Disease Resistance of Different Genetic Types of Channel Catfish, *Ictalurus punctatus*, Female × Blue Catfish, *I. furcatus*, Male Hybrid Catfish

by

Sheng Dong

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
May 7, 2016

Keywords: Disease Resistance; Hybrid Catfish; Channel Catfish; *Flavobacterium columnare*; *Edwardsiella ictaluri*;

Copyright 2016 by Sheng Dong

Approved by

Rex A. Dunham, Chair, Professor, School of Fisheries, Aquaculture and Aquatic Sciences
Jeffery S. Terhune, Associate Professor, School of Fisheries, Aquaculture and Aquatic Sciences
Charles Chen, Associate Professor, Department of Crop, Soil and Environmental Sciences

Abstract

Eleven genetic types, Kansas Random × Rio Grande, Kansas Select × Rio Grande, 103KS × Rio Grande, Marion Select × Rio Grande, 103 KS × B, Marion Select × B, Auburn-Rio Grande × B, 103KS × D&B, Kansas Random × D&B, Marion Random × D&B, and Kansas Select × D&B, of channel catfish, *Ictalurus punctatus*, female × blue catfish, *I.furcatus*, male hybrid catfish were challenged with virulent *Flavobacterium columnare* in tanks. Sex and the interaction between sex and genotype did not affect the disease resistance. The genetic type was the key factor associated with columnaris resistance of hybrid catfish. There was no apparent sire effect, but there was a significant ($P<0.05$) dam effect on mortality, median death time and average survival times. 103KS had the best combining ability to produce hybrids with the best columnaris resistance. The best strategy to improve disease resistance of hybrid catfish is to identify female strains with the best combining ability. Potential genetics of these dam effects requires further study.

Two genetic types of hybrids and one genetic type of channel catfish performed similarly in regards to mortality and survival time when challenged with *Edwardsiella ictaluri*, causative agent of enteric septicemia of catfish (ESC). However, the channel catfish had the largest death rate. The experiment was complicated by co-infections with both ESC and *Ichthyophthirius multifiliis* (Ich). The mortality and survival time of the two types of ich infected hybrid catfish were similar, while the death rate and extent of death for the channel catfish were the highest. The difference in ESC resistance of the hybrid catfish and channel catfish were not as great as seen in earlier experiments. This may be a result of genotype-environment interactions from the intensity of the infection or the multiple infections. Additionally, the channel catfish used in this study was an intraspecific crossbred in channel catfish and may have improved disease resistance compared to channel catfish in previous comparisons. Intraspecific crossbreeding may be an alternative to interspecific hybridization for enhancement of disease resistance and should be further explored.

Acknowledgements

First and foremost, I would like to express my deep gratitude to my advisor Dr. Rex Dunham for giving me the opportunity to join the Genetics Team and for his guidance and support on my research all of the time. I would also like to thank my committee members, Dr. Jeffery Terhune and Dr. Charles Chen for their time and expertise on this project.

I would like to thank Ahmed Elaswad, Zhi Ye, Ramjie Odin, David Drescher, Dalton Robinson, William Bugg and Kamal Gosh of our team. They made tremendous efforts in helping me to finish the challenge experiments. Thanks also go to other lab members, from past to present: Dr. Baofeng Su, Dr. Ahmed Alsaqufi, Dr. Zhenkui Qin, Hanbo Li, Dan Zhang, Guyu Qin, Khoi Vo, Dr. Gushein Waikhom, Karim Khalil, Nermeen Youssef and Chia Chen Weng Chen.

I am also grateful to students and staff, especially Ms. Karen Veverica and Ms. Renee Beam at the E. W. Shell Fisheries Research Center, Dr. Shikai Liu in Dr. John Liu's lab, as well as Dr. Haitham Mohammed, Francisca Burgos Valverde and Wenlong Cai in Dr. Cova Arias' lab, and Ningxin Yan in Department of Mathematics and Statistics, for their assistance and advice during my research and data analysis.

I would like also to convey my sincere thanks to all my friends in Auburn that made my graduate experience unforgettable. Particularly, many thanks to my boyfriend Mingjie Feng, and roommates Chengyu Si and Xiaoxiao Zhong for their care and support, not only on my study and research, but also in daily life.

I would also give appreciation to Shanghai Ocean University for offering me the fellowship to partially support my living expenses in the US.

Finally and most importantly, a special thank you to my family for their constant love and for supporting me to attend Auburn University and complete my Master of Science degree.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AUMPC	Area under Mortality Progress Curve
BHI	Brain Heart Infusion
C ×B	Channel Catfish, Female × Blue Catfish, Male
CFU	Colony Forming Unit
ESC	Enteric Septicemia of Catfish
FDA	Food and Drug Administration
IP	Intraperitoneal (injection)
MS	Modified Shieh

I. Literature Review

1.1 US catfish industry

In the United States, the production of ictalurid catfish plays an important role in the aquaculture industry. Currently, catfish are largely produced in Mississippi, Arkansas, Alabama and Texas. Catfish production constituted approximately 68% of the total domestic freshwater production in 2015 (NMFS, 2015). However, catfish production has had more than a 50% reduction from 2003 (Hanson and Sites, 2015). The factors causing the decline of catfish industry include intense competition from imported products from Asia, increased feed and labor costs, as well as fish disease control (FAO, 2011; Wagner et al. 2002).

The 2012 annual case summary report from the Aquatic Research & Diagnostic Laboratory indicated that bacterial disease cases dominated the total number of submitted cases in previous years (MSU, 2012). The most common bacterial infections in catfish are enteric septicemia of catfish (ESC) (*Edwardsiella ictaluri*), columnaris (*Flavobacterium columnare*), and motile *Aeromonas* septicemia (MAS) (*Aeromonas hydrophila* and related motile aeromonads) (Plumb and Hanson, 2011). To prevent disease in catfish industry, improving disease resistance by genetic enhancement and development of vaccination can be effective precautionary measures (Arias et al., 2012). Selection, crossbreeding, hybridization, and transgenic techniques can be applied in genetic enhancement programs for improving disease resistance.

1.2 Female channel catfish × male blue catfish hybrid catfish

Channel catfish, *Ictalurus punctatus*, is the most important freshwater species farmed in the US for human consumption (Small, 2006). It has desirable traits, including rapid growth, ease of spawning, good feed conversion and high dress-out weight (Kelly, 2004).

Fifty years ago, the first research on hybrid catfish of different crosses between seven distinct species of North American catfish was conducted (Dupree et al., 1969; Giudice 1966). However, only one interspecific hybrid, female channel catfish × male blue catfish *I. furcatus* (C×B hybrid) was proved to have better characteristics than either of its parents (Dunham et al., 2008). C×B hybrid catfish have superior performance with respect to

growth rate, survival rate, feed conversion ratio, tolerance to low oxygen (1.0 mg/L), harvestability by seining, vulnerability to angling and fillet yield (Argue et al., 2003; Dunham and Smitherman, 1983; Dunham et al., 1987; Dunham et al., 1998; Dunham et al., 2014; Dunham and Masser, 2012; Tave et al., 1981; Wolters et al., 1996; Yant et al., 1975). In additional, the C×B hybrid also exhibits increased resistance to Ich (*Ichthyophthirius multifilis*), channel catfish virus, columnaris (*Flexibacter columnare*), enteric septicemia of catfish (*Edwardsiella ictaluri*), and aeromonas (*Aeromonas hydrophila*) (Dunham and Masser, 2012).

1.3 *Flavobacterium columnare* infection

1.3.1 Columnaris disease

Columnaris disease was regarded as the second leading cause of mortality in pond raised catfish in the southeastern United States after enteric septicemia of catfish (ESC) caused by the bacterium *Edwardsiella ictaluri* (Durborow et al., 1998). However, according to 2012 annual case reports, columnaris disease has become the most frequently diagnosed bacterial disease in commercial catfish farms in the USA (MSU, 2012). It was first described by Davis (1922) but the pathogen was first isolated and characterized 70 years ago (Ordal and Rucker, 1944). The disease can cause acute to chronic infection in wild, cultured and ornamental freshwater fish, particularly for channel catfish and ictalurids (Plumb and Hanson, 2011). Columnaris is primarily an epithelial disease, but it can cause both surface (gill and skin) and systemic infections (Noga, 2010). It can sometimes occur as a primary or secondary infection, and be chronic, subacute, acute and per-acute, clinically (Plumb and Hanson, 2011). The mortality of tank-held channel catfish fingerlings can reach 90% under optimal disease conditions and the mortality of pond populations is usually lower than 50-60% (Plumb and Hanson, 2011). The economic losses caused by the disease are estimated at 30 million dollars, annually (Shoemaker et al., 2011).

1.3.2 Bacterial characteristcs

Flavobacterium columnare is considered to be the causative agent of columnaris disease (Noga, 2010), and is a gram negative bacterium made of slender flexible rods 0.5 wide × 4-12 μm in length (Roberts, 2012). Columnaris is a bacillus bacterium and its name

stems from the dome-shaped columns that columnaris arranges itself in wet mounts of infected tissue (Ordal and Rucker, 1944). The survival of *F. columnare* primarily depends upon water temperature, pH, water hardness, level of organic matter and status of host (Bullock et al., 1986). Its growth temperature ranges from 4 ℃ to 37 ℃, and the optimum temperature is 20-25 ℃ (Amend, 1982). Low pH and hardness can reduce the survival of *F. columnaris* (Fijan, 1968). *F. columnare* can survive in sterile water and mud (Bullock et al., 1986). It requires low levels of nutrients and agar for growth. When grown on agar plates, the bacterium exhibit flat, spreading, pale yellow and tightly adherent colonies with convoluted center, rhizoid edges, a specific odor, and gliding motility (Anacker and Ordal, 1955; Noga, 2010; Roberts, 2012). Griffin (1992) and Plumb and Hanson (2011) reported that, *F. columnare* isolates can produce hydrogen sulfide (H_2S), chondroitin lyases and extracellular galactosamine glycan, degrade gelatin, complex acidic polysaccharides of connective tissue. They also have catalase activity and cytochrome oxidase activity, and are resistant to neomycin sulfate and polymixin B.

1.3.3 Clinical signs and virulence factors

Columnaris is primarily an epithelial disease and causes external infections such as necrotic skin and gill, which may turn to systemic lesions (Noga, 2010). The disease typically has the appearance of white or yellow plaques with a red periphery on head and back of channel catfish, which is known as a saddleback lesion, fin rot, especially the caudal fin (Olivares-Fuster, 2010). Another typical symptom of columnaris is known as ‘cigar mouth’, due to the brown coloration along the lips and in the internal area of the mouth cavity (Durborow et al., 1998). The progression of lesions is rapid and the lesions become yellow or orange ulcers due to masses of bacteria (Noga, 2010). Erosion of the skin may expose the deep dermis and underlying skeletal muscle (Griffin, 1987). The damage to the cutaneous barrier can potentially result in disordered osmotic pressure and electrolyte balance (Tripathi et al., 2005). Necrosis of the gills is another common clinical sign of columnaris disease. *F. columnare* can attach to the gill surface, grow in spreading patches, and cover the gill filaments (Durborow et al., 1998). The gills can be eroded by protein and cartilage-degrading enzymes produced by the bacteria and fusion of gill filaments may eventually generate hemorrhaging, loss of the respiratory system, and circulatory collapse

(Durborow et al., 1998; Foscarini 1989.) Mouth rot is also lethal due to anorexia and starvation (Ferguson, 2006). Although external lesions are commonly seen in *F. columnare* infection, internal infections are seldom observed and systemic entry into the host is not required for the disease to occur (Bader et al., 2003). The bacteria can be detected in the blood, liver, kidney and even swelling of trunk kidney is present in some cases of columnaris infection, and gross internal clinical signs are often lacking (Bader et al., 2003; Hawke and Thune, 1992). However, the progression of columnarias is rapid and fish die before any of the signs of the disease were observed in some acute cases (Tripathi et al., 2005).

There are different virulence factors of *F. columnare* as a fish pathogen, including lipopolysaccharide (LPS), plasmids, adhesion capabilities and enzyme activities (Nematollahi et al., 2003).

In general, there are two phases of *F. columnare* pathogenesis. First, the bacteria attach to fish skin and gill tissues. The adhesion plays a major role in the virulence of the strain, because loss of adhesion is shown to dramatically reduce the virulence of *F. columnare*. Secondly, adhered bacteria divide and produce enzymes that can degrade fish connective tissue (Bader et al., 2005). In addition, LPS and other capsular polysaccharides, and biofilm formation are considered to play important roles in columnaris pathogenesis (Bader et al., 2005; Staroscik and Nelson, 2008). Twenty-three proteases were produced by isolates of *F. columnare* derived from channel catfish (Newton et al., 1997). The enzymatic activities of these proteases contribute to extensive necrotic lesions.

1.3.4 Epidemiology

Columnaris disease commonly occurs in fresh and brackish water habitats worldwide and most aquaculture environments, and *in vitro* experiments showed that *F. columnare* cannot tolerate 15 min exposure to 4% salt treatments, but the mucus layer of the host may provide a strong shield (Bullock et al.,1986; Durborow et al., 1998; Suomalainen et al., 2005a). Arias et al. (2006) found that *F. columnare* is one of the main catfish pathogens and it can be detected in absence of infective episodes in catfish ponds in Alabama. However, fish serve as the primary reservoir of *F. columnare* and the bacteria can be shed from gills and skin lesions, and even dead fish are able to spread the disease (Bullock et al., 1986;

Kunttu et al., 2009). The bacteria usually have adhesive capacity to the gills and mucus of the fish (Roberts, 2012). It can be latent during winter on the fish suffering a previous columnaris outbreak and become a source of infection in the next summer (Mohammed, 2015). The bacterium can transmit directly through contact with infected fish, or indirectly via the environment and by cohabitation with carrier fish (Welker et al., 2005).

For different bacterial strains, host species and conditions, it will take different incubation times from exposure to *F. columnare* to the emergence of visible clinical signs (Mohammed 2015). The genomovar II of *F. columnare* was considered to be the most dominant genomovar during the severe columnaris outbreaks in Auburn, AL, USA (Mohammed and Arias, 2014). Arias et al. (2012) demonstrated that genomovar II strains are more virulent than genomovar I strains. The incubation period of less virulent strains may be longer than that of high virulent strains (Farmer 2004).

F. columnare has a wide host range including almost all species of freshwater fishes and some amphibians (Durborow et al., 1998). No wild, cultured, or ornamental species in aquaria are totally resistant to columnaris. There are at least 36 species of fish susceptible to columnaris (Shoemaker et al., 2003). For instance, cultured eels (both fresh and brackish water) are highly susceptible under intensive culture conditions (Plumb and Hanson, 2011). The disease is also common in pond fishes, such as carp, ictalurids and salmonids (Bullock et al., 1986).

Columnaris disease is considered to be an opportunistic infection with stress of the host being a prerequisite for infection (Mohammed, 2015). Healthy fish without stress are generally not susceptible to columnaris disease (Durborow et al., 1998).

Typically, columnaris disease breaks out during spring, summer, and fall when preferred temperature occurs, and it can occur in colder temperatures but the mortalities and acuteness of disease decrease significantly (Mohammed, 2015). Durborow et al. (1998) found that the disease commonly occurs in many freshwater tropical aquarium fish due to the high temperature of aquaria (25-30 ℃). Channel catfish seem to have columnaris problem when water temperatures are in the range of 25 to 32 ℃ (Durborow et al., 1998). Moreover, some other adverse factors can aggravate the risk of *F. columnare* infection. Water quality such as high organic load, high ammonia, high nitrite, low salinity, and low

dissolved oxygen, and other physical stressors such as high densities, feed deprivation, excessive handling and physiological injury may also exacerbate the columnaris infection (Mohammed, 2015; Noga, 2010; Plumb and Hanson, 2011; Straus et al., 2015).

1.3.5 Diagnosis, prevention and treatment

Presumptive diagnosis of columnaris disease is based on the clinical signs which were mentioned above, and the presence of typical lesions containing long, thin and non-flagellated gram-negative rods with gliding motion and the formation of characteristic columns in wet mount preparations from skin lesions and infected tissues (Bullock et al., 1986; Noga, 2010). However, biochemical, serological or molecular methods after isolation and cultivation of the pathogen from lesions or infected tissues are necessary for definitive identification.

Biochemical tests such as gram staining, flexirubin pigment, Tween-20 starch, gelatin, casein, and lecithin hydrolysis, immunological techniques, such as agglutination, ELISA and immunofluorescence can be used for identification (MacFaddin, 2000; Mohammed, 2015; Panangala et al., 2006). Compared with first two methods, molecular techniques based on PCR with specific primers are more accurate and rapid (Panangala et al., 2006).

For most bacterial diseases, prevention is a critical factor of management to control the disease outbreaks (Wagner et al., 2002). However, it is difficult to eliminate columnaris disease because the bacterium is widespread in the aquaculture environments (Bullock et al., 1986).

Routine bacterial community management and good husbandry practices are useful for columnaris disease control (Arias et al., 2006). The study of Sung et al., (2001) indicated that a fluctuation of species diversity in a microbial community is suggestive of environmental stress and management practices should be carried out to avoid disease risk. Good management practice including monitoring of water quality, manipulating of feeding and stocking density, etc. Water temperature is a limiting factor of columnaris outbreaks. Suomalainen et al. (2005a) suggested that the pathogen carriers are not a disease risk when water temperature is not raised and decreasing the stocking density can prevent columnaris disease when water temperature is high. Additionally, good feeding practices can maintain the immunity of the fish for disease resistance (Shoemaker et al., 2003).

When the fish have to be handled or during early infection with columnaris, chemotherapeutics or antibiotic treatment can be implemented as preventive measures (Noga, 2010). Davis (1922) reported that adding copper sulfate to pond water at 0.5 mg/L or a 20 min copper sulfate bath at 37 mg/L could prevent epizootic episodes of columnaris. Darwish et al. (2009) suggested that potassium permanganate could be a favorable prophylactic therapy for columnaris for physically compromised fish. Conrad et al. (1975) found that ozone treatment of water could prevent columnaris as it reduces the numbers of *F. columnare*. Suomalainen et al. (2005b) recommended that salt and acid bath treatments could be applied for disinfection of *F. columnare* cells in the water to prevent transmission of columnaris. Some studies indicated that antibiotic treatments can also prevent columnaris disease. Nitrofurans can be applied as a bath or in oral administration to effectively treat and prevent columnaris disease (Bullock et al., 1986). Based on the results of Thomas-Jinu and Goodwin (2004), oxytetracycline or a combination of sulphadimethoxine and ormetoprim in feed and bath treatments with chloramine-T can reduce mortality of columnaris exposed channel catfish. However, antibiotic treatments require withdrawal periods before the fish can be sold to market as food.

Besides good management practices, chemotherapeutics and antibiotic treatment, vaccination can also be used as a prophylactic treatments. Fujihara and Nakatani (1971) first used an oral vaccine with heat-killed cells to establish active immunity to *F.columnaris* disease in coho salmon (*Oncorhynchus kisutch*). Schachte and Mora (1973) reported a heat-inactivated bacterin with adjuvant can stimulate the production of agglutinating antibodies by subcutaneous or intramuscular injection in channel catfish fingerlings. Moore et al. (1990) found annual immersion with formalin inactivated *F. columnaris* bacterins resulting in decreased mortality of pond-reared channel catfish. Shoemaker et al. (2005) developed a rifampicin modified live vaccine against columnaris disease. It was licensed under commercial name of AQUAVAC-COL™ (Merck Animal Health) for prevention of columnaris disease in catfish and largemouth bass (Shoemaker et al., 2011). Since *F. columnare* genomovar II strain is more virulent to catfish, new rifampicin mutants of *F. columnare* genomovar II were developed (Olivares-Fuster, 2011). This vaccine is safer and more stable, and it can provide more protection against columnaris diseases than the current

commercial vaccine (Mohammed et al., 2013).

Although prevention is important to control the disease outbreaks, after the onset of disease, treatment should commence. Since external surfaces like skin and gills are the primary sites which *F. columnare* attacks, therapeutic drugs can be administrated directly to the water as a dip, flush or bath (Wakabayashi, 1991). Immersion in a salt bath is a common therapy for *F. columnare.* A study showed the growth of *F. columnare in vitro* was inhibited by salt (NaCl) at a dose of 10 g/L (Bernardet, 1989). Another study indicated that copper sulfate ($CuSO_4$) has clear therapeutic value against columnaris infection, but this therapeutic is regulated by law in the USA because heavy metals accumulate in fish and the environment (Darwish et al., 2012; Wakabayashi 1991). Potassium permanganate ($KMnO_4$) is also commonly used to treat external columnaris in ponds at 2 mg/L or at a higher concentration if the water's organic load is high (Durborow et al., 1998). However, complementary to external therapy, medicated feed should also be used as an optimal treatment (Amend and Ross, 1970). Based on laboratory antibiotic sensitivity tests, Terramycin® (oxytetracyline HCl) and Romet® medicated feeds are effective in treating columnaris infections, but technically they are extra-labelled for use on other infections (Durborowet al., 1998). Gaunt et al. (2010) demonstrated that florfenicol was efficacious and safe against *F. columnare* infection in channel catfish. The U.S. Food and Drug Administration (FDA) fully approved Aquaflor® (florfenicol) type A medicated article as an aquaculture drug for treatment of columnaris disease (FDA, 2012). However, administration of antibiotics has problems including: expense, infected fish seldom feed well and reducing intake of medicated feed, drug resistance and residues in food fish.

1.4 *Edwardsiella ictaluri* infection

1.4.1 ESC disease

Enteric septicemia of catfish (ESC) caused by the bacterium *Edwardsiella ictaluri* has been considered to be the most prevalent and costly disease affecting commercial catfish farms (Hawke et al., 1981). It was first described in 1976 in sick catfish from Alabama and Georgia (Hawke, 1979). The disease results in dramatic economic losses, millions of dollars annually, to the catfish industry (Noga, 2010). More than half of catfish fry and fingerling

losses in the US are due to ESC (Williams and Lawrence, 2010). It occurs as a chronic or acute, primary infection. The host fish are infected via the gut in the former form and the nervous route in the latter form (Noga, 2010). Under artificial challenge conditions, ESC often leads to extremely high mortality as soon as a few days after onset of infection (Peatman et al., 2007).

1.4.2 Bacterial characteristics

Edwardsiella ictaluri as the causative agent of ESC first isolated in 1976 (Hawke, 1979). *E. ictaluri* is a gram negative bacterium that is a short rod with a size of 0.5 × 1-3 μm (Roberts, 2012). It is the most fastidious member in the *Edwardsiella* species and requires a 48 h incubation at 26 ℃ to form 1 mm diameter colonies on brain heart infusion (BHI) agar plate (Roberts, 2012). Optimal growth temperature for *E. ictaluri* is 25-30 ℃ and a fish population is at risk of infection when temperatures are in the 22 to 28 ℃ range (Hawke et al., 1981; Francis-Floyd et al., 1987).

E. ictaluri belongs to the genus *Edwardsiella* and it is most closely related to *E. tarda* than other members within the family *Enterobacteriaceae* based on biochemical characterization and DNA-DNA homology (Hawke et al., 1981). *E. ictaluri* is resistant to bile salt, but sensitive to NaCl at 2% concentrations (Waltman et al., 1986). It is able to produce gas from glucose at 25 ℃ and is negative indole and H_2S production from triple sugar iron agar (Hawke et al., 1986). *E. ictaluri* lacks proteases, lipases, esterases, and most other extracellular enzymes, but it can degrade chondroitin sulfate (Waltman et al., 1986).

1.4.3 Clinical signs and virulence factors

ESC has two forms with pathognomonic clinical signs in channel catfish (Hawke, 1979). One is an acute form, which is related to the gut route of infection. After *E. ictaluri* is ingested, it enters the bloodstream and colonizes organs to cause necrosis (Noga, 2010). This form of ESC often leads to acute mortality, but sometimes there are few external signs (Noga, 2010). The diseased fish has a head-up-tail-down posture and shows spinning swimming behavior (Plumb and Hanson, 2011). They may exhibit enlarged abdomen, exophthalmia, pale gills, hemorrhage and depigmented lesions on the jaw, dorsum and flanks (Roberts, 2012). The internal signs include petechial lesions in muscles, necrosis of liver and swollen trunk kidney (Noga, 2010). Another form of ESC is the chronic form that

is associated with nervous system route of infection. The bacteria invade from the nasal opening to the olfactory organ and brain (Noga, 2010). Typically, an open lesion develops on the central skull, hence it name “hole-in-the-head” disease (Plumb and Hanson, 2011). Hemorrhaging may also occur at the base of fins and skin under the jaw (Plumb and Hanson, 2011).

Little is known about the factors associated with ESC pathogenesis. Some studies indicated that potential virulence factors may include lipopolysaccharide (LPS), chondroitinase, GAPDH outer membrane protein (OMP) and other bacterial cell surface material (Yeh et al., 2005; Trung Cao et al., 2014).

1.4.4 Epidemiology

ESC is a seasonal disease that breaks out in ponds in the areas of southeast United States. It is prevalent primarily in May and June, as well as September and October because its pathogenicity is temperature-dependent (about 24-28 ℃) (Noga, 2010). Except during these periods, the disease may occur in chronic form with low mortality (Noga, 2010). In the ponds, ESC can recur because *E.ictaluri* can survive in mud at 25 ℃ for over one and half months (Plumb and Quinlan, 1986).

Different species, strains, and families of catfish have various resistances against ESC. In general, channel catfish has the lowest level of ESC resistance, and C×B hybrid catfish and blue catfish are less susceptible to ESC (Wolters and Jounson, 1994; Wolter et al., 1996). ESC infections were reported to occur not only in wild populations, but also in the farmed fish and under experimental challenge within many fish species, such as brown bullhead (*Ameiurus nebulosus*), white catfish (*Ameiurus catus*), danio (*Danio devario*), walking catfish (*Clarias batrachus*), Asian catfish (*Pangasius hypophthalmus*), rainbow trout (*Oncorhynchus mykiss*) (Hawke et al., 1981; Waltman et al. 1985; Kasornchandra et al., 1987; Crumlish et al., 2002; Keskin et al., 2004).

Under artificial challenge conditions, ESC develops rapidly and causes great mortality in the first few days after infection (Wolter et al., 1996). In ponds, ESC infection can cause substantial morbidity, and reduce the feed intake of infected fish (Manning et al., 2005). The mortality may increase with the development of the disease, or decrease or cease if the water temperature is out the optimal range (Hawke et al., 1998).

1.4.5 Diagnosis, prevention and treatment

ESC can be diagnosed by bacterial culture techniques followed by biochemical characterization of the isolates from internal organs on brain heart infusion agar (BHI), or tryptic soy agar (TSA) with 5 percent sheep's blood (Hawke et al., 1998). It typically takes a 48-hour culture to form very high numbers of colonies, which are whitish, smooth and circular (Zhang, 2007). Besides the classical identification, serology methods and molecular techniques have been developed for detecting *E. ictaluri*. Enzyme immunosorbent assay (ELISA), indirect fluorescent antibody test (IFAT) and fluorescent *in situ* hybridization (FISH) have been used for diagnosis, though some false-negative results were reported (Hanson and Rogers, 1989; Ainsworth et al., 1986; Speyerer and Boyle, 1987). Other useful tools for diagnosis include polymerase chain reaction (PCR), real-time PCR, and loop-mediated isothermal amplification (LAMP) which are more specific and sensitive (Mullis and Faloona, 1987; Bilodeau et al., 2003; Yeh et al., 2005).

Good management practices, such as relieving stress, providing sufficient nutrition and administering proper chemotherapy, can reduce the incidence of ESC. To prevent ESC, genetic improvement is also important (Hawke et al., 1998). The hybrids of Norris female channel catfish and blue catfish males showed improved resistance to ESC compared to pure Norris strain channel catfish (Hawke et al., 1998).

Vaccination is another approach to control the bacterial disease in aquaculture. Formalin killed vaccines are widely used in the trout and salmon industries to protect fish from some certain bacterial infections (Hawke et al., 1998). However, a formalin-killed *E. ictaluri* vaccine did not protect against ESC in channel catfish (Thune et al., 1997). A modified live *E. ictaluri* vaccine was developed by a rifampicin resistant strategy (Klesius and Shoemaker, 1999). It was effective and marketed by Intervet Inc. (Millsboro, DE, USA) as a licensed vaccine (AQUAVAC-ESC®) for 7-10 day post-hatch channel catfish (Shoemaker et al., 1999; Arias et al., 2003).

After the onset of disease, other treatments such as feeding the infected fish with antibiotics may be efficacious. Oxytetracycline and ormetoprim-sulfadimethoxine have been used and show different success rates against various *E. ictaluri* isolates (Noga, 2010). Although some isolates of *E. ictaluri* exhibit susceptibility to antibiotics including

kanamycin, streptomycin, neomycin and nitrofurantoin, few antibiotics have been approved for application in the catfish industry (Noga, 2010). Aquaflor® (florfenicol) was very efficacious in clinical trials and has been approved for treatment of ESC in all life stages of channel catfish by FDA (Noga, 2010; Olivares-Fuster, 2010). Terramycin and Romet are also approved by FDA and seem to be effective against ESC infection (Plumb and Hanson, 2011).

1.5 Genetic enhancement program for improvement of disease resistance

As a quantitative trait, disease resistance can be improved in some but not all cases by selection, which takes large effort and multiple generations. Much work to improve disease resistance has been done in oyster industry. Selection for resistance to *Bonamia ostreae* in *Ostrea edulis*, to *Bonamia roughleyi* and *Marteilia sydneyi* in *Saccostrea glomerata* and selection for resistance to *Roseovarius crassostrea* in *Crassostrea virginica*, have been successful (Dégremont et al., 2015).

Unlike selection, crossbreeding and hybridization are short-term programs. Combining abilities differ from strain to strain for disease resistance. Intraspecific crossbreeding has been used to improve relative disease resistance (Dunham and Smitherman, 1987; Dunham, 2011). Interspecific hybridization has also been used with several aquatic animals to increase disease resistance and improve many other traits (Dunham, 2011).

There are few studies of disease resistance for triploid fish. Lakhaanantakun (1992) indicated that diploid and triploid Thai walking catfish had similar resistance to *Aeromonas hydrophila*. However, according to physiological studies, triploids should have inferior disease resistance than the diploids (Dunham, 2011).

In 1985, the first successful genetic engineering fish was reported in China (Zhu et al., 1985). Henceforth, transgenic technology research has been investigated for many aquatic animal species. Although, numerous studies concentrated on transfer of GH-gene, transgenic techniques can also be applied to improve disease resistance. Channel catfish with cecropin B gene showed enhanced disease resistance to both columnaris and ESC, but no significant differences in growth rate compared to non-transgenic fish (Dunham et al., 2002).

1.6 Challenge methods

Injection and immersion with the bacteria are commonly used in bacterial challenge experiments of aquatic animals. For injection methods, two options exist, intraperoteneal (IP) and intramuscular (IM).

The disadvantages of the methods above include: the fish are exposed to additional stress of handling, the pathogen used for injection or immersion may be attenuated due to growing *in vitro*, the bacteria can bypass the natural barriers of fish by injection, such as skin and mucus, and the fish generally contact a very high concentration of the bacteria by immersion, which they would not normally experience (Alcorn et al., 2005). Compared to injection and immersion, cohabitation challenge seems to provide an approximately real interaction between pathogen and fish (Chettri et al., 2015).

The challenge methods affect the results of experiments (Nordmo and Ramstad, 1997). The time required to cause mortality from *Renibacterium salmoninarum* in chinook salmon by cohabitation was significantly longer than by injection or immersion challenges (Beacham and Evelyn, 1992; Murray et al., 1992). Columnaris disease developed more rapidly in channel catfish by immersion than by intramuscular injection (Welker et al., 2005). Additionally, challenge experiments are not able to completely simulate the environmental conditions in commercial farms, thus, the applicability of challenge experiments to commercial conditions may be reduced (Fjalestad et al., 1993).

1.7 Objectives

Information of the bacterial disease resistance of C×B hybrids is important. It can provide references to determine the suitability of different strains of brood stock for producing C×B hybrids in commercial applications. The objective of my study is to compare the disease resistance among different genetic types of C×B hybrid catfish and channel catfish against columnaris and ESC.

References

Ainsworth, A.J., Capley, G., Waterstreet, P., Munson, D., 1986. Use of monoclonal antibodies in the indirect fluorescent antibody technique for the diagnosis of *Edwardsiella ictaluri*. Journal of Fish Diseases 9:439–444.

Alcorn, S., Murray, A.L., Pascho, R.J., Varney, J., 2005. A cohabitation challenge to compare the efficacies of vaccines for bacterial kidney disease (BKD) in chinook salmon *Oncorhynchus tshawytscha*. Diseases of aquatic organisms 63(2-3):151–160.

Amend D., 1982. Columnaris (*Flexibacter columnaris*) disease of freshwater fishes and a brief review of other flexibacterial diseases of fish. Antigens of fish pathogens.:139–161.

Amend D.F., Ross A.J., 1970. Experimental control of columnaris disease with a new nitrofuran drug, P-7138, The Progressive Fish-Culturist 32:19–25.

Anacker, R.L., Ordal, E.J., 1955. Study of a bacteriophage infecting the myxobacterium *Chondrococcus columnaris*. Journal of Bacteriology 70:738–740.

Argue, B.J., Liu, Z., Dunham, R.A., 2003. Dress-out and fillet yields of channel catfish, *Ictalurus punctatus*, blue catfish, *Ictalurus furcatus*, and their F1, F2 and backcross hybrids. Aquaculture 228(1), 81–90.

Arias, C.R., Abernathy, J.W., Liu, Z., 2006. Combined use of 16S ribosomal DNA and automated ribosomal intergenic spacer analysis to study the bacterial community in catfish ponds. Letters in applied microbiology 43(3):287–292.

Arias C.R., Cai W., Peatman, E., Bullard S.A.,2012. Catfish hybrid *Ictalurus punctatus* × *I. furcatus* exhibits higher resistance to columnaris disease than the parental species. Diseases of aquatic organisms 100(1):77–81.

Arias, C.R., Shoemaker, C.A., Evans, J.J., Klesius, P.H., 2003. A comparative study of *Edwardsiella ictaluri* parent (EILO) and *E. ictaluri* rifampicin-mutant (RE-33) isolates using lipopolysaccharides, outer membrane proteins, fatty acids, Biolog, API 20E and genomic analyses. Journal of Fish Diseases 26(7): 415–421.

Bader, J., Nusbaum, K., Shoemaker, C., 2003. Comparative challenge model of *Flavobacterium columnare* using abraded and unabraded channel catfish, *Ictalurus*

punctatus (Rafinesque), Journal of Fish Diseases 26:461–467.

Bader, J.A., Shoemaker, C.A., Klesius, P.H., 2005. Production, characterization and evaluation of virulence of an adhesion defective mutant of *Flavobacterium columnare* produced by beta-lactam selection, Letters in Applied Microbiology 40:123–127.

Beacham, T.D., Evelyn, T.P.T., 1992. Population and genetic variation in resistance of chinook salmon to vibriosis, furunculosis, and bacterial kidney disease. Journal of Aquatic Animal Health 4:153–167.

Bernardet, J.F., 1989. "*Flexibacter columnaris*": first description in France and comparison with bacterial strains form other origins. Diseases of Aquatic Organisms 6: 37–44.

Bilodeau, A.L., Waldbieser, G.C., Terhune, J.S., Wise, D.J., Wolters, W.R., 2003. A real-time polymerase chain reaction assay of the bacterium *Edwardsiella ictaluri* in channel catfish. Journal of Aquatic Animal Health 15:80–86.

Bullock, G., Hsu, T.C., Shotts, Jr E., 1986. Columnaris disease of fishes, USFWS Fish Disease Leaflet 1986:1–9.

Chettri, J.K., Skov, J., Jaafar, R.M., Kross øy, B., Kania P.W., Dalsgaard, I., Buchmann, K., 2015. Comparative evaluation of infection methods and environmental factors on challenge success: *Aeromonas salmonicida* infection in vaccinated rainbow trout. Fish Shellfish Immunol 44(2):485–495.

Conrad, J.F., Holt, R.A., Kreps, T.D., 1975. Ozone disinfection of flowing water. The Progressive Fish-Culturist 37:134–136.

Crumlish, M., Dung, T.T., Turnbull, J.F., Ngoc, N.T.N., Ferguson, H.W., 2002. Identification of *Edwardsiella ictaluri* from diseased freshwater catfish, 110 *Pangasius hypophthalmus* (Sauvage), cultured in the Mekong Delta, Vietnam. Journal of Fish Diseases 25:733–736.

Darwish, A., Mitchell, A., Straus, D., 2009. Evaluation of potassium permanganate against an experimental subacute infection of *Flavobacterium columnare* in channel catfish, *Ictalurus punctatus* (Rafinesque), Journal of Fish Diseases 32:193–199.

Darwish, A.M., Mitchell, A., Straus, D.L., 2012. Evaluation of a 4-h static copper sulphate treatment against experimental infection of *Flavobacterium columnare* in channel catfish (*Ictalurus punctatus*). Aquaculture Research 43:688–695.

Davis, H.S., 1922. A new bacterial disease of fresh-water fishes: By HS Davis, Government Printing Office.

Dégremont, L., Garcia, C., Allen, S.K., 2015. Genetic improvement for disease resistance in oysters: a review. Journal of invertebrate pathology 131:226–241.

Dunham, R.A., 2011. Aquaculture and fisheries biotechnology: genetic approaches. CABI.

Dunham, R.A., Liu, Z., Argue, B.J., 1998. The effect of the absence or presence of channel catfish males on induced ovulation of channel catfish females for artificial fertilization with blue catfish sperm. The Progressive Fish-Culturist 60 (4), 297–300.

Dunham, R.A., Masser, M.P., 2012. Production of hybrid catfish. SRAC Publication No.190.

Dunham, R.A., Ramboux, A.C., Perera, D.A., 2014. Effect of strain on the growth, survival and sexual dimorphism of channel × blue catfish hybrids grown in earthen ponds. Aquaculture 420, S20-S24.

Dunham, R.A., Smitherman, R.O., 1983. Response to selection and realized heritability for body weight in three strains of channel catfish, *Ictalurus punctatus*, grown in earthen ponds. Aquaculture 33(1), 89–96.

Dunham, R.A., Smitherman, R.O., 1987. Genetics and Breeding of Catfish. Regional Research Bulletin 325, Southern Cooperative Series. Alabama Agricultural Experiment Station, Auburn University, Auburn, Alabama

Dunham, R.A., Smitherman, R.O., Goodman, R.K., 1987. Comparison of mass selection, crossbreeding, and hybridization for improving growth of channel catfish. The Progressive Fish-Culturist 49(4), 293–296.

Dunham R.A., Umali G.M., Beam R., Kristanto A.H., Trask M., 2008. Comparison of production traits of the NWAC-103 channel catfish, NWAC103 channel × blue hybrid catfish, Kansas select 21 channel catfish and blue catfish grown at commercial densities and exposed to natural bacterial epizootics. North American Journal of Aquaculture 70: 98−106.

Dunham, R.A., Warr, G.W., Nichols, A., Duncan, P.L., Argue, B., Middleton, D., Kucuktas, H., 2002. Enhanced bacterial disease resistance of transgenic channel catfish *Ictalurus punctatus* possessing cecropin genes. Marine Biotechnology 4(3): 338−344.

Dupree, H.K., Green, O.L., Sneed, K.E., 1969. Techniques for the hybridization of catfishes. U.S. Fish and Wildlife Service, Southeastern Fish Cultural Laboratory, Publication 221, Marion, Alabama.

Durborow, R.M., Thune, R.L., Hawke, J.P., Camus, A.C., 1998. Columnaris Disease: A Bacterial Infection Caused by Flavobacterium Columnare. South Regional Aquaculture Centre SRAC, Texas A & M University.

FAO, 2011. United States of America. National Aquaculture Sector Overview Fact Sheets. In FAO Fisheries and Aquaculture Department, Rome.

Farmer, B., Improved methods for the isolation and characterization of *Flavobacterium Columnare*. M.S. Thesis 2004. Louisiana State University.

Ferguson, H., 2006. Systemic pathology of fish: A text and atlas of normal tissue in teleosts and their responses in disease, Scotian Press London.

Fijan, N. 1968. The survival of Chondrococcus columnaris in waters of different quality. Bull. Off. Int. Epizoot. 69(7-8):1159–1166.

Fjalestad, K.T., Gjedrem, T., Gjerde, B., 1993. Genetic improvement of disease resistance in fish: an overview. Aquaculture 111(1), 65–74.

Food and Drug Administration, 2012. FDA Announces the Approval of Two New Indications for Aquaflor. US Food and Drug Administration, Rockville, MD, USA. (http://www.fda.gov/AnimalVeterinary/NewsEvents/CVMUpdates/ucm299443.htm)

Francis-Floyd, R., Beleau, M.H., Waterstrat, P.R., Bowser, P.R., 1987. Effect of water temperature on the clinical outcome of infection with *Edwardsiella ictaluri* in channel catfish. Journal of the American Veterinary Medical Association 191(11):1413–1416.

Fujihara, M.P., Nakatani, R.E., 1971. Antibody Production and Immune Responses of Rainbow Trout and Coho Salmon to *Chondrococcus Columnaris*. Journal of the Fisheries Research Board of Canada 28(9):1253–1258.

Foscarini, R., 1989. Induction and development of bacterial gill disease in the eel (*Anguilla japonica*) experimentally infected with *Flexibacter columnaris*: pathological changes in the gill vascular structure and in cardiac performance, Aquaculture 78:1–20.

Gaunt, P.S., Gao, D., Sun, F., Endris, R., 2010. Efficacy of Florfenicol for Control of

Mortality Caused by *Flavobacterium columnare* Infection in Channel Catfish. Journal of Aquatic Animal Health, 22:2, 115–122.

Giudice, J.J., 1966. Growth of a blue × channel catfish hybrid as compared to its parent species. The Progressive Fish-Culturist 28(3), 142–145.

Griffin, B.R, 1987. Columnaris disease: recent advances in research, Aquaculture 13:48–50.

Griffin, B.R, 1992. A simple procedure for identification of *Cytophage columnaris*. Journal of Aquatic Animal Health 4:63–66.

Hanson, L.A., Rogers, W.A., 1989. Enzyme immunoassay identification of *Edwardsiella ictaluri* in decomposing channel catfish. Journal of the World Aquaculture Society 20:279–280.

Hanson, T., Sites, D., 2015. 2014 U.S. Catfish Database. USDA National Agricultural Statistics Service (NASS), Mississippi Agricultural Statistics Service (MASS).

Hawke, J.P., McWhorter, A.C., Steigerwalt, A.G., Brenner, D.J., 1981. *Edwardsiella ictaluri* sp. nov., the causative agent of enteric septicemia of catfish. International Journal of Systematic Bacteriology 31:396–400.

Hawke, J.P., 1979. A Bacterium Associated with Disease of Pond Cultured Channel Catfish, *Ictalurus punctatus*. Journal of the Fisheries Research Board of Canada 36(12): 1508–1512.

Hawke, J.P., Thune, R.L., 1992. Systemic isolation and antimicrobial susceptibility of *Cytophaga columnaris* from commercially reared channel catfish. Journal of Aqu–ly, MA. Channel Catfish Broodfish Management. March 2004 SRAC Publication No. 1802.

Hawke, J.P., Durborow, R.M., Thune. R.L., Camus, A.C., 1998. ESC-enteric septicemia of catfish. SRAC Publication No. 477.

Kasornchandra, J., Rogers, W.A., Plumb, J.A., 1987. *Edwardsiella ictaluri* from walking catfish, *Clarias batrachus* L., in Thailand. Journal of Fish Diseases 10:137–138.

Kelly, A.M., 2004. Channel catfish broodfish management. SRAC Publication No.1802, Southern Regional Aquaculture Center, Stoneville, Mississippi.

Keskin, O., Secer, S., Izgur, M., Turkyilmaz, S., Mkakosya, R., 2004. *Edwardsiella ictaluri* infection in rainbow trout (*Oncorhynchus mykiss*). Turkish Journal of Veterinary and Animal Sciences 28:649–653.

Klesius, P.H., Shoemaker, C.A., 1999. Development and use of modified live *Edwardsiella ictaluri* vaccine against enteric septicemia of catfish. Advances in Veterinary Medicine 41:523–537.

Kunttu, H.M.T., Valtonen, E.T., Jokinen, E.I., Suomalainen, L. R., 2009. Saprophytism of a fish pathogen as a transmission strategy. Epidemics 1:96–100.

Mac Faddin, J.F., Biochemical tests for identification of medical bacteria, Williams & Wilkins. Baltimore, MD. (2000).

Lakhaanantakun, A., 1992. The effects of triploidy on survival rate, growth rate and feed conversation ratio of walking catfish (*Clarias microcephalus* Gunther). M.S. thesis, Kasetsart University, Bangkok, Thailand.

Manning, B.B., Terhune, J.S., Li, M.H., Robinson, E.H., Wise, D.J., Rottinghaus, G.E., 2005. Exposure to feedborne mycotoxins T-2 toxin or ochratoxin A causes increased mortality of channel catfish challenged with *Edwardsiella ictaluri*. Journal of aquatic animal health 17(2):147–152.

Mohammed, M.H., 2015. Studies on the epidemiology, vaccination, susceptibility, and treatment of columnaris disease in fishes. Ph.D. Dissertation. Auburn University, AL.

Mohammed H., Arias C.R., 2014. Epidemiology of columnaris disease affecting fishes within the same watershed. Diseases of aquatic organisms 109: 201–211.

Mohammed H., Olivares-Fuster O., LaFrentz S., Arias C.R., 2013. New attenuated vaccine against columnaris disease in fish: Choosing the right parental strain is critical for vaccine efficacy. Vaccine 31:5276–5280.

Moore A.A., Eimers M.E., Cardella M.A., 1990. Attempts to control *Flexibacter columnaris* epizootics in pond-reared channel catfish by vaccination, Journal of Aquatic Animal Health 2:109–111.

MSU, 2012 annual case summary report of the Aquatic Research and Diagnostic Laboratory. Mississippi State University, College of Veterinary Medicine, Thad Cochran, Stoneville, MS.

Mullis, K., Faloona, F., 1987. Specific synthesis of DNA in vitro via a polymerasecatalyzed chain reaction. Methods in Enzymology 155:335–350.

Murray, C.B., Evelyn, T.P.T., Beacham, T.D., Barner, L.W., Ketcheson, J.E., Prosperi-Porta, L., 1992. Experimental induction of bacterial kidney disease in chinook salmon by immersion and cohabitation challenges. Diseases of aquatic organisms 12:91–96.

Nematollahi, A., Decostere, A., Pasmans, F., Haesebrouck, F., 2003. Flavobacterium psychrophilum infections in salmonid fish. Journal of fish diseases 26(10): 563–574.

Newton, J.C., Wood, T.M., Hartley, M.M., 1997. Isolation and partial characterization of extracellular proteases produced by isolates of F*lavobacterium columnare* derived from channel catfish. Journal of Aquatic Animal Health 9:75–85.

NMFS (National Marine Fisheries Service). 2015. Fisheries of the United States 2014. NMFS, Office of Science and Technology, Silver Spring, Maryland.

Noga E.J., 2010. Fish disease: diagnosis and treatment, John Wiley & Sons.

Nordmo, R., Ramstad, A.,1997. Comparison of different challenge methods to evaluate the efficacy of furunculosis vaccines in Atlantic salmon, *Salmo salar* L, Journal of Fish Diseases 119–126.

Olivares-Fuster, Ó., 2010. Development, Characterization and Early Evaluation of New Modified Live Vaccines Against Columnaris Disease. M.S. thesis. Auburn University, AL.

Olivares-Fuster, O., Arias, C.R., 2011. Development and characterization of rifampicin‐resistant mutants from high virulent strains of *Flavobacterium columnare*. Journal of Fish Diseases 34(5):385–394.

Ordal, E.J., Rucker, R.R., 1944. Pathogenic myxobacteria. Exp Biol Med (Maywood) 56 (1), 15–18.

Panangalal, V., Shelby, R.A., Shoemaker, C.A., Klesius, P.H., Mitra, A., Morrison, E.E., 2006. Immunofluorescent test for simultaneous detection of *Edwardsiella ictaluri* and *Flavobacterium columnare*, Diseases of Aquatic Organisms. 68:197–207.

Peatman, E., Baoprasertkul, P., Terhune, J., Xu, P., Nandi, S., Kucuktas, H., Li, P., Wang, S., Somridhivej, B., Dunham, R.A., Liu, Z., 2007. Expression analysis of the acute phase

response in channel catfish (*Ictalurus punctatus*) after infection with a Gram-negative bacterium. Developmental and Comparative Immunology 31(11): 1183–1196.

Plumb, J.A., Hanson, L.A., 2011. Health maintenance and principal microbial diseases of cultured fishes, 3rd edition. John Wiley & Sons.

Plumb, J.A., Quinlan, E.E., 1986. Survival of *Edwardsiella ictaluri* in pond water and bottom mud. The Progressive Fish-Culturist 48(3), 212–214.

Roberts, R.J., 2012. Fish Pathology, 4th edition. John Wiley & Sons.

Schachte, J.H., Mora E.C., 1973. Production of Agglutinating Antibodies in Channel Catfish (*Ictalurus Punctatus*) against *Chondrococcus Columnaris*. Journal of the Fisheries Research Board of Canada 30(1):116–118.

Shoemaker, C.A., Klesius, P.H., Bricker, J.M., 1999. Efficacy of a modified live *Edwardsiella ictaluri* vaccine in channel catfish as young as seven days post hatch. Aquaculture 176, 189–193.

Shoemaker, C.A., Klesius, P.H., Lim, C., Yildirim, M., 2003. Feed deprivation of channel catfish, *Ictalurus punctatus* (Rafinesque), influences organosomatic indices, chemical composition and susceptibility to *Flavobacterium columnare*. Journal of Fish Diseases 26:1–9.

Shoemaker, C.A., Klesius, P.H., Evans, J.J., 2005. Modified live *Flavobacterium columnare* against columnaris disease in fish, USA. U.S. Patent Number 6881412.

Shoemaker, C.A., Klesius, P.H., Drennan, J.D,. Evans, J.J., 2011. Efficacy of a modified live *Flavobacterium columnare* vaccine in fish. Fish and Shellfish Immunology 30:304–308.

Small, CB. 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.

Speyerer, P.D., Boyle, J.A., 1987. The plasmid profile of *Edwardsiella ictaluri*. Journal of Fish Diseases 10:461–470.

Staroscik, A.M., Nelson, D.R., 2008. The influence of salmon surface mucus on the growth

of *Flavobacterium columnare*. Journal of Fish Diseases 31:59–69.

Straus, L.D., Farmer, D.B., Beck, H.B., Bosworth, G.B, Torrans, L.E., Tucker, S.C., 2015. Water hardness influences *Flavobacterium columnare* pathogenesis in channel catfish. Aquaculture 435: 252–256.

Sung, H.H., Hsu, S.F., Chen, C.K., Ting, Y.Y., Chao,W.L., 2001. Relationships between disease outbreak incultured tiger shrimp (*Penaeus monodon*) and the composition of *Vibrio* communities in pond water and shrimp hepatopancreas during cultivation. Aquaculture 192:101–110.

Suomalainen, L., Tiirola, M., Valtonen, E., 2005. Influence of rearing conditions on *Flavobacterium columnare* infection of rainbow trout, *Oncorhynchus mykiss* (Walbaum), Journal of Fish Diseases 28:271–277.

Suomalainen, L., Tiirola, M., Valtonen, E., 2005. Treatment of columnaris disease of rainbow trout: low pH and salt as possible tools? Diseases of aquatic organisms 65: 115–120.

Tave, D., Mcginty, A.S., Chappell, J.A., Smitherman, R.O., 1981. Relative harvestability by angling of blue catfish, channel catfish, and their reciprocal hybrids. North American Journal of Fisheries Management 1 (1), 73–76.

Thomas-Jinu, S., Goodwin, A., 2004. Acute columnaris infection in channel catfish, *Ictalurus punctatus* (Rafinesque): efficacy of practical treatments for warmwater aquaculture ponds, Journal of Fish Diseases 27:23–28.

Tripathi, N.K., Latimer, K.S., Gregory, C.R., Ritchie, B.W., Wooley, R.E., Walker, R.L., 2005. Development and evaluation of an experimental model of cutaneous columnaris disease in koi *Cyprinus carpio*, Journal of veterinary diagnostic investigation17:45−54.

Trung Cao, T., Tsai, M.A., Yang, C.D., Wang, P.C., Kuo, T.Y., Gabriel Chen, H.C., Chen, S.C., 2014. Vaccine efficacy of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from *Edwardsiella ictaluri* against *E. tarda* in tilapia. The Journal of general and applied microbiology 60(6):241−250.

Thune, R.L., Collins, L.A., Penta, M.P., 1997. A comparison of immersion, immersion/oral combination and injection methods for the vaccination of channel catfish *Ictalurus punctatus* against *Edwardsiella ictaluri*. Journal of the World Aquaculture Society 28(2):193−201.

Wagner, B.A., Wise, D.J., Khoo, L.H., Terhune, J.S., 2002. The epidemiology of bacterial diseases in food-size channel catfish. Journal of Aquatic Animal Health 14: 263−272.

Wakabayashi, H., 1991. Effect of environmental conditions on the infectivity of *Flexibacter columnaris* to fish, Journal of Fish Diseases 14:279−290.

Waltman, W., Shotts, E.B., Blazer, V.S., 1985. Recovery of *Edwardsiella ictaluri* from danio (*Danio devario*). Aquaculture 46:63−66.

Waltman, W.D., Shotts, E.B., Hsu, T.C., 1986. Biochemical characteristics of Edwardsiella ictaluri. Applied and environmental microbiology 51(1):101−104.

Welker, T.L., Shoemaker, C.A., Arias, C.R., Klesius, P.H., 2005. Transmission and detection of *Flavobacterium columnare* in channel catfish *Ictalurus punctatus*. Diseases of aquatic organisms 63(2-3):129−38.

Williams, M.L., Lawrence, M.L., 2010. Verification of an *Edwardsiella ictaluri*-specific diagnostic PCR. Letters in Applied Microbiology 50:153–157.

Wolters, W.R., Johnson, M.R., 1994. Enteric septicemia resistance in blue catfish and three channel catfish strains. Journal of Aquatic Animal Health 6:329–34.

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(3), 249–254.

Yant, R., Smitherman, R.O., Green, O.L., 1975. Production of hybrid (blue × channel) catfish and channel catfish in ponds. In Proceedings Annual Conference Southeast, Association of Game and Fish Commissioners, 29, 86–89.

Yeh, H.Y., Shoemaker, C.A., Klesius, P. H., 2005. Evaluation of a loop-mediated isothermal amplification method for rapid detection of channel catfish *Ictalurus punctatus* important bacterial pathogen *Edwardsiella ictaluri*. Journal of microbiological methods 63(1), 36–44.

Zhang, Y. Molecular characterization of *Edwardsiella* spp. and *flavobacterium columnare*. Ph.D. Dissertation. 2007. Auburn University, AL.

Zhu, Z., He, L., Chen, S., 1985. Novel gene transfer into the fertilized eggs of gold fish (*Carassius auratus* L. 1758). Journal of Applied Ichthyology 1(1): 31–34.

II. Columnaris resistance of different genetic types of channel catfish, *Ictalurus punctatus*, female × blue catfish, *I. furcatus*, male hybrid catfish

2.1 Introduction

In the United States, the production of ictalurid catfish is a significant part for the aquaculture industry counting for an estimated 68% of the domestic freshwater fish production (NMFS, 2015). In the past decades, catfish production had consistent growth from the 1980s to the early 2000s, however, the production in 2015 was only 54% of the peak value in 2003 (Hanson and Sites, 2015). The annual outbreak of fish disease contributed to the loss of market share of the US catfish industry (FAO, 2011).

Columnaris can infect different stages of catfish and cause economic losses of 30 million dollars in the US annually (Shoemaker et al., 2011). Based on 2012 annual case reports, columnaris disease was the most frequently diagnosed bacterial disease in commercial catfish farms (MSU, 2012). In the southeastern United States, columnaris is known as a disease that can cause very high mortality second only to enteric septicemia of catfish (ESC) (Durborow et al., 1998).

Columnaris was first found nearly one century ago (Davis, 1922). The causative agent of this disease is *Flavobacterium columnare*, a gram-negative, aerobic, rod-shaped bacterium, which was isolated and characterized by Ordal and Rucker (1944). The mortality of tank-held channel catfish fingerlings can reach 90% under optimal conditions for disease development while the mortality of pond populations is usually 50-60% (Plumb and Hanson, 2011).

To prevent the outbreak of this disease, genetic enhancement, such as interspecific hybridization, to effectively improve the fish disease resistance is one of the strategies that have been implemented. Channel catfish (*Ictalurus punctatus*) × blue catfish (*I. furcatus*) (C×B) hybrid catfish has several superior traits compared to channel catfish, including faster growth, higher production, higher survival rate and enhanced disease resistance in pond culture, and thus, it is widely cultured in commercial farms currently (Chatakondi et al., 2000; Dunham et al., 1998; Giudice, 1966).

Various genetic types of hybrids may differ due to the different disease resistances of

their parental fish. The objective of this study was to compare the disease resistances of different genetic types of C×B hybrid catfish and to identify the strains of channel catfish and blue catfish that have good combining ability for genetic enhancement of the resistance to columnaris.

2.2 Materials and methods

2.2.1 Experimental fish

Eleven genetic types of C×B hybrid catfish were used in this study. They were Kansas Random × Rio Grande, Kansas Select × Rio Grande, 103KS × Rio Grande, Marion Select × Rio Grande, 103 KS × B, Marion Select × B, Auburn-Rio Grande × B, 103KS × D&B, Kansas Random × D&B, Marion Random × D&B, Kansas Select × D&B.

Kansas Random (KR) is the oldest strain of channel catfish in the US, and was collected from the Ninnescha River, KS in 1911 (Dunham and Smitherman 1984). Kansas Select (KR) has been selected for 8 generations for increased body weight. 103 KS is an F_2 generation cross between NWAC-103 and KS. NWAC-103 was selected for growth for 2 generations and originated from a fast growing strain. Marion Random (MR) was originally collected from the Red River, OK in 1949, but several strains were mixed with these fish over time (Dunham and Smitherman 1984). Marion Select (MS) has been selected for 8 generations for increased body weight. Auburn-Rio Grande (AR) select line originated from crossing Auburn channel catfish females with Rio Grande channel catfish males in 1976. From that time forward, this line has been selected for growth rate. Auburn strain originated from several riverine sources and Rio Grande from the Rio Grande River, TX. Rio Grande blue catfish also originated from the Rio Grande River, TX and D&B is a commercial strain developed in Texas (Dunham and Smitherman, 1984). B was AR strain of blue catfish.

2.2.2 Preparation of experimental fish

All procedures involving the handling and treatment of fish in this study were approved by the Auburn University Institutional Animal Care and Use Committee (AU-IACUC).

All the fish were grown in pond R-2 for two years at the Auburn University E.W. Shell Fisheries Research Center. They were seined and transferred to the counting shed for 4 months, and then moved to the challenge facilities one week before challenge for

acclimation. Eighty fish (average size 0.85 ± 0.28 kg) were stocked in four replicate holding tanks (600 L water). Water temperature (25.99 ± 0.49 ℃), dissolved oxygen (6.10 ± 0.28 mg/L), pH (6.78 ± 0.16) were checked daily.

2.2.3 Bacterial challenge

The BGFS-27 isolate (genomovar II) of *F. columnare* was used in this challenge. It is a virulent *F. columnare* isolate that is known to cause heavy mortalities by two days in aquaculture settings. The bacteria were re-isolated from a symptomatic fish. The bacteria were then cultured in modified Shieh (MS) broth and incubated in a shaker incubator at 28 ℃, 100rpm for 24 h. The concentration of the bacteria was 6×10^8 CFU /mL as determined by plate colony-counting method after ten-fold gradient dilutions on MS agar plates. Bacterial culture of *F. columnare* was diluted 10 times by PBS solution to make bacterial solution for injection.

Eight carrier fish (0.70 ± 0.05 kg) were injected with 1 ml of bacterial solution (1×10^7 CFU/mL) into the peritoneal cavity. The IP injection was performed by injecting in the abdomen at a 45 degree angle between the pelvic fins and the anal vent. These eight fish were marked by fin clips and randomly assigned into the four tanks (each tank with two carrier fish). The numbers of moribund and dead fish were recorded daily which did not include the injected ones until 14^{th} day following challenge.

2.2.4 Data analysis

The mortality and average survival time by tank, genetic type and sex of C×B hybrid catfish were calculated. The mean mortality and mean survival time of genetic types with common sires pooled (RG, DB and B) and common dams pooled (KR, KS, 103KS, MR, MS and AR) were calculate to evaluate sire and dam effects on disease resistance. All the data of survival time and cumulative mortalities were analyzed by Statistical Analysis System (SAS® 9.3 Software, SAS Institute Inc., NC, USA). Two/three-way analysis of variance (ANOVA) was conducted to determine the significance of tank, sex, genetic type and tank × genetic types, sex × genetic types and tank × sex × genetic types effects.

Multiple comparisons of mortalities, survival times and median death time were conducted using t test with Fisher's least significant difference (LSD) at the 0.05 level. The same comparison method was utilized to compare the mortalities, survival times and median death time of the data pooled by sire genetic type and dam genetic type, as well as

determine the sire effect, dam effect and sire × dam effect on disease resistance.

2.3 Results

According to the outputs of ANOVA, the *P* values for tank, sex, genetic types, and interaction between tank and sex, tank and genetic type, genetic type and sex were 0.18, 0.22, 0.02, 0.26, 0.79 and 0.24, respectively. The *P* value of interaction among tank, genetic type and sex were 0.94. Therefore, genetic type was a major effect ($P<0.05$) for disease resistance. And the data of four tanks were combined for further analysis.

2.3.1 Mortality

The cumulative mortality of eleven genetic types of C × B hybrid catfish is reported in Table 1. The mortality of KS × RG、103KS × B、AR × B、KR × DB、MR × DB genetic types of C × B hybrid catfish were all 100%, which were 50.0% and 42.3% higher than those of 103KS × RG and MS × RG hybrid catfish. For hybrid catfish males, mortalities of six genetic types of hybrids (KS × RG, 103KS × B, AR × B, KR × DB, MR × DB and KS × DB) were 100%, while eight genetic types of hybrid females (KS × RG, 103KS × RG, 103KS × B, MS × B, AR × B, 103KS × DB, KR × DB and MR × DB) reached 100% mortality. The genetic type of hybrids with the lowest mortality were male 103KS × RG (25%) and female MS × RG (50%)。The results of ANOVA indicated that genetic type is the major factor that affected the mortality of hybrid catfish and the sex effect on mortality was not significant.

Table 1 Average weight an cumulative mortality of eleven genetic types of female channel catfish (*Ictalurus punctatus*) × male blue catfish (*I. furcatus*), hybrid catfish at the end of the challenge experiments with virulent *Flavobacterium columnare*.

Genetic type[1]	Weight[2] (kg)	Mortality[3] (%)	Weight[2] (kg)		Mortality[4] (%)	
			Male	Female	Male	Female
KR x RG	0.87 ± 0.31	72.7[ab] ± 9.4	0.75	0.99	67.8	80.0
KS x RG	0.86 ± 0.12	100.0[b] ± 0.0	0.83	0.87	100.0	100.0
103KS x RG	0.57 ± 0.15	50.0[a] ± 35.4	0.67	0.54	25.0	100.0
MS x RG	1.00 ± 0.36	57.7[a] ± 11.8	1.07	0.93	67.8	50.0
103KS x B	0.85 ± 0.56	100.0[b] ± 0.0	0.60	1.01	100.0	100.0
MS x B	0.88 ± 0.34	75.0[ab] ± 35.4	0.49	1.08	50.0	100.0
AR x B	0.67 ± 0.32	100.0[b] ± 0.0	0.43	0.91	100.0	100.0
103KS x DB	0.97 ± 0.29	83.3[ab] ± 17.7	1.10	0.89	67.8	100.0
KR x DB	0.63 ± 0.19	100.0[b] ± 0.0	0.47	0.75	100.0	100.0
MR x DB	0.76 ± 0.26	100.0[b] ± 0.0	0.88	0.64	100.0	100.0
KS x DB	0.79 ± 0.19	83.3[ab] ± 17.2	0.80	0.77	100.0	67.8

[1] Blue catfish sires were Rio Grande (RG), D&B (DB) and AR(B). Channel catfish lines were Kansas random (KR), Kansas select (KS, selected for 8 generations for increased body weight), Marion random (MR), Marion select (MS, selected for 8 generations for increased body weight), 103KS (an F2 generation cross between NWAC-103 and KS) and Auburn-Rio Grande (AR, Auburn channel catfish females cross with Rio Grande channel catfish males and selected for growth rate).

[2] No significant difference among weight of each genetic type of hybrid catfish and between weight of males and females of hybrid catfish.

[3] The values with the same superscript lowercase letters are not significantly different at 0.05 probability level with t test (Fisher's least significant difference method).

[4] No sex effect on mortality on each genetic type of hybrid catfish.

2.3.2 Survival time

The mean survival times of eleven genetic types of hybrid catfish are shown in Fig. 1. Survival times of MS × RG males and females, as well as AR × B males and females were equal (Fig. 1a). The values of survival times of male and female of other genetic type

hybrids had no significant difference (P>0.05). ANOVA results indicated that there were no sex effects or sex × genetic type interaction on survival times. Genetic type was the main effect on survival time.

The mean survival times of KR × RG, MS × RG, AR ×B and KR × DB hybrids were all less than five days, and lower (P<0.05) than those of other seven genetic types of hybrid catfish. MS × RG and KR × DB hybrid catfish had the shortest survival time, which was 3.5 day. The mean survival times of 103KS × B and 103KS × DB hybrid catfish were the longest, which were more than 7 days (Fig. 1b).

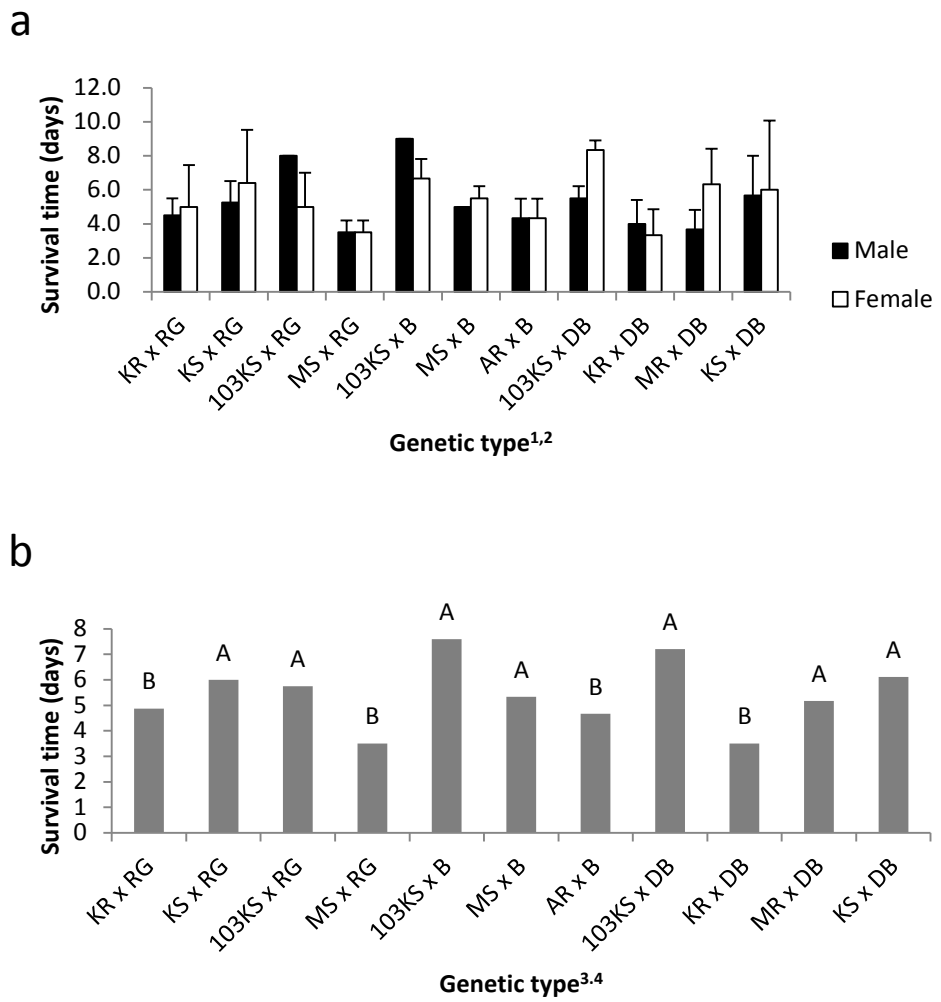

Fig. 1 Survival time of eleven genetic types of female channel catfish (*Ictalurus punctatus*) × male blue catfish (*I. furcatus*), (C ×B) hybrid catfish in the challenge experiments with virulent *F. columanre*: (a) average survival time of male and female C ×B hybrid catfish; (b) survival time of C ×B hybrid catfish, the values with the same capital letters are not significantly different at 0.05 probability levels with t test (Fisher's least significant difference method).

[1,3] Blue catfish sires were Rio Grande (RG), D&B (DB) and AR(B). Channel catfish lines were Kansas random (KR), Kansas select (KS, selected for 8 generations for increased body weight), Marion random (MR), Marion select (MS, selected for 8 generations for increased body weight), 103KS (an F2 generation cross between NWAC-103 and KS) and Auburn-Rio Grande (AR, Auburn channel catfish females cross with Rio Grande channel catfish males and selected for growth rate).

[2] No significant difference was observed between the survival times of males and females for each genetic type of hybrid catfish (P>0.05).

[4] The columns with the same capital letters are not significantly different at 0.05 probability level with t test (Fisher's least significant difference method).

2.3.3 Sire and dam effect on mortality and survival time

The cumulative mortalities of three sire genetic types and six dam genetic types are reported in Table 2. The mortality of hybrid catfish with the common sire and dam genetic type ranged from 73.5% to 93.3%, and from 72.7% to 100%, respectively. There were no sire, dam and sire × dam effects on mortalities.

Table 2 Cumulative mortalities of three sire genetic types and six dam genetic types of female channel catfish (*Ictalurus punctatus*) × male blue catfish (*I. furcatus*), hybrid catfish at the end of the challenge experiments with virulent *Flavobacterium columnare*.

	Genetic type[1]	Mortality (%)[2]	Survival time[3] (days)
Sire	RG	73.5 ± 33.3	5.5 ± 4.8
	DB	82.8 ± 11.9	5.2 ± 3.0
	B	93.3 ± 10.0	5.7 ± 3.1
Dam	KR	81.3 ± 21.3	4.3^{b} ± 4.6
	KS	90.0 ± 12.5	5.9^{ab} ± 3.8
	103KS	73.7 ± 18.9	6.9^{a} ± 4.0
	MR	100.0 ± 0.0	5.2^{ab} ± 2.1
	MS	72.7 ± 50.0	5.4^{ab} ± 5.2
	AR	100.0 ± 0.0	4.8^{b} ± 1.1

[1] Blue catfish sires were Rio Grande (RG), D&B (DB) and AR (B). Channel catfish lines were Kansas random (KR), Kansas select (KS, selected for 8 generations for increased body weight), Marion random (MR), Marion select (MS, selected for 8 generations for increased body weight), 103KS (an F2 generation cross between NWAC-103 and KS) and Auburn-Rio Grande (AR, Auburn channel catfish females cross with Rio Grande channel catfish males and selected for growth rate).

[2] No sire or dam effects were observed on mortalities ($P>0.05$).

[3] The values with the same superscript lowercase letters are not significantly different at 0.05 probability level with t test (Fisher's least significant difference method).

For survival time, no sire effect and no sire × dam effects were observed. However, there were differences ($P<0.05$) among six dam genetic types of hybrids (Table 2). The hybrid catfish with dam genetic type of 103KS has the longest mean survival duration, which was almost seven days. The dam genetic types with the shortest survival times were KR (4.5 days) and AR (4.7 days).

2.3.4 Median death time

The median death time of eleven genetic types of C × B hybrid catfish is reported in Table 3, which ranges from 4 days to 10 days. The median death time of 103KS × RG, 103KS ×B and 103KS × DB genetic types of C × B hybrid catfish were significantly longer (P<0.05) than MS × RG, KR × DB and KS × DB. For hybrid catfish males, the median death time ranged from 3 days to 15 days and for females, it ranged from 4 days to 8 days. There was no difference between the median death time of males and females for each genetic type of C × B hybrid catfish.

Table 3 Median death time of eleven genetic types of female channel catfish (*Ictalurus punctatus*) × male blue catfish (*I. furcatus*), hybrid catfish in the challenge experiments with virulent *Flavobaterium. Columanre.*

Genetic type[1]	Median death time[2] (days)	Median death time (days)[3]	
		Male	Female
KR x RG	5^{ab}	5	5
KS x RG	5^{ab}	5	5
103KS x RG	10^{a}	15	5
MS x RG	4^{b}	4	4
103KS x B	8^{ab}	9	6
MS x B	5^{ab}	5	5
AR x B	5^{ab}	5	5
103KS x DB	7^{ab}	6	8
KR x DB	4^{b}	3	4
MR x DB	6^{ab}	4	7
KS x DB	5^{b}	4	5

[1] Blue catfish sires were Rio Grande (RG), D&B (DB) and AR (B). Channel catfish lines were Kansas random (KR), Kansas select (KS, selected for 8 generations for increased body weight), Marion random (MR), Marion select (MS, selected for 8 generations for increased body weight), 103KS (an F2 generation cross between NWAC-103 and KS) and Auburn-Rio Grande (AR, Auburn channel catfish females cross with Rio Grande channel catfish males and selected for growth rate).

[2] The values with the same superscript lowercase letters are not significantly different at 0.05 probability level with t test (Fisher's least significant difference method).

[3] No significant difference between median death time of males and females of hybrid catfish (P>0.05).

The median death time of three sire genetic types and six dam genetic types are

reported in Table 4. There was no significant difference (P>0.05) among the sire genetic type of C × B hybrid catfish. The median death time of 103KS genetic type C × B hybrid catfish was the longest as 8 days and significantly longer (P<0.05) than that of other five dam genetic types of C × B hybrid catfish.

Table 4 Median death time of three sire genetic types and six dam genetic types of female channel catfish (*Ictalurus punctatus*) × male blue catfish (*I. furcatus*), hybrid catfish in the challenge experiments with virulent *Flavobacterium. Columanre*

	Genetic type[1]	Median death time (days)
Sire[2]	RG	5.5
	DB	5.3
	B	6
Dam[3]	KR	4.5[b]
	KS	5[b]
	103KS	8[a]
	MR	4[b]
	MS	4.5[b]
	AR	5[b]

[1] Blue catfish sires were Rio Grande (RG), D&B (DB) and AR (B). Channel catfish lines were Kansas random (KR), Kansas select (KS, selected for 8 generations for increased body weight), Marion random (MR), Marion select (MS, selected for 8 generations for increased body weight), 103KS (an F2 generation cross between NWAC-103 and KS) and Auburn-Rio Grande (AR, Auburn channel catfish females cross with Rio Grande channel catfish males and selected for growth rate).

[2] No sire effect were observed on median death time (P>0.05).

[3] The values with the same superscript lowercase letters are not significantly different at 0.05 probability level with t test (Fisher's least significant difference method).

2.4 Discussion

Several studies compared the columnaris resistance of C × B hybrid catfish and the parental fish of hybrids (Arias et al., 2012; Dunham et al., 2008). However, there are no reports on the resistance of various genetic types of C × B hybrid catfish against columnaris.

In current study, there was no apparent trend in the disease resistance against columnaris based on either sex or interaction between sex and genetic type. Genetic type affected disease resistance of C × B hybrid catfish. However, the relationship between total mortality and average survival time were not consistent. Although the mortality of KR × DB

and AR ×B hybrid catfish was the highest,100.0%, and they had the second and third lowest survival time at 3.5 days and 4.7 days, respectively. The trends of mortality and survival time of MS × RG, KR × RG, KS × DB, 103KS × DB and 103KS × B seemed to be the opposite. The mortalities of MS × RG and KR × RG hybrid catfish were low but their survival durations were short, while the mortalities of KS × DB, 103KS × DB and 103KS × B hybrid catfish were high with relative longer survival duration. Looking at both traits simultaneously, the latter group was the most resistant to columnaris.

The C × B hybrid catfish used in this experiment were all grown in the same pond for two years, thus having uniform exposure to the ubiquitous *F. columnare* in the pond, other pathogen and water quality conditions prior to experimentation. Although not naïve, this type of preparation might produce results that are more relevant for commercial industry.

In an experimental challenge with columnaris, mortality in C×B hybrids was observed from day 4 post-challenge and the mortality of C×B hybrids was less than 50% (Arias et al., 2012), which was less than all genetic types of C × B hybrid catfish in the current study. The current experiment was conducted in winter. The infection may have been more severe as the current experiment may have mimicked what occurs during the spring, one of the peaks of disease season. Fish were being held at cold temperature and then were acclimated to warmer temperatures for the challenge. This is similar to temperatures warming in spring, which results in increases in disease incidence as the catfish immune system apparently does not activate as quickly as the pathogens. Genotype-environment interactions may also impact results when the genetic type of experimental fish and challenge methods differ.

No apparent sire effect on mortality and average survival times was observed. The dam effect was significant for average survival time. This partially explains why the hybrid catfish whose dam genetic types were 103KS and MS with longer average survival duration also had lower mortality, and the hybrid catfish whose dam genetic types were AR with the shorter average survival duration had higher mortality as well. This result appears to be consistent with the fact that the mortality of 103KS × RG hybrid catfish was lowest and the mortality of AR × B hybrid catfish was one of the highest when overall survival, median survival time and survival time are considered simultaneously, 103KS was the best dam type followed by MS.

The strongest effect on columnaris resistance was the dam effect. The best strategy to improve disease resistance of hybrid catfish is to identify female strains with the best combining ability. Potential genetics of these dam effects requires further study.

References

Arias C.R., Cai W., Peatman, E., Bullard S.A.,2012. Catfish hybrid *Ictalurus punctatus* × *I. furcatus* exhibits higher resistance to columnaris disease than the parental species. Diseases of aquatic organisms 100(1):77–81.

Chatakondi, N.G., Benfer, J., Jackson, L.S., Yant, D.R., 2000. Commercial evaluation of channel x blue hybrid catfish production and their performance in ponds. In Catfish Farmers of America Research Symposium at Albuqerque, NM, USA.

Davis, H.S., 1922. A new bacterial disease of fresh-water fishes: By HS Davis, Government Printing Office.

Dunham, R.A., Liu, Z., Argue, B.J., 1998. The effect of the absence or presence of channel catfish males on induced ovulation of channel catfish females for artificial fertilization with blue catfish sperm. The Progressive Fish-Culturist 60 (4), 297–300.

Dunham, R.A., Smitherman, R.O., 1984. Ancestry and breeding of catfish in the United States. Circ. 273. Alabama Agricultural Experiment Station, Auburn University, Alabama, USA. 100p.

Dunham, R.A., Umali, G.M., Beam, R., Kristanto, A.H., Trask, M., 2008. Comparison of production traits of NWAC103 channel catfish, NWAC103 channel catfish × blue catfish hybrids, Kansas Select 21 channel catfish, and blue catfish grown at commercial densities and exposed to natural bacterial epizootics. North American Journal of Aquaculture 70(1):98–106.

Durborow, R.M., Thune, R.L., Hawke, J.P., Camus, A.C., 1998. Columnaris Disease: A Bacterial Infection Caused by Flavobacterium Columnare. South Regional Aquaculture Centre SRAC, Texas A & M University.

FAO, 2011. United States of America. National Aquaculture Sector Overview Fact Sheets. In FAO Fisheries and Aquaculture Department, Rome.

Giudice, J.J., 1966. Growth of a blue × channel catfish hybrid as compared to its parent species. The Progressive Fish-Culturist 28(3), 142–145.

Hanson, T., Sites, D., 2015. 2014 U.S. Catfish Database. USDA National Agricultural Statistics Service (NASS), Mississippi Agricultural Statistics Service (MASS).

MSU, 2012 annual case summary report of the Aquatic Research and Diagnostic Laboratory. Mississippi State University, College of Veterinary Medicine, Thad Cochran, Stoneville, MS.

NMFS (National Marine Fisheries Service). 2015. Fisheries of the United States 2014. NMFS, Office of Science and Technology, Silver Spring, Maryland.

Ordal, E.J., Rucker, R.R., 1944. Pathogenic myxobacteria. Exp Biol Med (Maywood) 56 (1), 15–18.

Plumb, J.A., Hanson, L.A., 2011. Health maintenance and principal microbial diseases of cultured fishes, 3rd edition. John Wiley & Sons.

Shoemaker, C.A., Klesius, P.H., Drennan, J.D,. Evans, J.J., 2011. Efficacy of a modified live *Flavobacterium columnare* vaccine in fish. Fish and Shellfish Immunology 30:304–308.

III. Concomitant resistance to enteric septicemia of catfish and *Ichthyophthirius multifiliis* of channel catfish, *Ictalurus punctatus*, female × blue catfish, *I. furcatus*, male hybrid catfish and channel catfish

3.1 Introduction

Enteric septicemia of catfish (ESC) was first reported to infect catfish in Alabama and Georgia in 1976 (Hawke, 1979). More than fifty percent of catfish fry and fingerling losses in the US result from ESC infection, and the disease leads to great economic losses of millions of dollars in the catfish industry every year (Noga, 2010; Williams and Lawrence, 2010). Thus, it has been regarded as one of the most prevalent diseases affecting commercial catfish farm.

The causative agent of ESC is the bacterium *Edwardsiella ictaluri* (Hawke et al., 1981). It can infect fish via the gut route and nervous route in acute and chronic forms, respectively (Noga, 2010).

In artificial challenges, ESC can cause the acute form of infection that leads to high mortality in a few days after infection (Peatman et al., 2007). In the ponds, outbreaks of ESC often happen and cause relatively high mortality in spring and fall during the optimal 24-28 ℃ water temperature for the pathogenicity of *E. ictaluri* (Noga, 2010). In other seasons of a year, the disease can also occur in the chronic form and lead to low mortality (Noga, 2010). Plumb and Quinlan (1986) indicated that *E. ictaluri* can survive in mud at 25 ℃ for over one and half months. ESC may recur in the pond, particularly in the southeastern farms and ponds because of the high temperatures.

Disease resistances of catfish against ESC vary with different strains and families of catfish. Generally, disease resistance against ESC of blue catfish is stronger than that of channel catfish, *Ictalurus punctatus*, female × blue catfish, *I. furcatus*, male (C×B) hybrid catfish, followed by channel catfish (Wolters and Johnson, 1994; Wolters et al., 1996). In catfish industry, both intraspecific crossbreeding and interspecific hybridization have been applied to improve disease resistance (Dunham and Smitherman, 1987; Dunham, 2011).

Various genetic types of hybrids may differ due to the different disease resistances of their parental fish. The objective of this study was to compare the disease resistance to ESC

of different genetic types of C×B hybrid catfish and channel catfish.

3.2 Materials and methods

3.2.1 Experimental fish

Two genetic types of C×B hybrids catfish and one strain of channel catfish were used in this study. A large number of channel catfish females from several strains were hybridized with two blue catfish sire types, Rio Grande (RG) and D&B × Rio Grande (DR). These hybrids were compared to a single crossbreed channel catfish, is mix female × (ARMK family195 × 15) male.

3.2.2 Preparation of experimental fish

The channel catfish female × Rio Grande blue catfish male (C × RG) hybrid catfish and mix female × (ARMK family195 × 15) male (U × ARMK) channel catfish were reared in R-21 and M-9 ponds at Auburn University E.W. Shell Fisheries Research Center. The channel catfish female × (D&B × Rio Grande) blue catfish male (C × DR) hybrids were reared in G-14 pond at Auburn University Fish Genetics Research Unit. They were seined and transferred to the challenge facilities one week before challenge for acclimation. Fish were challenged in three replicate tanks (300 L water). Sixty fish were placed in each tank, twenty U × ARMK channel catfish (average size 25.33 ± 12.80 g), twenty C × RG hybrids (average size 26.38 ± 11.64 g) and twenty C × DR hybrids (average size 25.21 ± 9.26 g) each. Water temperature (27.40 ± 0.59 ℃), dissolved oxygen (8.78 ± 0.35 mg/L), pH (6.76 ± 0.09) were checked daily.

3.2.3 Bacterial challenge

E. ictaluri MS-S97-773 was isolated from a natural outbreak and utilized in the experimental challenge. Bacteria were re-isolated from a single symptomatic fish and biochemically confirmed to be *E. ictaluri*, before being inoculated into brain heart infusion (BHI) medium and cultured in a shaker incubator at 28 ℃ overnight. The concentration of the bacteria culture was measured by 10-fold serial dilutions on BHI agar plates as 1.8×10^{10} CFU/mL. The challenge experiments were performed by immersion. The water inlet was turned off in each tank for 1 h. Bacterial culture was added to each tank to make the final concentration at 2×10^{7} CFU/mL. After 1 hour of the immersion exposure, water was

turned on again. Dying fish with typical ESC clinic signs were collected. The numbers of moribund and dead fish were recorded every 12 hours during 108 hours after immersion challenge.

3.2.4 Data analysis

According to the mortality records, survival time, median death time and cumulative mortalities of C × RG and C × DR hybrid catfish and U × ARMK channel catfish at different time-points of challenge process were calculated, respectively. Survival time equals to the sum of survival time of each fish divided by the total number of fish. Median death time equals duration of time to reach 50% mortality.

All the data of average survival time, median death time and cumulative mortalities were analyzed by Statistical Analysis System (SAS® 9.3 Software). Average survival times, median death time and mortalities of the two genetic types of hybrid catfish and one channel catfish were compared using t test with Fisher's least significant difference (LSD).

In the area of crop research, AUWPC (area under wilt progress curve) and AUDPC (area under disease progress curve) are often used to evaluate quantitative disease resistance of different cultivars and measure the disease severity (Jeger and Viljanen-Rollinson, 2001). In our study, area under the mortality progress curve (AUMPC) was used to characterize the rate of disease progress. The measurement was adapted from the index of area under wilt progress curve (AUWPC) used to estimate the rate of expression of slow mildewing in Knox wheat (Schaner and Finney, 1977). In our research, the AUMPC was calculated using the following formula suggested by Schaner and Finney (1977):

$$\mathrm{AUMPC} = \sum_{i=1}^{n-1} [(y_i + y_{i+1})/2]\,(t_{i+1} - t_i)$$

where y_i is the number of moribund fish and dead fish at time t_i and n is the total number of time points.

AUMPC index of the three genetic types of catfish on each time point was calculated with the data of cumulative mortalities and the formula above. Different regression models were fitted to the change pattern of AUMPC index. Multiple comparison procedures with Tukey method were used to compare the AUMPC at different time-points. The slopes of AUMPC between 12 hour intervals were calculated and compared with two-sample t test.

In the *Edwardsiella ictaluri* challenge experiment, *Ichthyophthirius multifiliis* (Ich) also occurred. The mortality due to Ich was also recorded and analyzed.

3.3 Results

3.3.1 Results for ESC infection

Average survival time and cumulative mortalities of C × RG hybrid catfish, C × DR hybrid catfish and U ×ARMK channel catfish were shown in Table 5. The mean survival time of R × G hybrid catfish, DB × RG hybrid catfish, and U × (ARMK 195×15) channel catfish were 68.9 h, 73.9 h and 74.3 h, respectively. The median death times of the three genetic types of catfish were 80 h, 84 h and 84 h, respectively. The mean mortalities of these three genetic types of catfish from ESC were 73.3%, 75% and 73.3%, respectively. One-hundred percent of the fish died, however, as the fish also became infected with *Ichthyophthirius Multifiliis*. The *P* values of comparison of survival time, median death time and mortality were 0.79, 0.37 and 0.98, respectively. Thus, there were no significant differences of the average survival time, median death time and cumulative mortalities from ESC ($P>0.05$).

Table 5 Survival time, median death time and mortalities of two genetic types of female channel catfish (*Ictalurus punctatus*) × male blue catfish (*I. furcatus*), hybrid catfish and one genetic type of channel catfish (*I. punctatus*) when challenged with *Edwardsiella ictaluri* in tanks.

Genetic type[1]	Survival time[2] (hours)	Median death time[3] (hours)	Mortality[4] (%)
C x DR	68.9 ± 8.2	80 ± 7	73.3 ± 10.4
C x RG	73.9 ± 4.0	84 ± 0	75.0 ± 18.0
U x ARMK	74.3 ± 12.9	84 ± 12	73.3 ± 5.7

[1] Blue catfish sires were D&B × Rio Grande (DR) and Rio Grande (RG), channel catfish dams were a mixture of several strains (C). Channel catfish line was mix female × (ARMK family195 × 15) male (U x ARMK).

[2,3,4] No significant differences were observed among survival time, median death time and mortality for three groups of catfish at a probability level of 0.05 with a t test (Fisher's least significant difference method).

The AUMPC indexes over times were illustrated in Fig. 2. At each time point, the *P* values of comparison of AUMPC from 12-h time-point to 96-h time-point were 0.77, 0.88, 0.96, 0.92, 0.86, 0.81, 0.82 and 0.86, respectively. Therefore, no significant difference was found among the AUMPC indexes ($P>0.05$), also indicating no difference in mortality rates.

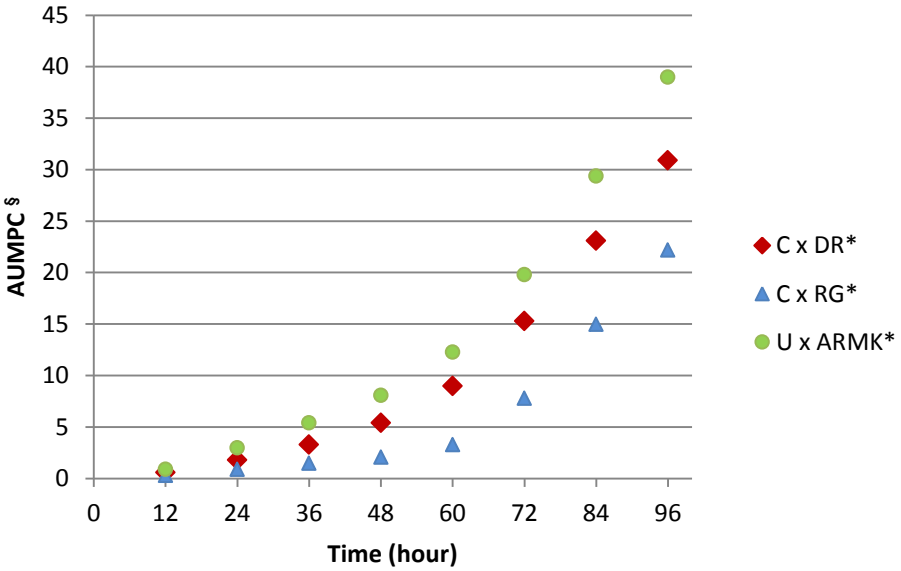

Fig. 2 AUMPC of two genetic types of female channel catfish (*Ictalurus punctatus*) × male blue catfish (*I. furcatus*), hybrid catfish and one genetic type of channel catfish (*I. punctatus*) during a *Edwardsiella ictaluri* challenge experiment in tanks.

* Blue catfish sires were D&B × Rio Grande (DR) and Rio Grande (RG), channel catfish dams were a mixture of several strains (C). Channel catfish line was mix female × (ARMK family195 × 15) male (U x ARMK).

§ No significant difference among AUMPC values of three groups of catfish at different time-points with multiple comparison procedures (Tukey method) at the 0.05 probability level.

Linear model and polynomial model were used to fit the AUMPC index. The equations, values of multiple R-squared and adjusted R-squared were shown in Table 6. R-squared is a statistical index to measure how close the data are to the fitted regression line. According to the values of multiple R-squared and adjusted R-squared, polynomial model were much better fit the AUMPC change patterns.

Table 6 Regression equations for AUMPC change patterns in a *Edwardsiella ictaluri* challenge experiment for two genetic types of female channel catfish (*Ictalurus punctatus*) × male blue catfish (*I. furcatus*), hybrid catfish and one genetic type of channel catfish (*I. punctatus*) in tanks.

Genetic type*	Model	Equation§	Multiple R-squared	Adjusted R-squared
C x DR	Linear	Y(1)=-8.0143+0.3554X	0.9005	0.8839
	Polynomial	$Y(1) = 0.0049X^2 - 0.1696X + 2.4857$	0.9976	0.9966
C x RG	Linear	Y(2)=-6.4286+0.2420X	0.7963	0.7623
	Polynomial	$Y(2)= 0.0049X^2 - 0.2871X + 4.1518$	0.9843	0.978
U x ARMK	Linear	Y(3)=-9.1607+0.4426X	0.9142	0.8999
	Polynomial	$Y(3)= 0.0055X^2 - 0.1561X + 2.8125$	0.9968	0.9955

* Blue catfish sires were D&B × Rio Grande (DR) and Rio Grande (RG), channel catfish dams were mixed several strains (C). Channel catfish line was mix female × (ARMK family195 × 15) male (U x ARMK).

§ Y=AUMPC index, X=time in hours.

The three polynomial curves were shown in Fig. 3. From the three curves, the three tendency of mortality change were almost the same. In polynomial model equation ($Y = a + b_1X + b_2X^2$), there are three parameters, a, b_1 and b_2. The parameter a, indicates the expected value of Y when X equals to zero. The parameter b_1 is the rate of change when X equals zero. The parameter b_2 reveals the direction and steepness of the curve

95% confidence limits of a, b_1 and b_2 in the polynomial curve equations for three genetic types of catfish were revealed in Table 7. No significant differences were observed among the three curves because overlaps were existed among the 95% confidence limits of each parameter within the three groups of catfish.

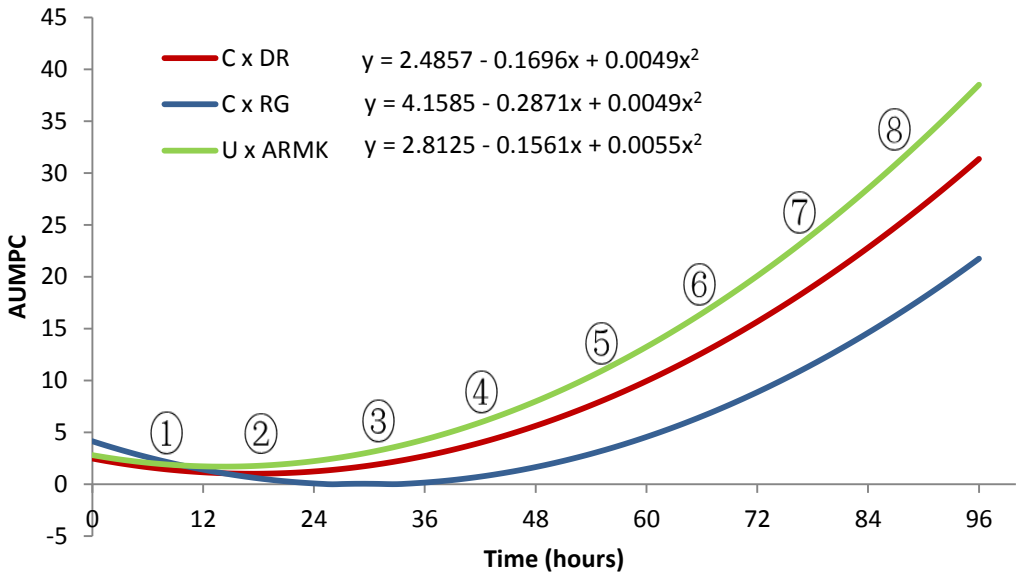

Fig. 3 Polynomial regression curves for AUMPC in a *Edwardsiella ictaluri* challenge experiment of two genetic types of female channel catfish (*Ictalurus punctatus*) × male blue catfish (*I. furcatus*), hybrid catfish and one genetic type of channel catfish (*I. punctatus*) in tanks.

Table 7 Comparison of 95% confidence limits of three parameters in the polynomial curve equations for a *Edwardsiella ictaluri* challenge experiment with two genetic types of female channel catfish (*Ictalurus punctatus*) × male blue catfish (*I. furcatus*), hybrid catfish and one genetic type of channel catfish (*I. punctatus*) in tanks.

Genetic Type*	Parameter a†		Parameter b_1‡		Parameter b_2§	
	Estimate	95% confidence limits	Estimate	95% confidence limits	Estimate	95% confidence limits
C x DR	2.4857	0.6806 ~ 4.2908	- 0.1696	-0.2463 ~ -0.0929	0.0049	0.0041 ~ 0.0056
C x RG	4.1518	0.8246 ~ 7.4789	- 0.2871	-0.4284 ~ -0.1457	0.0049	0.0036 ~ 0.0062
U x ARMK	2.8125	0.2595 ~ 5.3655	- 0.1561	-0.2646 ~ -0.0476	0.0055	0.0046 ~ 0.0065

* Blue catfish sires were D&B × Rio Grande (DR) and Rio Grande (RG), channel catfish dams were mixed several strains (C). Channel catfish line was mix female × (ARMK family195 × 15) male (U x ARMK).

† a is the expected value of Y when X = 0.

‡ b1 is the rate of change when X = 0.

§ b2 tells the direction and steepness of the curvature.

There was no significant genetic type effect when the data of survival time, median

death time and mortality were analyzed.

The *P*-values of two-sample t test for slopes of AUMPC between 12 hour intervals are shown in Table 8. From 0 h to 96 h post challenge, the duration was divided in to 8 intervals. The intervals were illustrated in Fig. 4. Among the three groups, only the tendency change between 5^{th} interval (48~60 hours post challenge) and 6^{th} interval (60~72 hours post challenge) was all significantly different ($P<0.05$). Other tendency changes between the adjacent time periods were not significant different ($P>0.05$) or not significant different at the same time. Two-sample t test was also conducted to compare the slopes between each pairs of genetic types, but no significant difference were observed ($P>0.5$).

Table 8 *P* values of interval slope comparison for AUMPC curves for mortality of two genetic types of female channel catfish (*Ictalurus punctatus*) × male blue catfish (*I. furcatus*), hybrid catfish and one genetic type of channel catfish (*I. punctatus*) by two sample t-test when challenged with *Edwardsiella ictaluri* in tanks.

Interval	*P* value		
	C x DR	C x RG	U x ARMK
1-2	0.0494	0.0572	0.3206
2-3	0.0669	0.2539	0.1994
3-4	0.0848	0.1835	0.2079
4-5	0.2601	0.0669	0.2167
5-6[§]	0.0057	0.0048	0.0109
6-7	0.2495	0.0219	0.1384
7-8	1.0000	0.4226	0.4226

[§] *P* values of three groups of catfish were all less than 0.05 with two-sample t test.

3.3.2 Results for Ich infection

Approximately, 25% of the fish became infected with ich and died during the ESC challenge. Average survival time and cumulative mortalities of C × RG hybrid catfish, C × DR hybrid catfish and U ×ARMK channel catfish were shown in Table 9. The mean survival time of C × RG hybrid catfish and C × DR hybrid catfish were the same as 101.2 h, and that of U × ARMK channel catfish was 98.4 h. The mortalities of the three genetic types of catfish were 20%, 25% and 30%, respectively. There were no significant differences of the average survival time and cumulative mortalities among them ($P>0.05$).

Table 9 Survival time and mortalities of two genetic types of female channel catfish (*Ictalurus punctatus*) × male blue catfish (*I. furcatus*), hybrid catfish and one genetic type of channel catfish (*I. punctatus*) due to *Ichthyophthirius multifiliis* in the challenge experiment with *Edwardsiella ictaluri* in tanks.

Genetic type[1]	Survival time[2] (hours)	Mortality[3] (%)
C x DR	101.2 ± 1.0	20.0 ± 15.0
C x RG	101.2 ± 5.0	25.0 ± 18.0
U x ARMK	98.4 ± 6.6	30.0 ± 10.0

[1] Blue catfish sires were D&B × Rio Grande (DR) and Rio Grande (RG), channel catfish dams were mixed several strains(C). Channel catfish line was mix female × (ARMK family195 × 15) male (U x ARMK).

[2,3] No significant differences among survival time, median death time and mortality among three groups of catfish at 0.05 probability level with t test (Fisher's least significant difference method).

The AUMPC indices over time are illustrated in Fig. 4. The *P* values of comparison of AUMPC at each time point were 0.42, 0.53, 0.46, 0.31, 0.27, 0.42, 0.52 and 0.52. Thus, no significant difference was found among the AUMPC indices at each time point (*P*>0.05).

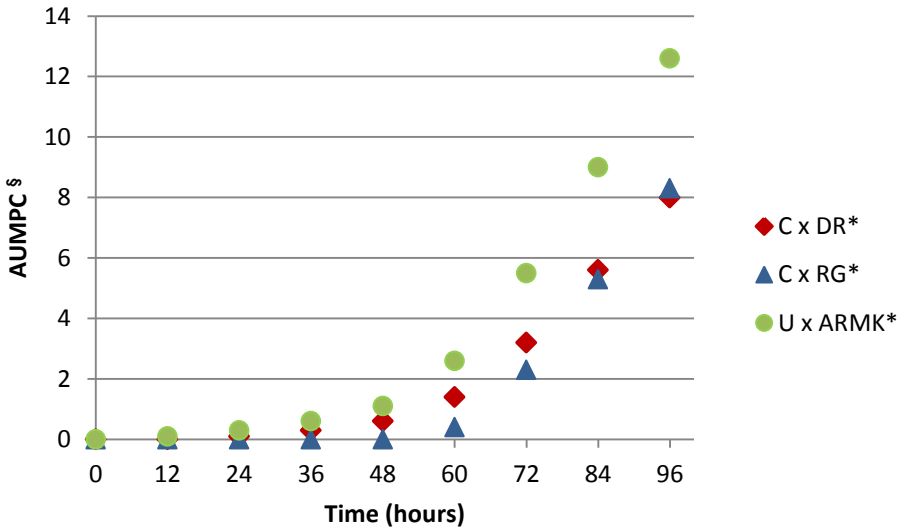

Fig. 4 AUMPC of two genetic types of female channel catfish (*Ictalurus punctatus*) × male blue catfish (*I. furcatus*), hybrid catfish and one genetic type of channel catfish (*I. punctatus*) due to *Ichthyophthirius multifiliis* during an *Edwardsiella ictaluri* challenge experiment in tanks.

[*] Blue catfish sires were D&B × Rio Grande (DR) and Rio Grande (RG), channel catfish dams were mixed several strains (C). Channel catfish line was mix female × (ARMK family195 × 15) male (U x ARMK).

[§] No significant difference among AUMPC values of three groups of catfish at different time-points with multiple comparison procedures (Tukey method) at 0.05 probability level.

Linear, polynomial and logistic modelling were used to fit the AUMPC index. The equations, values of multiple R-squared and adjusted R-squared are shown in Table 10. According to the values of adjusted R-squared and pseudo R-squared, logistic models were a much better fit for the AUMPC change patterns than linear and polynomial models.

Table 10 Regression equations for AUMPC change patterns due to *Ichthyophthirius multifiliis* infection in an *Edwardsiella ictaluri* challenge experiment for two genetic types of female channel catfish (*Ictalurus punctatus*) × male blue catfish (*I. furcatus*), hybrid catfish and one genetic type of channel catfish (*I. punctatus*) in tanks.

Genetic type[*]	Model	Equation[§]	Adjusted R-squared	Pseudo R-squared
C x DR	Linear	$Y(1)= -1.45556+0.07153X$	0.7613	-
	Polynomial	$Y(1) = 0.00131X^2 - 0.05452X + 0.30909$	0.9842	-
	Logistic	$Y(1) = 1/(0.1098+102.8e^{-0.0852X})$	-	0.9996
C x RG	Linear	$Y(2)=-1.79556+0.07514X$	0.6218	-
	Polynomial	$Y(2)= 0.00184X^2 - 0.10112X + 0.67212$	0.9525	-
	Logistic	$Y(2) = 1/(0.1017+2645.4e^{-0.1233X})$	-	0.9987
U x ARMK	Linear	$Y(3)=-2.43333+0.12431X$	0.7758	-
	Polynomial	$Y(3)= 0.00221X^2 - 0.08760X + 0.53333$	0.9883	-
	Logistic	$Y(3) = 1/(0.0585+28.8e^{-0.0753X})$	-	0.9997

[*] Blue catfish sires were D&B × Rio Grande (DR) and Rio Grande (RG), channel catfish dams were mixed several strains (C). Channel catfish line was mix female × (ARMK family195 × 15) male (U x ARMK).

[§] Y=AUMPC index, X=time in hours.

The three logistic curves are shown in Fig. 5. In the logistic model equation ($Y = 1/[1/b_1+(b_1-b_2)/(b_1*b_2)*\exp(-b_3)X)]$, and there are three parameters, b_1, which is the height of the horizontal asymptote when X tends to be infinite, b_2, which is the expected value of Y when X equals to zero, and b_3, which is a measurement of increase rate. The 95% confidence limits of b_1, b_2 and b_3 in the logistic curve equations for three genetic types of catfish are reported in Table 10.

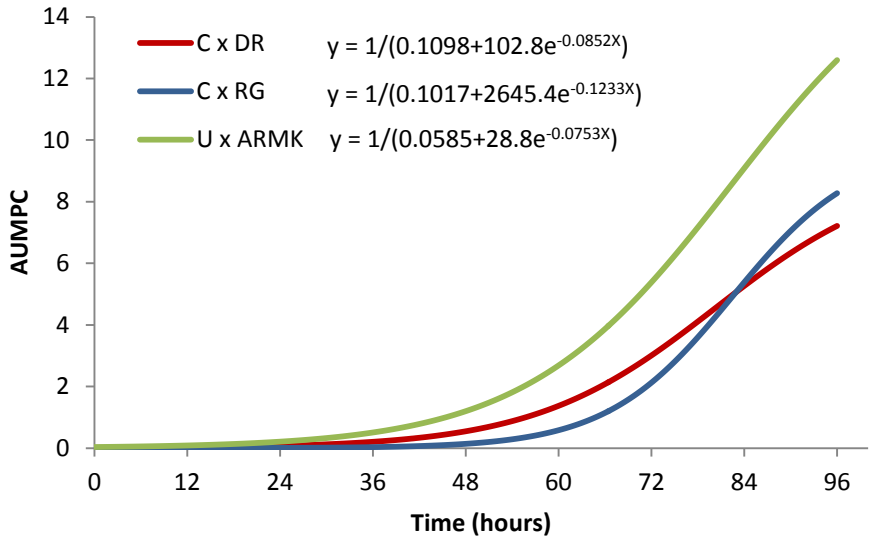

Fig. 5 Logistic curves for AUMPC due to *Ichthyophthirius multifiliis* infection in an *Edwardsiella ictaluri* challenge experiment of two genetic types of female channel catfish (*Ictalurus punctatus*) × male blue catfish (*I. furcatus*), hybrid catfish and one genetic type of channel catfish (*I. punctatus*) in tanks.

Table 11 Comparison of 95% confidence limits of three parameters in the logistic curve equations due to *Ichthyophthirius multifiliis* infection for an *Edwardsiella ictaluri* challenge experiment with two genetic types of female channel catfish (*Ictalurus punctatus*) × male blue catfish (*I. furcatus*), hybrid catfish and one genetic type of channel catfish (*I. punctatus*) in tanks.

Genetic Type*	Parameter b_1†		Parameter b_2‡		Parameter b_3§	
	Estimate	95% confidence limits	Estimate	95% confidence limits	Estimate	95% confidence limits
C x DR	9.1051	8.4901 ~ 9.7200	0.00971	0.00516 ~ 0.0143	0.0852	0.0783 ~ 0.0921
C x RG	9.8306	8.8067 ~ 10.8544	0.000378	-0.00020 ~ 0.000952	0.1233	0.1031 ~ 0.1435
U x ARMK	17.0858	15.7092 ~ 18.4624	0.0347	0.0212 ~ 0.0482	0.0753	0.0694 ~ 0.0812

* Blue catfish sires were D&B × Rio Grande (DR) and Rio Grande (RG), channel catfish dams were mixed several strains (C). Channel catfish line was mix female × (ARMK family195 × 15) male (U × ARMK).

† b_1 is the height of the horizontal asymptote when X tends to be infinite.

‡ b_2 is the expected value of Y when X = 0.

§ b_3 is a measurement of increase rate.

The parameter b_1 of the logistic curve of U × ARMK channel catfish was much larger than the other two curves for hybrid catfish. The estimation of b1 of the channel curve is greater than other two b_1 of hybrids, and their 95% confidence intervals do not overlap, so there is 95% probability this b_1 is greater than the other two. The parameter b_2 of the logistic curve of the three genetic types of catfish were different. The estimations of b_2 of the three curves are different, and their 95% confidence intervals do not overlap, thus there is 95% probability that these three b_2 are diverse from each other. The logistic curve of C × RG hybrid catfish had the minimum b_2 and U × ARMK channel catfish had the maximum b_2. The parameter b_3 of logistic curve of C × RG was greater than the other two curves. Thus, the mortality of U × ARMK channel catfish appears to be greater than that of C × DR and C × RG hybrid catfish when the time of infection is sufficient. However, the mortality of C × RG hybrid catfish may increase the most in a relative shorter time.

3.4 Discussion

Differences in ESC mortality among the two hybrid types and the channel catfish were subtle. The AUPMC analysis indicated that the rate of death for the channel catfish was more rapid than the hybrids and the C × RG hybrids had the slowest death rate. Rate of death is important as the longer the fish can survive the longer the culturist has to treat and potentially stop the infection. Based on the results of slope comparisons, the time before 48-60 hours of ESC infection would be critical to initial treatment. Similarly, although subtle, the extent of death and the rate of death were highest for channel catfish from ich infection.

Observations on commercial farms indicate that hybrid catfish would be expected to have greater ESC resistance than channel catfish. In the studies of Wolters et al. (1966) and Dunham et al. (2008), channel catfish × blue catfish hybrids demonstrated greater ESC resistance than channel catfish, and the differences were more substantial than in the current study.

Several factors might explain the differences in the current study and that of Wolters et al. (1996) and Dunham et al. (2008). Genotype-environment interactions may occur when there is concomitant infection with ESC and *Ichthyophthirius multifiliis* infection,

exacerbating the infection and accelerating the mortality. The multiple infections may produce results that are not be representative of that of a normal ESC infection and may make it more difficult to evaluate the impact of ESC infection on different genetic types of catfish. The onset of infection was much more rapid than what is normally seen for ESC infections. Ich may have caused epithelial damage, allowing more rapid entrance of *E. ictaluri.* Further experiments on mixed infections are needed as they may represent what is more likely to be the true commercial environment.

The current experiment was conducted in winter. The infection may have been more severe as the current experiment may have mimicked what occurs during the spring, one of the peaks of disease season. Fish were being held at cold temperature and then were acclimated to warmer temperatures for the challenge. This is similar to temperatures warming in spring, which results in increases in disease incidence as the catfish immune system apparently does not activate as quickly as the pathogens.

Genotype-environment interactions may also occur because the two genetic types of hybrids and one line of channels used in this experiment were reared separately in three different ponds before they were transferred to the challenge facilities. Potentially, different exposure dose may give different level of immune stimulation and immune response to ESC.

Another explanation, which could also be classified as a genotype-environment interaction is the intensity of the infection. The strain of *E. ictaluri*, the exposure dose and intensity of infection may have been too severe, making it more difficult for the genetic differences to be observed.

Another alternative is that U × ARMK channel catfish, a crossbreed, has high resistance similar to the hybrid catfish. Intraspecific crossbreeding of channel catfish strains has resulted in an increase in ESC resistance in 5 of 7 crosses evaluated (Wolters and Johnson 1995; Padi, 2003; Padi and Dunham 2009). Therefore, the strategy of intraspecific crossbreeding in channel catfish should also be seriously considered for improvement of disease resistance as well as interspecific hybridization.

Ich is typically a warm water disease, but it can break outccur in spring when fish are stressed from overwintering (Noga, 2010), and a warmwater type of ich appears to exist at

Auburn. The fish used in ESC challenge were seined from ponds in January and transferred to the environment with warmer temperature. This may explain the unexpected ich infection. The observed survival of the hybrids was higher than that of channel catfish. This result was consistent with the study of Xu et al. (2011), for which C×B hybrid catfish were as susceptible to also had observed higher survival when challenged with Ich than channel catfish or blue catfish, and these differences would be significant with increased replication. The concomitant infection may be a major reason that mortality rose so rapid in the current experiment as *Ichthyophthirius multifiliis* acts as a potential vector to transmit *E. ictaluri* in channel catfish (Xu et al., 2012a). In the cases of co-infection with ESC and ich in channel catfish, no matter which was the primary infection, the mortality was significantly higher than that of the fish infected with only one pathogen (Shoemaker et al., 2012; Ku et al., 2012b).

References

Dunham, R.A., Smitherman, R.O., 1987. Genetics and Breeding of Catfish. Regional Research Bulletin 325, Southern Cooperative Series. Alabama Agricultural Experiment Station, Auburn University, Auburn, Alabama.

Dunham R.A., Umali G.M., Beam R., Kristanto A.H., Trask M., 2008. Comparison of production traits of the NWAC-103 channel catfish, NWAC103 channel × blue hybrid catfish, Kansas select 21 channel catfish and blue catfish grown at commercial densities and exposed to natural bacterial epizootics. North American Journal of Aquaculture 70: 98−106.

Dunham, R.A., 2011. Aquaculture and fisheries biotechnology: genetic approaches. CABI.

Hawke, J.P., 1979. A Bacterium Associated with Disease of Pond Cultured Channel Catfish, *Ictalurus punctatus*. Journal of the Fisheries Research Board of Canada 36(12): 1508–1512.

Hawke, J.P., McWhorter, A.C., Steigerwalt, A.G., Brenner, D.J., 1981. *Edwardsiella ictaluri* sp. nov., the causative agent of enteric septicemia of catfish. International Journal of Systematic Bacteriology 31:396–400.

Jeger, M.J., Viljanen-Rollinson, S.L.H., 2001. The use of the area under the disease-progress curve (AUDPC) to assess quantitative disease resistance in crop cultivars. Theoretical and Applied Genetics 102(1): 32–40.

Noga E.J., 2010. Fish disease: diagnosis and treatment, John Wiley & Sons.

Padi, J.N., 2003. Genetic studies on reproduction, bacterial disease resistance and tolerance of adverse water quality in channel catfish, *Ictalurus punctatus*, PhD Dissertation. Auburn University, AL.

Padi, J.N., Dunham R.A., 2009. Genetic differences in resistance of farmed channel catfish (*Ictalurus punctatus*) crossbreeds to enteric septicemia and columnaris diseases. Ghana Journal of Science 49:27–34.

Peatman, E., Baoprasertkul, P., Terhune, J., Xu, P., Nandi, S., Kucuktas, H., Li, P., Wang, S., Somridhivej, B., Dunham, R.A., Liu, Z., 2007. Expression analysis of the acute phase response in channel catfish (*Ictalurus punctatus*) after infection with a Gram-negative bacterium. Developmental and Comparative Immunology 31(11): 1183–1196.

Plumb, J.A., Quinlan, E.E., 1986. Survival of *Edwardsiella ictaluri* in pond water and bottom mud. The Progressive Fish-Culturist 48(3):212–214.

Shaner, G., Finney, R.E., 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67(8):1051–1056.

Shoemaker, C.A., Martins, M.L., Xu, D.H., Klesius, P.H., 2012. Effect of *Ichthyophthirius multifiliis* parasitism on the survival, hematology and bacterial load in channel catfish previously exposed to *Edwardsiella ictaluri*. Parasitology research 111(5):2223–2228.

Williams, M.L., Lawrence, M.L., 2010. Verification of an *Edwardsiella ictaluri*-specific diagnostic PCR. Letters in Applied Microbiology 50:153–157.

Wolters, W.R., Johnson, M.R., 1994. Enteric septicemia resistance in blue catfish and three channel catfish strains. Journal of Aquatic Animal Health 6:329–334.

Wolters, W.R, Johnson, M.R., 1995. Analysis of a diallel cross to estimate effects of crossing on resistance to enteric septicemia in channel catfish, *Ictalurus punctatus*. Aquaculture 137(1): 263–269.

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(3):249–254.

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(1):25–30.

Xu D.H., Shoemaker, C.A., Klesius, P.H., 2012. *Ichthyophthirius multifiliis* as a potential vector of *Edwardsiella ictaluri* in channel catfish. FEMS microbiology letters, 2012, 329(2):160–167.

Xu, D.H., Shoemaker, C.A., Martins, M.L., Pridgeon, J.W., Klesius, P. H., 2012. Enhanced susceptibility of channel catfish to the bacterium *Edwardsiella ictaluri* after parasitism by *Ichthyophthirius multifiliis*. Veterinary microbiology 158(1):216–219.