theronts fish^{-1}, the mortality for USDA 102 strain was 78.5%, significantly lower than USDA 101 strain (92.7%) or D&B strain (100%).

Table 3 The infection level of *Ichthyophthirius multifiliis* (Ich) on body surface of blue catfish 5 days post-exposure to theronts. The infection level was assessed by assigning scores of 0, 1, 2 and 3 to fish that showed no infection, <50, 50–150 and 151–300 trophonts fish^{-1}, respectively. The infection score is the average infection score of 14 fish sampled from each blue catfish strain exposed to different concentrations of theronts

Theronts per fish	Blue catfish strains	Numbers of fish with infection level				Infection score
		None	<50	50–150	151–300	
200	D&B	1	13	0	0	0.93 ± 0.07^{a}
	USDA 101	2	12	0	0	0.86 ± 0.10^{ab}
	USDA 102	3	11	0	0	0.79 ± 0.11^{b}
1000	D&B	0	12	2	0	1.14 ± 0.09^{c}
	USDA 101	0	14	0	0	1.00 ± 0^{cd}
	USDA 102	1	13	0	0	0.93 ± 0.07^{d}
5000	D&B	0	10	4	0	1.29 ± 0.13^{e}
	USDA 101	0	9	5	0	1.36 ± 0.13^{e}
	USDA 102	0	11	3	0	1.21 ± 0.11^{e}
25 000	D&B	0	0	0	14	3.00 ± 0^{e}
	USDA 101	0	0	0	14	3.00 ± 0^{e}
	USDA 102	0	0	0	14	3.00 ± 0^{e}

Within a column for each theront concentration, means (±standard error) followed by the same lower case letter are not statistically different ($P > 0.05$). No infection was observed for control fish exposed to no theronts.

Table 4 The cumulative mortality and median days to death (MDD) of blue catfish after exposure to theronts of *Ichthyophthirius multifiliis* (Ich) in Trial I. Three strains of blue catfish (seven fish per strain) were cohabited in the same tank and subjected to the same treatments. Four replicated tanks were used for fish exposed to each concentration of theronts, and two tanks were used as non-infection controls

Theronts per fish	Blue catfish strains	Number of dead fish	Mortality (%)	MDD
0	D&B	0 (14)	0 ± 0^{a}	NA
0	USDA 101	0 (14)	0 ± 0^{a}	NA
0	USDA 102	0 (14)	0 ± 0^{a}	NA
200	D&B	10 (28)	35.5 ± 20.5^{b}	18.5 ± 0.2^{a}
200	USDA 101	5 (28)	18.0 ± 10.8^{c}	18.4 ± 0.5^{a}
200	USDA 102	5 (28)	17.8 ± 7.8^{c}	18.3 ± 0^{a}
1000	D&B	28 (28)	100 ± 0^{d}	15.1 ± 1.1^{b}
1000	USDA 101	26 (28)	92.7 ± 7.3^{d}	14.9 ± 1.2^{b}
1000	USDA 102	22 (28)	78.5 ± 12.4^{e}	14.6 ± 0.9^{b}
5000	D&B	28 (28)	100 ± 0^{d}	11.0 ± 0.4^{c}
5000	USDA 101	28 (28)	100 ± 0^{d}	10.7 ± 0.5^{c}
5000	USDA 102	28 (28)	100 ± 0^{d}	12.5 ± 1.6^{c}
25 000	D&B	28 (28)	100 ± 0^{d}	8.1 ± 0.5^{d}
25 000	USDA 101	28 (28)	100 ± 0^{d}	6.7 ± 1.1^{d}
25 000	USDA 102	28 (28)	100 ± 0^{d}	7.9 ± 0.4^{d}

NA, not available.

Total number of blue catfish from each strain exposed to different theront concentration is shown in parentheses. Within a given column for each theront concentration, means (±standard error) followed by different superscript letters are statistically different ($P < 0.05$).

Susceptibility of blue catfish to *I. multifiliis* in Trial II using separate-tank method

There was no statistical difference ($P > 0.05$) in the length and weight among three strains of blue catfish in Trial II. Most fish were infected and had an infection level with <50 spots fish^{-1} when exposed to 200 theronts fish^{-1}. The infection scores were lower in fish exposed to 200 theronts (0.93–0.97) per fish than those exposed to 1000 theronts (1.26–1.40) per fish (Table 5). The infection level increased greatly from 5 days post-infection to 10 days post-infection. No difference was noted on infection levels among three strains of blue catfish when exposed to the same concentration of Ich theronts except that the USDA 102 strain showed lower infection scores

Table 5 The infection level of *Ichthyophthirius multifiliis* (Ich) on body surface of blue catfish 5 or 10 days post-exposure to theronts in Trial II. The infection level was assessed by assigning scores of 0, 1, 2, 3 and 4 to fish that showed no infection, <50, 50–150, 151–300 and >300 trophonts fish^{-1}, respectively. The mean infection score is the average infection score of (*N*) fish sampled from each blue catfish strain exposed to the same concentration of theronts

Theronts per fish	Blue catfish strain	5 days post-infection		10 days post-infection	
		N	Infection score	*N*	Infection score
0	D&B	20	0 ± 0^{a}	20	0 ± 0^{a}
	USDA 101	20	0 ± 0^{a}	20	0 ± 0^{a}
	USDA 102	20	0 ± 0^{a}	20	0 ± 0^{a}
200	D&B	30	0.97 ± 0.03^{b}	30	2.93 ± 0.05^{b}
	USDA 101	30	0.97 ± 0.03^{b}	30	3.10 ± 0.09^{b}
	USDA 102	30	0.93 ± 0.05^{b}	30	2.90 ± 0.06^{b}
1000	D&B	30	1.40 ± 0.09^{c}	30	4.00 ± 0^{c}
	USDA 101	30	1.30 ± 0.09^{c}	30	3.97 ± 0.03^{c}
	USDA 102	30	1.26 ± 0.08^{c}	30	3.77 ± 0.08^{d}

Within a column for each theront concentration, means (±standard error) followed by the same lower case letter are not statistically different ($P > 0.05$).

Published 2012.

Table 6 The cumulative mortality and median days to death (MDD) of blue catfish after exposure to theronts of *Ichthyophthirius multifiliis* (Ich) in Trial II. The strains of blue catfish were kept in separate tanks and fish mortality was observed for 28 days post-challenge with Ich

Theronts per fish	Blue catfish strain	Number of dead fish	Mortality (%)	MDD
0	D&B	0 (30)	0 ± 0[a]	NA
0	USDA 101	0 (30)	0 ± 0[a]	NA
0	USDA 102	0 (30)	0 ± 0[a]	NA
200	D&B	10 (45)	22.3 ± 11.8[b]	18.3 ± 0.4[a]
200	USDA 101	26 (45)	57.7 ± 29.8[c]	17.2 ± 1.3[a]
200	USDA 102	8 (45)	18.0 ± 9.0[b]	17.8 ± 1.0[a]
1000	D&B	45 (45)	100 ± 0[d]	12.9 ± 0.3[b]
1000	USDA 101	45 (45)	100 ± 0[d]	11.8 ± 0.2[b]
1000	USDA 102	26 (45)	57.7 ± 12.2[c]	12.9 ± 1.4[b]

NA, not available.

Total number of blue catfish from each strain exposed to different theront concentration was shown in parentheses. Within a given column for each theront concentration, means (±standard error) followed by different superscript letters are statistically different ($P < 0.05$).

than the other two strains 10 days post-exposure to 1000 theronts fish^{-1}.

When exposed to 1000 theronts fish^{-1}, all D&B and USDA 101 blue catfish died with a MDD 12–13 days (Table 6). 42.3% of USDA 102 strain survived the infection at 1000 theronts fish^{-1}. When exposed to 200 theronts fish^{-1}, USDA 102 strain and D&B stain exhibited lower mortality (18% and 22.3%) than USDA 101 blue catfish (57.7%).

Discussion

All strains of blue catfish died when exposed to 5000 or 25 000 theronts fish^{-1} in Trial I. The fish exposed to 25 000 theronts fish^{-1} showed heavy infection with 151–300 visible trophonts fish^{-1} in the first infection cycle (5 days post-theront exposure). Fish were only exposed to two low concentrations of theronts (200 or 1000 theronts fish^{-1}) in Trial II because all fish died in Trial I when exposed to 5000 theronts fish^{-1} or higher. The USDA 102 blue catfish strain had 78.5% mortality in the cohabitation challenge Trial I and 57.7% mortality in the separate infection Trial II when exposed to 1000 theronts fish^{-1}. Almost all fish ($\geq$92.7%) from D&B or USDA 101 strains died in both trials when exposed to 1000 theronts per fish. The fish of USDA 102 were slightly larger than the other two strains in Trial II, but did not reach a statistical difference ($P > 0.05$). Differences in fish size may have influence on parasite resistance and larger fish may be more resistant to Ich infection. However, it is unlikely that the size of the fish led to the low mortality of USDA 102 blue catfish in this study. The USDA 102 blue catfish showed similar length and weight compared to D&B and USDA 101 blue catfish in Trial I. Among three blue catfish strains, only the USDA 102 strain had survivors in two different challenge trials when exposed to 1000 theronts fish^{-1}.

The MDD was inversely related to theront concentrations. When exposed to high theront concentrations, fish showed a short MDD. In this study, blue catfish showed an MDD of MDD 6.7–8.1 days when exposed to 25 000 theronts fish^{-1}. Most fish in this group died in the beginning of the second Ich infection cycle. At water temperature of 22–25 °C, Ich can complete an infection cycle in 5–6 days (Nigrelli *et al.* 1976; Dickerson 2006). Mature trophonts from the first cycle dropped off fish and developed into infective theronts in water. When large number of theronts attached to fish and penetrated into fish skin and gills, the re-infection of the parasite caused most fish to die. When recently dead fish were examined for Ich infection using wet mounts from skin mucus and gill filaments, almost all fish contained heavy parasite infection with few matured trophonts and many young trophonts. The MDD increased to 10.7–12.5 days when fish were exposed to 5000 theronts fish^{-1}. Most fish in this group died at the end of the second Ich infection cycle. Blue catfish could get infected by 200 theronts fish^{-1} but most of the fish survived the infection. The blue catfish demonstrated a much longer MDD in both trials (17.2–18.5 days) when exposed to 200 theronts fish^{-1}.

Two methods were used in current trials to compare the susceptibility of D&B, USDA 101 and USDA 102 strains to Ich. In Trial I, the cohabitation method was used and three strains of blue catfish were put in the same tank. As three strains of blue catfish had the same treatment in each tank, the cohabitation method decreased the chance for variation between experimental groups. The cohabitation method has been successfully used for evaluating protective immunity between immunized and non-immunized fish held in the same rearing groups (Nordmo 1997; Klesius *et al.* 2006; Xu *et al.* 2007). The cohabitation method is considered one of the best models for evaluating protective immunity, because immunized and

non-immunized fish are held in the same rearing group (Nordmo 1997; Klesius *et al.* 2006), thereby decreasing the chance for variation between experimental group, such as the number of infective pathogens, exposure time, temperature, water quality, volume of flowing water and amount of feed provided. The cohabitation method had many advantages in this trial, but the Ich infection levels were not easy to monitor among strains of blue catfish in the same tank. Fish had to be taken out from each tank to identify the strain relative to the marker. This problem can be addressed by using the separate-tank method. This trial took advantages of both methods and made evaluation of susceptibility to Ich more objective.

Several chemicals are available for mass marking of fish by immersion: calcein, tetracycline, alizarin, strontium and lanthanides (Bashey 2004). Marking fish using calcein or alizarin red S by immersion is easily performed for large numbers of fish within a short time (Bashey 2004; Xu *et al.* 2007). Both calcein and alizarin did not affect the appearance of fish and could be detected on the skeleton of live, anaesthetized or dead fish (Bashey 2004). In this study, theronts showed no difference in survival *in vitro* test when exposed to alizarin at concentrations from 25 to 200 mg L^{-1} for 4 h compared to control theronts (not exposed to alizarin). Using the markers calcein and alizarin, three strains of blue catfish could be put in the same tank to evaluate the susceptibility to Ich in the cohabitation method.

The information on the susceptibility of blue catfish to Ich would be helpful to blue catfish culture and the breeding programme of catfish hybrids. Ich is a significant parasite of many fish species including catfish. However, as noted earlier, effective chemical or physical means of treating Ich on large populations of farmed catfish are either ineffective or not economically feasible. Therefore, an alternative approach to reducing Ich in catfish is to breed for fish with improved resistance to the parasite. The results of this study demonstrate differences among strains of blue catfish relative to infection levels and mortality following Ich challenges. The data from two different Ich challenge methods were consistent, which strengthens the validity of the observed strain differences in fish relative to mortality. It is possible the differences in Ich susceptibility observed among blue catfish strains will be transmitted to their hybrid offspring, which is particularly important as hybrids are rapidly becoming a significant proportion of US catfish production.

Acknowledgements

This work was supported by USDA-ARS CRIS no. 6420-32000-024-00D. The authors thank Debbie Boykin, USDA Agricultural Research Service and Dr John Zhang, Centers for Disease Control and Prevention for their assistance on statistical analysis. We also thank Curtis Days and Jana Mladek for technical support and caring of fish. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

References

Bashey F. (2004) A comparison of the suitability of alizarin red S and calcein for inducing a nonlethally detectable mark in juvenile guppies. *Transactions of the American Fisheries Society* **133**, 1516–1523.

Bosworth B.G., Wise D.J., Terhune J.S. & Wolters W.R. (2003) Family and genetic group effects for resistance to proliferative gill disease in channel catfish, blue catfish and channel catfish × blue catfish backcross hybrids. *Aquaculture Research* **34**, 569–573.

Chatakondi N., Yant D.R. & Dunham R.A. (2005) Commercial production and performance evaluation of channel catfish × blue catfish F_1 hybrids. *Aquaculture* **247**, 1–4.

Dickerson H.W. (2006) *Ichthyophthirius multifiliis* and *Cryptocaryon irritans* (Phylum Ciliophora). In: *Fish Diseases and Disorders, Vol. 1, Protozoan and Metazoan Infections*, 2nd edn (ed. by P.T.K. Woo), pp. 116–153. CAB International, Wallingford, UK.

Dickerson H. & Clark T. (1998) *Ichthyophthirius multifiliis*: a model of cutaneous infection and immunity in fishes. *Immunological Reviews* **166**, 377–394.

Dunham R.A. & Argue B.J. (2000) Reproduction among channel, blue catfish and their F1 and F2 hybrids. *Transactions of the American Fisheries Society* **129**, 222–231.

Dunham R.A., Hyde C., Masser M., Smitherman R.O., Perez R., Plumb J. & Ramboux A.C. (1993) Comparison of culture traits of channel catfish, *Ictalurus punctatus* and blue catfish, *I. furcatus*. *Journal of Applied Aquaculture* **3**, 257–267.

Dunham R.A., Umali G.M., Beam R., Kristanto A.H. & Trask M. (2008) Comparison of production traits of the NWAC103 channel catfish, NWAC103 channel × blue hybrid catfish, Kansas select 21 channel catfish and blue catfish grown at commercial densities and exposed to natural

Published 2012.

bacterial epizootics. *North American Journal of Aquaculture* **70**, 98–106.

Farley D. & Heckmann R. (1980) Attempts to control *Ichthyophthirius multifiliis* Fouquet (Ciliophora: Ophryoglenidae) by chemotherapy and electrotherapy. *Journal of Fish Diseases* **3**, 203–212.

Fjalestad K.T., Gjedrem T. & Gjerde B. (1993) Genetic improvement of disease resistance in fish: an overview. *Aquaculture* **111**, 65–74.

Hawke J.P. & Khoo L.H. (2004) Infectious diseases. In: *Biology and Culture of Channel Catfish* (ed. by C.S. Tucker & J.A. Hargreaves), pp. 387–443. Elservier Publishers, Amsterdam, The Netherlands.

Hines R.S. & Spira D.T. (1973) *Ichthyophthirius multifiliis* (Fouquet) in the mirror carp, *Cyprinus carpio* L. I. Course of infection. *Journal of Fish Biology* **5**, 385–392.

Houghton G. & Matthews R.A. (1990) Immunosuppression in juvenile carp, *Cyprinus carpio* L.: the effects of the corticosteroids triamcinolone acetonide and hydrocortisone 21-hemisuccinate (cortisol) on acquired immunity and the humoral antibody response to *Ichthyophthirius multifiliis* Fouquet. *Journal of Fish Diseases* **13**, 269–280.

Klesius P.H., Evans J.J., Shoemaker C.A. & Pasnik D.J. (2006) A vaccination and challenge model using calcein marked fish. *Fish & Shellfish Immunology* **20**, 20–28.

Li M.H., Robinson E.H., Tucker C.S., Oberle D.F. & Bosworth B.G. (2009) Comparison of channel and blue catfish fed diets containing various levels of protein. *Journal of the World Aquaculture Society* **39**, 646–655.

MacLennan R.F. (1935) Observations on the life cycle of *Ichthyophthirius,* a ciliate parasitic on fish. *Northwest Science* **9**, 12–14.

Matthews R.A. (2005) *Ichthyophthirius multifiliis* Fouquet and ichthyophthiriosis in freshwater teleosts. *Advances in Parasitology* **59**, 159–241.

McCartney J.B., Fortner G.W. & Hansen M.F. (1985) Scanning electron microscopic studies of the life cycle of *Ichthyophthirius multifiliis*. *Journal of Parasitology* **71**, 218–226.

Nigrelli R.F., Pokorny K.S. & Ruggieri G.D. (1976) Notes on *Ichthyophthirius multifiliis*, a ciliate parasitic on freshwater fishes, with some remarks on possible physiological races and species. *Transactions of the American Microscopical Society* **95**, 607–613.

Nordmo R. (1997) Strengths and weaknesses of different challenge methods. In: *Fish Vaccinology* (ed. by R. Gudding, A. Lillehaug, P.J. Midtlyng & F. Brown), pp. 303–309. Karger Publishers, Basel.

Paperna I. (1972) Infection by *Ichthyophthirius multifiliis* of fish in Uganda. *Progressive Fish Culturist* **34**, 162–164.

SAS Institute. (1989) *SAS/Stat User's Guide, Version 6*, 4th edn. SAS Institute, Cary, NC.

Shinn A.P., Wootten R., Cote I. & Sommerville C. (2003) Efficacy of selected oral chemotherapeutants against *Ichthyophthirius multifiliis* (Ciliophora: Ophyroglenidae) infecting rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms* **55**, 17–22.

Sigh J. & Buchmann K. (2001) Comparison of immobilization assays and enzyme linked immunosorbent assays for detection of rainbow trout antibody-titres against *Ichthyophthirius multifiliis* Fouquet. *Journal of Fish Diseases* **24**, 49–51.

Silverstein P.S., Bosworth B.G. & Gaunt P.S. (2008) Differential susceptibility of blue catfish, *Ictalurus furcatus* (Valenciennes), channel catfish, *I. punctatus* (Rafinesque), and blue × channel catfish hybrids to channel catfish virus. *Journal of Fish Diseases* **31**, 77–79.

Small B.C. (2006) Improvements in channel catfish growth after two generations of selection and comparison of performance traits among channel catfish, blue catfish, and hybrid catfish fingerlings in an aquarium rack system. *North American Journal of Aquaculture* **68**, 92–98.

Stokes M.E., Davis C.S. & Koch G.G. (2000) *Categorical Data Analysis Using the SAS System*, 2nd edn. SAS Institute, Cary, NC.

Tidwell J.H. & Mims S.D. (1990) A comparison of second-year growth of blue and channel catfish in Kentucky. *Progressive Fish-Culturist* **52**, 203–204.

Tieman M. & Goodwin A.E. (2001) Treatments for Ich infections in channel catfish evaluated under static and flow-through water conditions. *North American Journal of Aquaculture* **63**, 293–299.

Traxler G.S., Richard J. & McDonald T.E. (1998) *Ichthyophthirius multifiliis* (Ich) epizootics in spawning sockeye salmon in British Columbia, Canada. *Journal of Aquatic Animal Health* **10**, 143–151.

Wolters W.R., Wise D.J. & Klesius P.H. (1996) Survival and antibody response of channel catfish, blue catfish, and channel catfish female × blue catfish male hybrids after exposure to *Edwardsiella ictaluri*. *Journal of Aquatic Animal Health* **8**, 249–254.

Xu D.H., Klesius P.H. & Shelby R.A. (2004) Immune responses and host protection of channel catfish, *Ictalurus punctatus* (Rafinesque), against *Ichthyophthirius multifiliis* after immunization with live theronts and sonicated trophonts. *Journal of Fish Diseases* **27**, 135–141.

Xu D.H., Klesius P. & Shoemaker C. (2007) Evaluation of a cohabitation challenge model in immunization trials for channel catfish, *Ictalurus punctatus* (Rafinesque), against *Ichthyophthirius multifiliis*. *Diseases of Aquatic Organisms* **74**, 49–55.

Xu D.H., Klesius P.H., Peatman E. & Liu Z. (2011) Susceptibility of channel catfish, blue catfish and channel × blue catfish hybrid to *Ichthyophthirius multifiliis*. *Aquaculture* **311**, 25–30.

Received: 27 July 2011
Revision received: 31 January 2012
Accepted: 6 February 2012