Evaluation of Disease Resistance in Selectively Bred Channel Catfish (*Ictalurus punctatus*) and Hybrid Catfish (Channel Catfish ♀ x Blue Catfish (*I. furcatus*) ♂)

By

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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 9, 2025

Keywords: Channel catfish (*Ictalurus punctatus*), Hybrid catfish, Blue catfish (*I. furcatus*), Selective breeding, Genetic improvement, Crossbreeding, Channel Catfish Virus (CCV), *Aeromonas hydrophila*, *Flavobacterium covae*, Motile Aeromonas Septicemia (MAS), Disease resistance

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Abstract

Selectively bred channel catfish (*Ictalurus punctatus*) and hybrid catfish (channel catfish ♀ x blue catfish (*I. furcatus*) ♂) were evaluated for resistance to channel catfish virus (CCV) and *Flavobacterium covae* (FC). In the CCV challenge, survival following CCV exposure varied significantly among families. Overall analysis showed slightly higher resistance for the hybrid catfish. Blue catfish paternal effects were more impactful than channel catfish maternal effects. These results contradicted earlier studies that indicated the resistance of hybrid catfish to CCV was less than or equal to channel catfish.

Two FC challenges were conducted, one high dose and one low dose, resulting in extremely rapid and slower mortality, respectively. In the high-dose challenge, overall survival fell below 10 % within 72 h, and channel catfish families had longer survival than the hybrid catfish (mean 19.9 h vs 16.8 h; log-rank $p = 0.01$), Family effects were also observed ($p < 0.05$). In the low-dose trial, median survival reached the 176-h study maximum for all families, and channel catfish still averaged longer survival than hybrid catfish (157.4 h vs 138.3 h; $p = 0.056$).

These findings contradict earlier reports that hybrid catfish are more resistant to columnaris than channel catfish (Arias et al., 2012). Genotype-environment interactions may occur due to strain differences in the pathogen, *F. covae* and/or the confined conditions of the experiments potentially causing more stress on the hybrid catfish leading to greater disease susceptibility in this specific challenge environment.

Keywords: Channel catfish (*Ictalurus punctatus*), Hybrid catfish, Blue catfish (*I. furcatus*), Selective breeding, Genetic improvement, Crossbreeding, Channel Catfish Virus (CCV), *Aeromonas hydrophila*, *Flavobacterium covae*, Motile Aeromonas Septicemia (MAS), Disease resistance

Acknowledgments

This research project would not have been possible without the support of many people. My appreciation and thanks to those who have helped along the way and influenced the ideas and insights that made this endeavor a success.

Special thanks to Dr. Rex Dunham, Dr. Timothy Bruce, Dr. Ian Butts, Dr. Sushan Ru, Dr. Baofeng Su and Kate Pottle for their support.

My sincerest appreciation goes to my lab mates, Dr. Darshika Hettiarachchi, Dr. Mei Shang, Misha Soman, Hamza Dilawar, Nadeen Abdo, Dhanuka Bombaranda, Parker Rae Menefee, Homnath Dhakal, Teresa David.

I also wish to express love and gratitude to my family for their understanding and endless love, my fiancée for her being there for me, and my father for allowing me to come and study. Those who supported me in any way during the completion of the study also deserve appreciation.

Above all, the Almighty ALLAH, for the knowledge, wisdom and life.

Table of Contents

List of Tables

Chapter II

List of Figures

List of Abbreviations

CCV	Channel Catfish Virus
FC	*Flavobacterium columnare*
MAS	Motile Aeromonas Septicemia
GWAS	Genome-Wide Association Study
QTL	Quantitative Trait Locus (or Loci)
RAS	Recirculating Aquaculture System
DO	Dissolved Oxygen
ppt	Parts per thousand (salinity)
h/hours	Hours (time unit used for survival recording)
KMIX	Code for selectively bred catfish families (e.g., KMIX 62 × KMIX 1)
K	Short for KMIX (e.g., K62 × K1)
B	Short for Blue Catfish (e.g., B3 × B7)
HR	Hazard Ratio
F1	First Filial Generation (parent generation for F2)
F2	Second Filial Generation (offspring from F1 crosses)
GCA	General Combining Ability
SCA	Specific Combining Ability
DNA	Deoxyribonucleic Acid
SNP	Single Nucleotide Polymorphism
ANOVA	Analysis of Variance

H^2	Broad-sense Heritability
h^2	Narrow-sense Heritability
PI3K	Phosphoinositide 3-Kinase (pathway associated with columnaris resistance)
CFU	Colony-Forming Unit (bacterial dose measure, mentioned in FC challenge context)
U.S.	United States
IVF	in vitro fertilization (IVF)

Chapter 1: Introduction

1.1 Evolution of aquaculture

Fish farming practices have evolved through the centuries with each culture and civilization having some capacity to farm fish based on fish species availability, catchability, and edibility. Since fish spoil quickly, a logical choice was to keep the fish alive as long as possible before consumption or trade. This motivated man to keep fish alive, confine them in closed areas by moving them from the open environments, such as shores, rivers, and lagoons, to nearby closed areas that had a similar environment to the original environment. This led to the evolution of other practices based on observations regarding poor water quality that could be visually detected or obvious from foul odors, leading to the development of corrective actions such as water exchange. Other problems, such as weight loss or the need to have bigger fish, led to the introduction of feeding and the need for more fish availability, likely leading to the closing of the life cycle.

The diversity of fish species led to each civilization having different fish to domesticate. The first evidence of early practices that can be described as aquaculture can be dated back 8,000 years ago (6000 BC) in China. The Chinese co-cultured common carp (*Cyprinus carpio)* with rice (Harland, 2019). Ancient China cultured common carp, and ancient Egypt grew tilapia (Sahrhage, 2008)

Ancient Egyptians farmed gilthead bream (*Sparus aurata)* around 3,500 years ago (1500 BC) based on analysis of fish teeth found in southern levant, which originated from Bardawil lagoon, which is also evidence for early aquaculture practices and trade during the late bronze age (Guy et al., 2018). Romans farmed or kept fish for commercial purposes in confined environments, based upon excavation of a Roman port, dating back to 100 BC. The excavations revealed an ancient fishing lagoon, fish tanks and a freshwater spring container (McCann, 1979).

1.2 Aquaculture and breeding

Successful breeding of fish will change the fish or any animal from a finite resource to a renewable resource (Harvey & Hoar, 1979), leading to large-scale cultivation and the commercialization of fish. Historically, fry had to be collected from the wild, as fishermen

usually knew the spawning season and the specific location of spawning or migration route, enabling fry collection for farming. This practice is still being used in some places where fish spawning is not possible such as collecting grey head mullet (*Mugil cephalus)* in Egypt (Saleh, 2008) and European eel (*Anguilla anguilla*), which still has some challenges during rearing in early life stages (Butts et al., 2014). This approach has many considerations, for example, the impact on captured fry for the future wild population, adaptability of fry in new confined environments and the expected differences in size, age, and even species.

The sustainable way to obtain fry includes breeding fish in captivity for which factors such as temperature, daylight hours and other environmental stimuli will affect gamete release. Sometimes breeding fish in captivity can be easy and natural by achieving the right environmental conditions. Nile tilapia, *Oreochromis niloticus*, is a good example, while many others can be more complicated and require intervention to obtain gametes

Having fish in captivity does not necessarily prevent gonads from developing to the final stages of maturation, however, the problem mainly lies with the release stage (Harvey & Hoar, 1979). There are many ways to induce gamete release in fish, and the reproduction of fish in captivity can often be regulated through environmental manipulations, such as adjusting photoperiod, water temperature or providing suitable spawning substrates. However, for some species, limited knowledge of their reproductive ecology or the inability to replicate natural environmental cues such as spawning migrations, specific depths, or riverine flow conditions makes natural spawning impractical or impossible. In such cases, using exogenous hormones offers an effective alternative to induce reproductive maturation and obtain fertilized eggs. Additionally, in all aquaculture species, hormonal treatments serve as valuable management tools to enhance egg production, stimulate spermiation, and improve hatchery efficiency. Hormonal therapies also play a critical role in enabling artificial gamete collection, facilitating interspecific hybridization, chromosome manipulation, and artificial fertilization as part of selective breeding programs (Mylonas et al., 2010).

1.3 US Catfish industry

Catfish growers in the United States had sales of 358 million dollars during 2024, being the most produced fish in the USA with 146.71 million kilograms produced in 2021 (Vilsack & Hamer, 2022). Around 187,891,954 m2 of land is utilized for catfish alone. The southern

United States is the primary contributor to catfish production with Alabama and Mississippi accounting for 85% of the land area devoted to catfish production (USDA, 2022) However, catfish production has been fluctuating as after a period of rapid growth in the 1970s through the 1990s, a dramatic contraction occurred between 2003–2013 followed by a steady, stable rebound of growth from 2014 to 2019 (Engle et al., 2022). The industry stagnated from 2017 to 2022 as the number of fish farms decreased from 921 in 2017 to 646, meaning surviving farmers were producing more fish as the decrease in farms was more dramatic than the decrease in production. Catfish production decreased from 155.24 million kilograms in 2017 to 146.70 million kilograms in 2021 (USDA, 2022). The catfish industry faces many challenges, mainly from intense competition from imported products from Asia, increased feed and labor costs (FAO, 2011), as well as fish disease outbreaks such as enteric septicemia of catfish (ESC) and columnaris (Wagner et al., 2002).

1.3.1 Channel catfish strains

Channel catfish, *Ictalurus punctatus*, was the most important freshwater species farmed in the US for human consumption (Small, 2006). Now the catfish industry produces a combination of channel catfish and hybrid catfish (channel catfish ♀ x blue catfish (*I. furcatus*) ♂. There are many strains of channel catfish, such as the Kansas strain, Rio Grande strain, NWAC 103 strain, Auburn strain and Norris strain with each varying for traits such as rapid growth, ease of spawning, seinablity, good feed conversion ratio (FCR) and high dress out % (Dunham and Smitherman, 1984; Kelly, 2004).

Dunham and Smitherman (1984) documented the origins of catfish strains and breeding. The oldest domestic strain of channel catfish, Kansas, was collected from the Ninnescah River, Kansas, 114 years ago in 1911. Many channel catfish strains used in aquaculture across the United States during the 1970s-1990s had partial ancestry from the Red River near Denison Dam, Lake Texoma, Oklahoma. In 1949, fish were collected from pools formed in the Red River after the construction of Denison Dam by the Arkansas Game and Fish Commission. These fish were then propagated in Arkansas state hatcheries, forming the foundation of broodstock for some of the earliest commercial catfish farms, such as Leon Hill, Edgar Farmer, Anderson-Nelson, and War Eagle Minnow Farms. Many of these original stocks were subsequently distributed to federal hatcheries and research institutions in Alabama, Arkansas, Louisiana, and Mississippi. Auburn University, Marion National Fish Hatchery, and

Stephens, Inc., distributed these fish further f to catfish farms, particularly in Alabama, where a significant portion of U.S. catfish production is centered. Additional stocks that contributed to genetic diversity in commercial catfish farming include those from the Yazoo River (Mississippi), Mississippi River, Rio Grande River (Texas), and Kansas River. By diversifying the gene pool, these introductions helped minimize inbreeding in commercial operations.

The Mississippi River strain was widely distributed and utilized in commercial farms of California, Missouri, and Arkansas, particularly through the efforts of Osage Fisheries in Missouri. Similarly, blue catfish stocks in U.S. aquaculture originate primarily from the Alabama River, Rio Grande River, Arkansas River, Mississippi River and Red River with some influence from Texas and Oklahoma river systems. Primary strains used today are D &B and Rio Grande strains of blue catfish. These historical introductions and selective breeding programs have shaped modern channel catfish aquaculture, influencing traits such as growth rate, disease resistance, and overall performance in farmed populations. The ancestry needs to be revisited as two major germplasm releases have occurred since the work of Dunham and Smitherman (1984).

Today, 80% of brood stock used for production of channel catfish is Delta Select (Eric Peatman, personal communication). These fish were derived from 12 commercial farms in Arkansas and Mississippi to form the base of Delta Select and undergone selection for growth and dressout percentages. Regular releases of this USDA line began in 2015 and continue.

One of the main research lines at Auburn University is the Ksas MIX line derived from the Kansas strain, which is the oldest domestic strain of channel catfish collected from the Ninnescah River, Kansas by the Kansas Fish and Game in 1911. The Kansas strain was obtained by Auburn University in 1970 from the first catfish farm, Krebiel Farm, Kansas. It later proved to be one of better growing and disease-resistant strains of channel catfish, but it has a late maturation. Jesse Chappell conducted the first body weight selection of these fish in 1977, and Rex Dunham completed the first response to selection in 1980, resulting in the Kansas Select line. After 6-7 generations of mass selection, these fish showed severe inbreeding depression and significantly reduced reproduction. They were backcrossed with Kansas random strain, to reinvigorate their reproduction and selection for body weight begun again for another 3 generations and renamed KMIX.

1.3.2 Status of genetic enhancement programs for ictalurid catfish

Strain selection, interspecific hybridization, crossbreeding, and mass selection have been all part of the genetic enhancement programs for catfish since 1966 that can be applied without government approval. Giudice (1966) found that the hybrid between channel catfish females and blue catfish males had heterobeltiosis for growth, and then Yant et al. (1976) demonstrated this heterobeltiosis at commercial densities. Growth, feed conversion ratio (FCR) (Yant et al., 1976; Li et al., 2004), survival, disease resistance (Ella, 1984; Wolters et al., 1996; Dunham et al., 2008; Arias et al., 2012), dressout percentage and fillet yield (Yant, 1975; Chappell, 1979; Huang et al., 1994; Argue et al., 2003; Bosworth et al., 2004; Bosworth, 2012) and seinability (Yant et al., 1976; Chappell, 1979; Dunham and Argue, 1998; Odin, 2017) were studied for hybrid catfish and the hybrid proven to be superior to the parent species.

Many farmers started adopting hybrid production techniques as part of dissemination efforts of Auburn University (Dunham and Perera, 2014). Now, 50-70 % of all catfish produced hybrids with about 200 million hybrid fry produced in 2014 alone (Multistate Research Project, 2015), which has grown to around 300 million fry produced in 2024 (Nagaraj Chatakondi, personal communication).

1.3.3 Selective breeding programs in aquaculture

Selective breeding programs were implemented in plants and animals as early as 1880 (Nilsson, 1898). Traits such as growth performance, survival, disease resistance and tolerance to environmental stressors are among the most common traits being selected.

Selective breeding programs have advanced greatly over the years. Nearly all poultry, swine and cattle are now based on selected lines with poultry growth rate increased by over 400% and FCR decreased by 50% in the period between 1950 and 2005 (Zuidhof et al., 2014) while in cattle, new improvement areas are being developed, reducing the methane production through selective breeding programs that are expected to reduce methane intensity by 24% in 2050 (de Haas et al., 2021).

Selection in fish breeding aims to enhance desirable traits like growth, reproduction traits disease resistance, and body shape. Fish targeted for selective breeding are of high economic such as salmonids (Kanis et al., 1976; Refstie et al., 1977; Gunnes and Gjedrem,

1978; Jonasson, 1996; Quinn et al., 2002 ;Unwin et al., 2003), rainbow trout, *Oncorhynchus mykiss* (Kincaid, 1981; Okamoto et al., 1993; Overturf et al., 2003; Quinton et al., 2004), striped bass, Morone saxatilis (Jacobs et al. 1999), Nile tilapia, *Oreochromis niloticus* (Eknath et al., 1993;; Tran et al., 2021; Trinh et al., 2021), common carp, *Cyprinus carpio* L. (Hulata, 1995; Vandeputte et al., 2004), rohu, *Labeo rohita* (Reddy et al., 2002) and channel catfish (Dunham and Smitherman, 1984; Smitherman and Dunham, 1985; Dunham et al., 1987; Rezk et al. ,2003; Bosworth et al., 2004; Dunham et al., 2014a, 2014b).

Selective breeding programs have been conducted for channel catfish to improve growth (length and weight), time of spawning, viability, disease resistance, dressout percentage (El-Ibiary et al., 1976; El-Ibiary and Joyce, 1978; Reagan, 1979; Dunham, 1981; Bondari, 1983, Dunham and Smitherman, 1983, 1985; Dunham et al., 1987; Rezk ,1993; Dunham and Brummett, 1999; Rezk et al., 2003; Small, 2005; Gima et al., 2014). The initial genetic enhancement programs conducted for catfish were strain selection, intraspecific crossbreeding, mass selection, and interspecific hybridization (Yant et al., 1976; Green et al., 1979; Dunham and Smitherman, 1983a, 1983b; Bosworth et al., 2020).

Although catfish represent a large portion of global aquaculture production, commercial application of pedigree-based selection programs has lagged behind that for species like Atlantic salmon, *Salmo salar* (Houston and Macqueen, 2019), rainbow trout (Sae-Lim et al., 2013) and Nile tilapia (Trinh et al., 2021; Tran et al., 2021). One reason for this is the widespread use of interspecific hybrid catfish, although reciprocal recurrent selection could improve performance of hybrid progeny.

For some species, like tra, *Pangasiandon hypophthalmus*, catfish, the increase in production has been extremely rapid and the focus has been on expanding farming operations, improving production techniques, developing feeds, and controlling diseases (Bosworth et al., 2020). For example, Vu et al. (2019) published a review of a long-term, pedigree-based selection program for tra catfish.

1.3.4 Quantitative genetics

Early genetics statistical tools were developed by Sir Francis Galton in 1875 who worked on inherited characteristics of sweet peas, as reported by his biographer Karl Pearson in 1930, who described linear regression and its slope (Stanton, 2001). Ronald A. Fisher was a

founder of modern statistics and quantitative genetics. His work from 1912 to 1924 was foundational and led to the development of the analysis of variance (ANOVA) (Rao, 1992).

The basic defining component for genetic improvement is that the phenotype (P) has the components, genetic makeup or genotype (G), environment (E), which refers to the external factors that have a direct or indirect impact on the phenotype, and the interaction between the genotype and environment (G_E), which is the change in rank or value of a genotype relative to other genotypes when the environment changes.

$P = G + E + GE$

Phenotypic variation (V_P) is essential for genetic enhancement and some portion of that variation must be genetic variation (V_P). The components of V_P are genetic variance (V_G), environmental variance (V_E), and the genotype- environment interaction variance (V_{GxE}).

$VP = V_G + V_E + V_{GE}$

Other components can also contribute to phenotypic variance. Epigenetics is the heritable change in gene function without any change in DNA sequence. The change is triggered by environmental stimuli, leading to transcriptional impact of epigenetic modifications, which influence the organism's phenotype. This will not only help in understanding the changes in gene function but also can be used as a tool for genetic enhancement programs, especially in disease resistance and other economical traits (Granada et al., 2018). Epigenetic modification techniques were used to improve disease resistance (González-Recio, 2012), to masculinize females (Navarro-Martín et al., 2011; Chen et al. ,2014 ; Shao et al., 2014), increase tolerance to different levels of heat stressors (Norouzitallab et al. 2014; Campos *et al*. ,2013a); improve growth under continues light regimes (Giannetto et al., 2013), improve tolerance to salinity (Móran et al., 2013), improve male reproduction (Wang *et al*., 2016), and improve embryo mortality and development (Dorts et al., 2016; Lai et al., 2016).

Maternal effects, although mostly temporary, can prove critical, especially during early life stages for embryos and fry. Environmental maternal effects are non-genetic contributions of the female to its offspring (Green, 2008). Maternal effects can also be genetic. Crosses between cold-resistant and cold-sensitive blue tilapia (*Oreochromis aureus*) were challenged under cold environment, revealing that cold tolerance was maternally inherited (Nitzan et al., 2016). Maternal effects can also contribute to disease resistance, as yolk proteins, phosvitin,

lipovitellin IgM, lysozymes, lectin, cathelicidin and complement components were all maternally transferred to offspring, and these factors were part of the innate and adaptive immune systems (Zhang et al., 2013).

Epistasis is another important type of gene action that is defined as the interaction of alleles between two or more loci. For example, in Atlantic Salmon, 18 chromosomes contribute to both length and weight but the presence of only one of these chromosomes will have no genetic effect while the combined presence of two loci did result in a genetic effect upon phenotypic variance (Besnier et al., 2020).

Epistatic variance (V_I) along with additive variance (V_A), dominance variance (V_D) are considered the most important factors to consider in a breeding program, however epistatic variance is very difficult to measure which is why breeders focus on dominance and additive variances.

$V_G = V_A + V_D + V_I$

Additive variance (V_A) contributes significantly to traits such as lipid content with studies showing variation among different strains of rainbow trout while other traits, such as disease resistance and tolerance, exhibit dominance effects. For example, resistance to viral hemorrhagic septicemia in salmonids showed partial dominance, meaning heterozygous individuals had intermediate resistance which was closer to one parent than the other (Kinghorn, 1983). Growth rate, reproduction and disease resistance often respond to selection in aquaculture species (Dunham, 2023).

Selection is not universally successful. Selective breeding in common carp has had limited success for growth rate but has been more effective for disease resistance and body shape. Many heritability estimates are unreliable due to environmental biases, though genetic studies suggest potential for improvement if base populations are sufficiently variable (Vandeputte, 2003).

1.3.5 Combining ability: concepts and principles

Combining ability refers to the potential of a parent (individual, strain, or line) to pass on desirable traits to its offspring when crossed with other parents. It is widely used in

quantitative genetics and breeding programs to assess genetic contributions to hybrid performance.

There are two main types of combining ability. General combining ability (GCA) measures the average performance of a parent across multiple crosses. It indicates the presence of additive genetic effects, meaning the genes contribute consistently to the offspring. GCA is crucial for recurrent selection in breeding programs, where long-term genetic improvement is desired. The other type is specific combining ability (SCA), which measures the unique performance of a specific parental combinations. It reflects non-additive genetic effects such as dominance and epistasis, which contribute to heterosis. SCA is especially important in crossbreeding and hybridization strategies, where certain parental combinations yield superior crossbreeds or hybrids.

1.4 Diseases in catfish farms

Catfish fish farms face persistent and emergent threats from a diverse range of pathogens, leading to substantial economic losses and posing complex management challenges. Bacterial diseases remain the most documented and consequential causes of morbidity and mortality in American catfish aquaculture. Notably, in recent years, motile Aeromonas septicemia (MAS) caused by hypervirulent *Aeromonas hydrophila (vAh)* has been identified as a primary driver of catastrophic losses. Several studies detail the clonal emergence, Asian origin and epidemic spread of vAh in U.S. catfish farms with single outbreaks resulting in mass mortalities and multi-million-kg losses of marketable fish (Hossain et al., 2014; Rasmussen-Ivey et al., 2016; Xu et al., 2023) Genomic analyses reveal that vAh strains responsible for these outbreaks constitute a distinct lineage, characterized by acquiring novel virulence factors and secretion systems (Hossain et al., 2014; Rasmussen-Ivey et al., 2016). Field studies and transmission experiments demonstrate that vAh not only persists in pond environments such as biofilms and sediments but is also efficiently spread between ponds by fish-eating birds, further complicating control efforts (Cunningham et al., 2018; Cai et al., 2019; Tuttle et al., 2023).

Other bacterial pathogens continue to exert significant economic pressure. *Edwardsiella ictaluri*, the causative agent of enteric septicemia of catfish (ESC), is identified as a long-standing threat with annual losses frequently estimated in tens of millions of

dollars (Wagner et al., 2002; Peterman & Posadas, 2019; Wise et al., 2021). Recent research further highlights the emergence of *Edwardsiella piscicida*, particularly in hybrid catfish, emphasizing the dynamic evolution of the *Edwardsiella* complex and its implications for diagnosis, pathology, and control strategies (Armwood et al., 2022). *Flavobacterium covae*, responsible for columnaris disease, remains a recurrent cause of outbreaks, especially under high-stress and high-temperature conditions, with ongoing challenges related to antimicrobial resistance and disease management (Wagner et al., 2002; Wise et al., 2021).

Complexity is added by frequent co-infections—particularly among vAh, *E. ictaluri*, and *F. covae*—which aggravate clinical outcomes and economic losses, while complicating diagnostic and treatment protocols (Wise et al., 2021; Armwood et al., 2022). Several reviews synthesize these patterns, emphasizing the underestimated burden of polymicrobial diseases and persistent challenges in effective disease control (Wise et al., 2021). Management strategies evaluated in the literature include vaccination (especially for ESC), probiotic applications, chemical treatments, and emergent immune-nutrition approaches; however, practical implementation of many of these interventions remains limited (Armwood et al., 2022).

In addition to bacterial pathogens, recent studies address the roles of viral agents such as Ictalurid herpesvirus 1 (IcHV1/CCV) (Venugopalan et al., 2024), and parasitic or fungal threats such as proliferative gill disease caused by *Henneguya ictaluri* (Griffin et al., 2020) and *Saprolegnia* spp. (Ran et al., 2012). However, compared to bacterial disease, these topics appear less deeply investigated in the surveyed literature.

1.4.1 Channel Catfish Virus infection

Channel Catfish Virus (CCV) is a member of the Alloherpesviridae family, which includes herpesviruses that infect fish and amphibians. It is a double-stranded DNA virus that primarily affects juvenile channel catfish (Davison et al., 2009). The virus causes high mortality rates in aquaculture settings and spreads rapidly in warm water conditions, typically affecting fish in the summer months when water temperatures exceed 25°C. CCV it is considered a major threat to commercial catfish farming, leading to significant economic losses due to high mortality that can reach 100% in severe cases and reduce production efficiency, The virus can infect every major organ, including the liver, kidney, and spleen, causing

widespread hemorrhaging and tissue necrosis (Hao et al., 2021). Besides organ damage, fish exhibit lethargy, erratic swimming and hemorrhaging. CCV has been shown to induce apoptosis (programmed cell death) in infected fish cells, specifically through the extrinsic apoptosis pathway. This response is mediated by tumor necrosis factor receptor (TNF-R) and the Fas-associated death domain (FADD) protein, which activate caspase-8 and caspase-3, key enzymes involved in cell death. Unlike some other viral infections, CCV does not rely on the intrinsic mitochondrial apoptosis pathway, making its mechanism unique among herpesviruses (Dawar et al., 2017).

Heritability for resistance to CCV in channel catfish is zero (Dunham et al., unpublished) Some strains of channel catfish exhibit lower mortality rates after CCV exposure, indicating the potential for resistance-based breeding programs(Plumb et al., 1975) One major approach to improving CCV resistance has been hybridizing channel catfish with blue catfish. Channel catfish female X blue catfish male hybrid offspring demonstrate increased growth rates and improved resistance to stress, and their specific resistance to CCV continues to be studied (Plumb et al., 1975; Plumb & Chappell, 1975; Hanson et al., 2004; Silverstein et al., 2008; Wang et al., 2022).

1.4.2 Aeromonas infection

Aeromonas hydrophila is a Gram-negative, motile, facultatively anaerobic bacterium responsible for motile Aeromonas septicemia (MAS) in fish, including channel catfish. The disease can manifest in two forms, acute form, acute hemorrhagic septicemia that is characterized by edema, hemorrhage, and diffuse necrosis in fish tissues, and a chronic form, chronic ulcerative syndrome that is characterized by formation of deep dermal ulcers (Huizinga et al., 1979; Cipriano et al., 1984). The virulence of *Aeromonas hydrophila* is associated with multiple extracellular proteins and virulence factors, including 1) hemolysins and cytotoxins, 2) proteases, lipases, and enterotoxins, 3) fimbriae and flagella for adhesion, and 4) iron-binding systems and secretion systems (Daskalov, 2006).

Since 2009, a highly virulent strain of *Aeromonas hydrophila* (vAh) has caused severe epidemic outbreaks in catfish farms in Alabama, Mississippi, and Arkansas, leading to the loss of millions of kg of fish annually (Hemstreet, 2010; Da Silva et al., 2012). The disease primarily affects food-sized, marketable fish, typically during warm summer months (up to

35°C). Histopathological studies indicate that aeromonas infections lead to severe 1) splenitis with fibrinoid necrosis and leukocyte infiltration 2) gastrointestinal damage, including necrotic gastric glands and extensive intestinal epithelial destruction and 3) gill and kidney lesions, which contribute to systemic failure and high mortality. These outbreaks have significantly harmed catfish production, making *Aeromonas* one of the most economically damaging bacterial pathogens in the U.S. aquaculture industry (Abdelhamed et al., 2017).

Recent research suggests that the virulent U.S. epidemic strain of *Aeromonas hydrophila* originated from Asia, as whole-genome sequencing and phylogenetic analysis indicate that *Aeromonas* isolates from diseased grass carp in China and catfish in the U.S. share highly similar genomes. Another isolate from Mississippi in 2004 demonstrated intermediate genetic characteristics between Chinese and U.S. epidemic strains, suggesting a potential introduction of *Aeromonas* from imported fish stocks (Hossain et al., 2014).

1.4.3 Flavobacterium covae infection

Flavobacterium covae is a Gram-negative, rod-shaped, filamentous bacterium that belongs to the Flavobacteriaceae family. It is the causative agent of columnaris disease, a significant threat to freshwater fish worldwide. The bacterium is known for its gliding motility, a characteristic that aids in its colonization and infection of fish tissues (Davis 1922; Anderson & Conroy, 1969).

Columnaris disease primarily affects the fish's gills, skin, and fins, leading to necrotic lesions, ulcers, and respiratory distress. The infection is often fatal, particularly in warm water conditions (above 25°C), which favors bacterial proliferation. The pathogen can form biofilms, which enhance its resistance to antibiotics and contribute to its persistence in aquatic environments (Wolke, 1975).

The transmission of *F. covae* occurs through direct contact with infected fish, exposure to contaminated water, or horizontal transmission via bacterial shedding in the water column. The bacterium has been detected in asymptomatic carrier fish, allowing it to persist undetected in aquaculture settings (Austin & Austin, 1993; Wakabayashi, 1993).

Columnaris disease is a major cause of mortality in farmed channel catfish, second only to *Edwardsiella ictaluri* regarding economic impact. Outbreaks of columnaris disease have

resulted in millions of dollars in losses annually, affecting hatcheries and grow-out operations (Wagner et al., 2002).

A key factor influencing the virulence of *F. covae* is its genetic diversity. Studies have identified multiple genomovars (genetic variants) of *F. covae*, with genomovar II being significantly more virulent than genomovar I. Experimental challenges with genomovar II isolates resulted in 92–100% mortality in channel catfish fry, while genomovar I isolates caused significantly lower mortality (0–46%) (Shoemaker et al., 2008).

At low bacterial density, *F. covae* is not a serious problem. Thus, environmental stressors, such as high stocking densities, poor water quality and fluctuating temperatures play a greater role in exacerbating disease outbreaks. The bacterium thrives in low dissolved oxygen levels and can form protective biofilms, making it difficult to eradicate with conventional treatments (Chowdhury & Wakabayashi, 1990; Decostere et al., 1999a,b,c; Altinok & Grizzle, 2001; Shoemaker et al., 2003; Suomalainen et al., 2006a).).

F. covae is very adaptable and continuously expands its host range, posing an increasing threat to new fish species in aquaculture. The first and recent documented case of columnaris disease in black carp (*Mylopharyngodon piceus*) in Northern Vietnam highlights the pathogen's adaptability and potential for further spread. The black carp outbreak resulted in 20–40% mortality within 7–10 days with similar clinical signs to other species such as saddleback lesions, fin erosion, gill necrosis, and mucus loss (Hoai et al., 2025).

Channel catfish generally exhibit greater resistance to *Flavobacterium covae* infection compared to blue catfish, indicating a species-level difference in susceptibility (Dunham et al., 1993). Intraspecific crossbreeds of channel catfish often exhibit enhanced resistance to columnaris (Padi & Dunham, 2009). This inherent higher resistance of channel catfish can be further enhanced through hybridization as channel catfish × blue catfish hybrids have been shown to possess significantly greater resistance to columnaris disease, particularly when challenged with highly virulent strains of *F. covae* (Arias et al., 2012). The observed heterosis suggests that both additive and non-additive genetic factors contribute to disease resistance, making hybridization a promising approach for reducing columnaris-related losses in aquaculture.

In addition to hybridization, selective breeding has proven effective in improving resistance to columnaris within catfish populations. Waters (2001) reported successful

selection in approximately 60% of strains tested, demonstrating that genetic gain for resistance is achievable. More recently, a genome-wide association study (GWAS) identified four quantitative trait loci (QTLs) linked to columnaris resistance in catfish. Notably, candidate genes within QTLs on linkage groups 7 and 12 were found to cluster functionally, many being involved in the PI3K signaling pathway, highlighting its likely importance in mediating resistance. These findings support the integration of molecular markers into breeding programs to enhance selection precision and durability of resistance in future generations (Geng et al., 2015), and these QTLs might be used for marker-assisted selection

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Chapter II: Resistance to Channel Catfish Virus among Families of Channel Catfish (*Ictalurus punctatus*) and Hybrid Catfish (Channel Catfish ♀ x Blue Catfish ♂ (*I. furcatus*))

Abstract

This experiment evaluated survival differences among 13 channel catfish (*Ictalurus punctatus*) and hybrid catfish families (5 channel catfish and 8 hybrid catfish) following exposure to channel catfish virus (CCV). A total of 130 fish were utilized, with 10 individuals representing each genotype in communal evaluation. Fish were subjected to a standardized CCV challenge (2.32 x 10^6 CFU/mL) through a one-hour immersion bath, after which mortalities and clinical symptoms were recorded. Kaplan-Meier survival analysis indicated significant survival differences among families ($p < 0.0001$). However, pairwise log-rank tests revealed only a few significant differences among genotypes, suggesting isolated family-level influences on survival. The genotype (KMIX 121 x KMIX 47) X (Blue 7) demonstrated the highest median survival (93 hours), while genotype (KMIX 68 x KMIX 3) X (N/A) had the lowest (39 hours). The analysis revealed significant paternal (sire) effects ($p < 0.0001$), with multiple pairwise comparisons showing strong differences among sires, particularly between Blue 3 and several hybrid sires. Maternal (dam) effects were marginally significant ($p = 0.042$), though fewer differences were detected in pairwise comparisons, suggesting reduced maternal influence at the fingerling stage. Overall, hybrid catfish had better resistance to CCV compared to channel catfish in contrast to previous studies. CCV resistance appears complicated from both a genetic and environmental perspective, and further experimentation is needed to clarify these effects, including examination of different doses and strain of CCV, strains of parents, method of virus delivery and other factors that could further explain the importance of both genetic effects as well as genotype-environment interactions.

Keywords: Channel catfish (*Ictalurus punctatus*), Hybrid catfish, Blue catfish (*Ictalurus furcatus*), Selective breeding, Genetic improvement, Crossbreeding, Channel Catfish Virus (CCV), Disease resistance

1.0 Introduction

Channel Catfish Virus (CCV) is a member of the Alloherpesviridae family, which includes herpesviruses that infect fish and amphibians. It is a double-stranded DNA virus that primarily affects juvenile channel catfish (*Ictalurus punctatus*) (Davison et al., 2009). The virus causes high mortality rates in aquaculture settings and spreads rapidly in warm water conditions, typically affecting fish in the summer months when water temperatures exceed 25°C. CCV is considered a major threat to commercial catfish farming, leading to significant economic losses due to high mortality rates, which can reach up to 100% in severe outbreaks, and reduced production efficiency. The virus infects major organs, including the liver, kidney, and spleen, causing widespread hemorrhaging and tissue necrosis (Hao et al., 2021). In addition to organ damage, infected fish exhibit lethargy, erratic swimming, hemorrhaging, and external lesions.

CCV has been shown to induce apoptosis (programmed cell death) in infected fish cells, specifically through the extrinsic apoptosis pathway. This response is mediated by tumor necrosis factor receptor (TNF-R) and the Fas-associated death domain (FADD) protein, which activate caspase-8 and caspase-3, key enzymes involved in cell death. Unlike some other viral infections, CCV does not rely on the intrinsic mitochondrial apoptosis pathway, making its mechanism unique among herpesviruses (Dawar et al., 2017).

Latent CCV persists in broodstock populations, complicating selective breeding for resistance. PCR-based detection confirms high carrier prevalence on farms (Gray et al., 1999; Thompson et al., 2005).

Wang et al. (2022) reported that hybrid vigor (heterosis and heterobeltiosis) in catfish may be environment-dependent, noting that advantages in traits such as disease resistance, feed conversion, carcass yield, and harvestability were observed in pond systems but not in small culture units. Their comparison of channel catfish and reciprocal F1 hybrids reared in tanks suggests that the expression of heterosis may be limited under controlled, intensive conditions.

Several studies showed that there is a difference in strains for resistance to CCV, which appears to be at least have a partial genetic basis with variation noted among channel catfish strains and families (Plumb et al., 1975; Ourth et al., 2017). However, interspecific hybrids consistently display lower mortality under CCV challenge compared to channel catfish, driven

by heterosis and genetic contributions from blue catfish, *I. furcatus* (Plumb et al., 1975; Silverstein et al., 2008; Wang et al., 2022).

Age is considered a key factor as generally, older fish are less resistant to CCV. Plumb et al. (1975) subjected six strains of 1-, 2-, and 3-month-old channel catfish from different geographical areas to CCV and there was no significant difference in mortality between age groups. However, Hanson et al., (2004) showed that 3–8 day-old, post-hatch fry were most resistant, whereas older fish (60 days post-hatch) had higher susceptibility but can develop immunity.

The primary objective of this experiment was to evaluate survival differences among KMIX channel catfish. families and channel catfish female X blue catfish, I. furcatus, male hybrid families following controlled exposure to Channel Catfish irus (CCV). Another objective was to compare the resistance of the channel catfish and hybrid catfish.

2.0 Materials and Methods

All investigations and experimental studies on animals were conducted according to the Institutional Animal Care and Use Committee (IACUC) and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) protocols and guidelines for Auburn University Institutional Animal Care and Use Committee (AU-IACUC # 2023-5149).

2.1 Broodstock management

Broodstock were housed at the Auburn University E.W. Shell Fisheries Center in Auburn, Alabama from 2019 to 2024. Channel catfish and blue catfish were cultured in earthen ponds and fed five days a week with 32% protein pelleted feed during the summer and three days per week during the winter. Leading up to the spawning season, feed was shifted to a 36% protein broodstock feed provided five days per week. In June, during peak spawning season, mature (7- -year-old) channel catfish females (N = 14, mean body weight = 2.95 kg), channel catfish males (N = 18, mean body weight = 2.12 kg) and blue catfish males (N = 7, mean body weight = 3.06 kg) were harvested from the earthen ponds by seining, using a 3.8 cm mesh net.

2.2 Experimental fish

Channel catfish were KMIX line derived from the Kansas strain, which is the oldest domestic strain of channel catfish collected from the Ninnescah River, Kansas by the Kansas Fish and Game in 1911 Dunham and Smitherman, 1984). The Kansas strain was obtained by Auburn University in 1970 from the first catfish farm, Krebiel Farm, Kansas. It later proved to be one of better growing and disease-resistant strains of channel catfish, but it has a late maturation. After 6-7 generations of mass selection (Dunham & Smitherman, 1983; Dunham & Brummett, 2019; Rezk et al., 2003; Padi, 1995; Dunham 2007), these fish showed severe inbreeding depression and significantly reduced reproduction. They were backcrossed with Kansas random strain, to reinvigorate their reproduction and selection for body weight begun again for another 3 generations and renamed KMIX.

Parents for the experimental fish were produced by a series of full-sib and half-sib matings (Johnson, 2021). Twelve of these channel catfish females were mated with 20 channel catfish and blue catfish males to produce a series of full-sib and half-sib families. These spawns had poor hatch, and for the current experiment 13 families, 5 channel catfish and 8 hybrid families, representing 6 dams and 9 sires were used in the challenges. Families produced are listed in Table 1.

2.3 In vitro fertilization procedures to produce the families

Upon harvest, gravid channel catfish females were administered luteinizing hormone releasing hormone analogue (LHRHa) at 90 μg/kg body weight via intraperitoneal implantation. Following implantation, the females were placed into individual mesh bags (38 cm × 56 cm) and held ~30 cm apart in 670 to 750 L flow through (30 L/min) pond water holding tanks. Temperature in the tanks ranged from 26 to 28^{o}C. At 36 h post-implantation, bags were checked every 4 h for eggs attached to the spawning bag, which indicates that the female is ovulating. Once eggs were detected, the females were anesthetized with 100 mg/L tricaine methanesulfonate (MS-222, Ferndale, WA) and 100 ppm NaHCO3 solution. Once anesthetized, the female fish were rinsed with pond water and dried thoroughly. Crisco® vegetable shortening was carefully rubbed on the underside of the females and eggs were hand-stripped into Crisco® coated metal pans (~25 g of eggs/pan). Blue catfish and channel catfish males were euthanized and their sperm were collected for *in vitro* fertilization (IVF) following

protocols by Dunham and Masser (2012) and Hettiarachchi et al. (2022). After euthanasia, testes were removed from the body cavity with a sterile scalpel and forceps. Testes were rinsed with 0.9% saline solution to remove any blood. After rinsing, the testes were gently dried and then minced with a scalpel blade. Following mincing, the testes were filtered with a 100 μm mesh. Thereafter, 10 mL of 0.9% saline was added for each 1 g of testes (w/v). Following filtration, the sperm solution was ready for fertilization.

Table 1.1: Mean and median survival times for five families of channel catfish (*Ictalurus punctatus*) and eight families of hybrid catfish (channel catfish ♀ × blue catfish (*I. furcatus*) ♂) following exposure to channel catfish virus (CCV). Endpoint survival, expressed as mean survival percentage, demonstrated that only four hybrid families and one channel catfish family retained any surviving individuals at 200 hours post-challenge. Each family was represented by n = 10 individuals. Statistically significant differences in survival were observed among certain families using Log-rank (Mantel-Cox) test. Descriptive statistics presented in the table—including mean survival percentage, mean survival time, and median survival time—were calculated using standard arithmetic functions in R mean, median, and survivor proportion.

Family genotype	Mean Survival %	Mean Survival Time (h)	Median Survival Time (h)
(K121 X K47) * (B7)	30	99	45
(K194 X K71) * (B1)	20	87.6	57
(K89 X K5) * (K117 X K55)	20	84.6	45
(K113 X K55) * (B7)	10	73.2	51
(K62 X K1) * (B1)	0	84.6	93
(K62 X K1) * (B3)	0	58.8	51
(K62 X K1) * (K121 X K47)	0	48	45
(K68 X K3) * (B1)	0	59.4	54
(K68 X K3) * (B3)	0	59.4	57
(K68 X K3) * (K214/209 X K81/78)	0	67.8	60
(K68 X K3) * (N/A)	0	36	39
(K89 X K5) * (B6)	0	49.2	42
(K89 X K5) * (KS 64 X M2)	0	51	45

*K + KMIX, B + blue catfish. One sire is a random unknown channel catfish (N/A), and one family has two potential sires (K 214 X K 81 or K 209 X K 78) as the brand for this male was hard to read.

All IVF was conducted at 27 to 28^{o}C. The hand-stripped eggs were first mixed in a metal pan with freshly collected KMIX or blue catfish sperm at a rate of 2 mL of sperm solution

(10 mL of 0.9% saline per 1 g of testes) per 25 g of eggs for 2 min (Hettiarachchi et al., 2023a). Temperature in the hatching trough ranged from 26 to 28^{o}C and the flow rate was held at 3.79 L/min. After 1 h, eggs were moved into hanging mesh baskets (7.0 m × 0.4 m × 0.2 m), which were suspended in a flow-through pond water hatching trough with paddlewheel agitation and compressed aeration. Temperature remained between 26 to 28^{o}C and the flow rate was 15 L/min in the hatching trough.

2.4 Fry rearing

Following hatchery and yolk sac absorption, fry from different families were reared separately in a recirculating aquaculture system (RAS). Dimensions for the RAS tanks were 30 cm × 60cm m × 20 cm (depth). Each family was stocked into a separate tank at an average stocking density of 1.8 fry/L.

Fry were initially fed ad-libitum with a powdered 50% Protein fish feed. Once they reached the appropriate size (0.5-1 grams), fry were transitioned to a 300 mm pelleted feed. Feed was administered multiple 4-5 times daily to ensure uniform growth and minimal competition. At two months of age, fry reached an average weight of approximately 2.4 grams.

Water quality was monitored daily in both systems. In the RAS, water temperature was maintained between 28–30°C, dissolved oxygen (DO) was kept above 5 mg/L, and salinity was maintained at 2–3 ppt. Other monitored parameters included ammonia, pH, and salinity, with nitrite, nitrate, GH, and KH also checked as needed which remained within narrow, fish-safe limits, pH ranged from 7.0 to 8.0, salinity held at 2–3 ppt, nitrite stayed below 0.25 mg L^{-1} and nitrate below 5 mg L^{-1}, while general hardness (GH) and carbonate hardness (KH) each remained between 71.6 and 89.5 mg L^{-1} as $CaCO_3$.

2.5 CCV challenge

Channel catfish fingerlings and hybrid catfish were challenged with channel catfish virus (CCV). The virus was prepared by thawing two cryostocks previously propagated and diluted to 10^5 in minimum essential medium (MEM10). After thawing, a total volume of 61 mL was prepared with 1 mL reserved for TCID50 calculation. Virus titer (TCID50) was determined by serial dilution and inoculation into channel catfish ovary (CCO) cell cultures.

Cytopathic effect (CPE) was recorded at 24-hour intervals. The calculated virus titer was 2.25×10^8 TCID50/mL. Fish were exposed via a static bath by adding 60 mL of virus to 100 L of water, resulting in an exposure dose of 1.35×10^5 TCID50/mL. Mortality was recorded every 6 hours post-challenge for 219 hrs. A mock control group without virus exposure was included to ensure that mortalities observed were exclusively due to CCV exposure and not environmental or other unrelated stressors.

Catfish were transferred into two 330 L shallow, rectangular tanks (L x W x H; 1.2m x 0.34 m" x 0.1 m"). These independent flow-through systems were supplied with carbon-filtered, heated municipal water with supplemental aeration via air stones. An aquarium pump was added to two corners of the tank to ensure water circulation. Each family was stocked in a mesh cage with 10 fish per cage. Prior to virus inoculation, the water volume of both tanks was reduced to 100 L, and water flow to the tanks was ceased. One tank served as the virus challenge tank, receiving the CCV inoculum, while the other tank received sterile media to serve as the mock control. Fish were exposed to CCV or sterile media for a 1-hour immersion bath with supplemental aeration and circulation. After this period, water flow was restored to 2 L/min, and tank volume was returned to 330 L. Circulating pumps were used to mix the water constantly to ensure that all families were equally exposed to the same water quality and virus titer in the tank. The experiment lasted for a total of 219 hrs or around 9 days.

2.6 Statistical analyses

All statistical analyses were performed using R software (version [4.4.2]). Survival analysis was conducted using the Kaplan-Meier estimator to evaluate differences in survival probability among families, dams, sires, and family types (channel catfish vs. hybrid catfish). The survival and survminer packages were used to generate survival curves and conduct log-rank tests. A global log-rank test was applied to assess overall survival differences across groups, followed by pairwise log-rank tests with Bonferroni correction to identify specific group comparisons showing significant differences.

Mean survival percentage, mean and median survival times were calculated for each family, and families were ranked accordingly. Additional stratified analyses were performed to assess the influence of shared parentage (dam or sire) on survival outcomes. Graphical representations of survival curves were generated using ggsurvplot, with confidence intervals

included or excluded as needed. Data visualization and summary statistics were also performed using packages from the tidyverse suite.

Additionally, all survival statistics were independently verified in GraphPad Prism (version 10.4.2, Windows 10). The raw data were entered into a Prism survival table containing three columns—time (h), event status (1 = death, 0 = censored), and the grouping variable (family, sire, dam, or treatment). Within Prism, Kaplan–Meier survival curves were generated for each analysis stratum, overall log-rank (Mantel–Cox) tests were calculated, and pair-wise log-rank comparisons were performed using the "compare all pairs of groups" option with Bonferroni adjustment of p-values. All numerical p-values from Prism were cross-checked against the R output to ensure consistency.

2.7 Data stratification for survival analyses

For every statistical comparison, fish were regrouped from the master data set (130 individuals) into progressively finer analytical strata. Overall survival – a single Kaplan-Meier curve that pooled all 13 families (N = 130) to characterize the global time-to-mortality pattern after CCV challenge.

Family-genotype level was illustrated as 13 Kaplan-Meier curves, one per family genotype (10 fish each). This grouping captured full-sibling effects and provided the basis for the pair-wise log-rank matrix.

To evaluate parental contributions to CCV resistance, progeny were regrouped by sire and dam. Offspring were collapsed into 10 paternal groups,four blue-catfish sires and six channel-catfish sires,each represented by 10 to 30 fingerlings (total N = 130). Blue-catfish male 1 was mated to three different females, while B males 3 and 7 were each mated to two females, creating multiple half-sibling families, for the dam level. All progenies were also collapsed into six maternal groups (all channel catfish dams), with 10 to 40 fish per dam (total N = 130). The most frequently used dam, KMIX 68 × KMIX 3, contributed to four separate family genotypes (overall N = 40). Other dams, such as KMIX 62 × KMIX 1 and KMIX 113 × KMIX 55, were likewise paired with multiple sires.

Fish were divided into hybrid (channel ♀ × blue ♂; eight families, N = 80) and channel catfish (five families, N = 50). This two-group comparison tested the conventional expectation

of heterosis. All subsequent Kaplan-Meier estimations, log-rank tests and pair-wise comparisons were run separately within these predefined strata.

3.0 Results

3.1 Overall survival analysis

Overall survival dynamics of all fish subjected to the CCV challenge is shown in Fig. 1.1. The first mortality was observed around 15 hours post-infection. Most mortalities occurred between approximately 15 and 60 hours, and only a few mortalities occurred beyond 100 hours. Resulting in overall survival of 7.85%.

All fish, irrespective of genotype, displayed uniform clinical symptoms such as faded body color, fin erosion, reddish discoloration, and swollen abdomens. This uniform symptomatology underscores the severity and virulence of the CCV strain utilized, reinforcing the conclusion that mortality events were directly attributable to the viral challenge. Further validating this finding, the mock control tank, subjected to identical environmental conditions minus the pathogen exposure, exhibited no mortalities throughout the study period, confirming that observed mortalities were a direct consequence of CCV exposure.

3.2 Survival analysis by family

Significant differences in survival probabilities were observed among family genotypes ($p < 0.0001$; Fig. 1.2; Table 1.1). To identify specific differences, a pairwise log-rank test was conducted with a Bonferroni adjustment for multiple comparisons (Table 1.2). the majority of genotypes did not differ from each other ($p > 0.05$). However, a few exceptions were noted, which were primarily responsible for the overall observed significance. Survival of genotype KMIX 68 X KMIX 3) X (N/A) was significantly lower than the hybrid families and two channel catfish genetic types, highlighting their poor resistance to CCV and indicating better resistance for the hybrid catfish. All other comparisons were not significantly different.

For (KMIX 62 X KMIX 1) X (B1), more than half the fish survived longer than 93 hours, resulting in median survival time of 84.6 hrs. Mean and median survival times varied noticeably among the different family genotypes. The genotype (KMIX 62 × KMIX 1) × (B1)

exhibited the highest median survival time of 93 hours, indicating strong resilience to CCV infection. It was followed by (KMIX 68 × KMIX 3) × (KMIX 214 × KMIX 81 / KMIX 209 × KMIX 78) showing a median survival of 60 hours. Several other genotypes such as (KMIX 194 × KMIX 71) × (B1) and (KMIX 68 × KMIX 3) × (B3) also had median survival times exceeding 54 hours, suggesting moderate resistance, it can also be noted that of these 4 genotypes, three were hybrids and one was channel catfish.

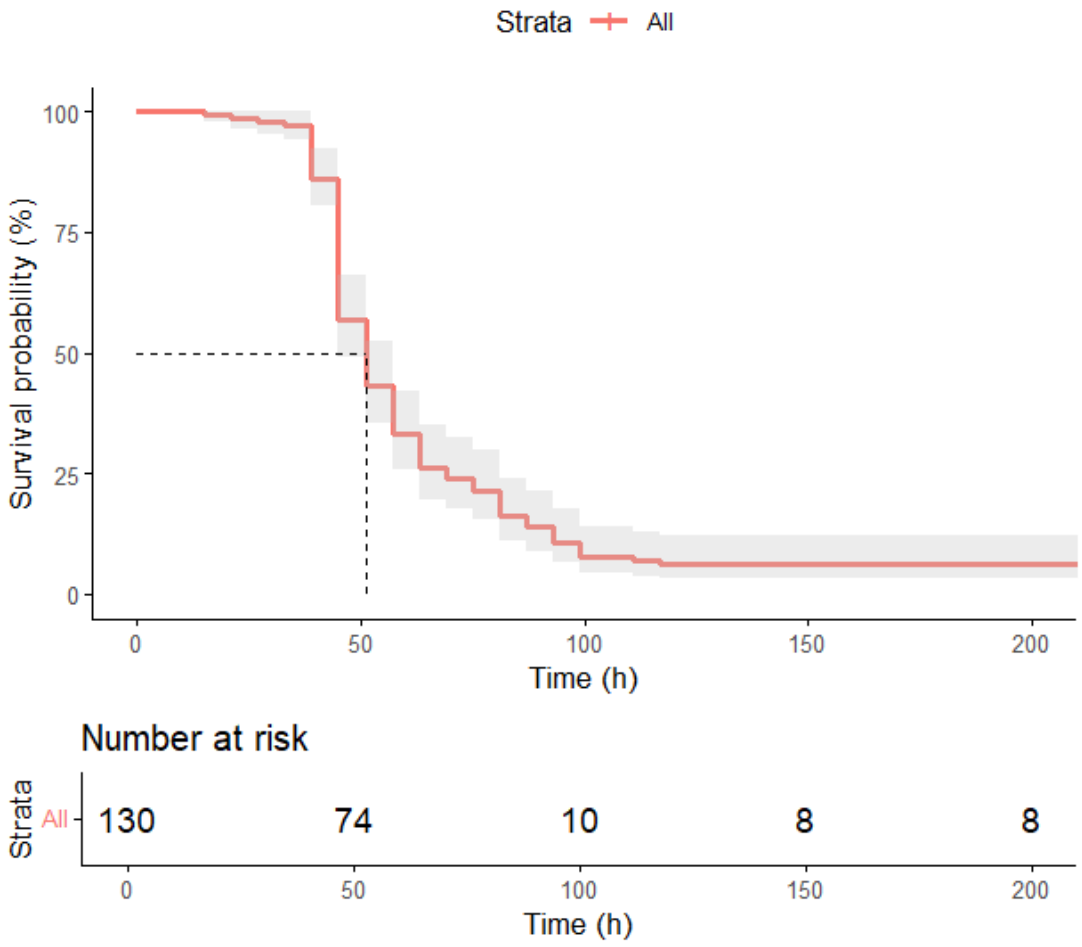

Figure 1.1. The combined survival curve of 130 CCV-challenged channel catfish (*Ictalurus punctatus*) and hybrid catfish (channel catfish ♀ x blue catfish (I. furcatus) ♂) over a 200-hour observation period. In the upper panel, the red step curve depicts the estimated survival probability (y-axis, in percent) as a function of time (hours) on the x-axis. Shaded in light gray around the curve are the 95 % confidence intervals. A horizontal dashed line at 50 % survival

and a vertical dashed line at the corresponding time (~50 h) mark the median survival time. The lower panel is the "Number at risk" table, which reports how many fish remain under observation (i.e., not yet dead or censored) at key time intervals.

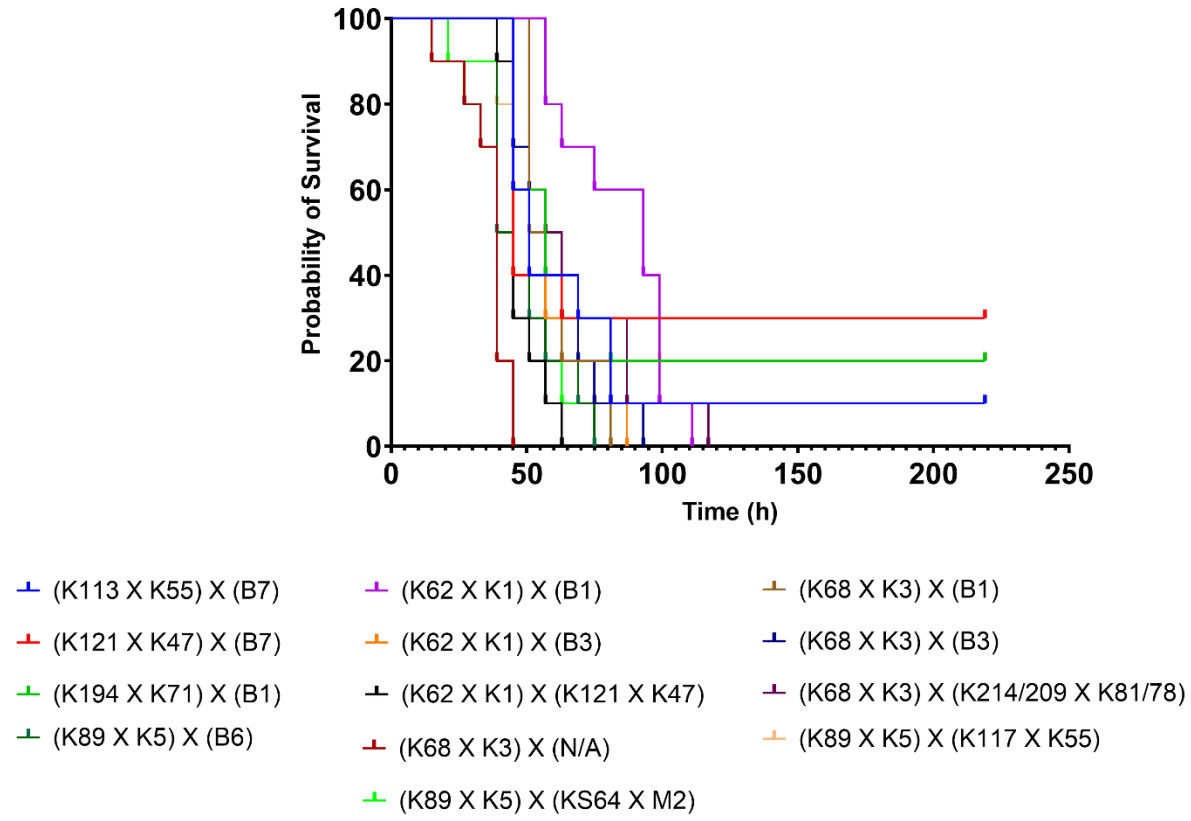

Figure 1.2. The Kaplan-Meier survival curve for families of channel catfish (*Ictalurus punctatus*) and hybrid catfish (channel catfish ♀ x blue catfish (*I. furcatus*) ♂) challenged with channel catfish virus. Each line represents a distinct family genotype. Highly significant differences (log-rank test, $p < 0.0001$) in survival were observed among the genotypes.

Table 1.2: Summarizes the pair-wise survival comparisons among the 13 experimental families of channel catfish (Ictalurus punctatus) and hybrid catfish (channel catfish ♀ x blue catfish (I. furcatus) ♂) challenged with channel catfish virus.The p-values shown are raw (unadjusted). To control the family-wise error rate a Bonferroni correction was applied by dividing the nominal $\alpha = 0.05$ by 78. Consequently, an individual comparison is considered significant only if $p < 0.00064$.

Genotype	(K113 X K55) X (B7)	(K121 X K47) X (B7)	(K194 X K71) X (B1)	(K62 X K1) X (B1)	(K62 X K1) X (B3)	X (K121 X K47)	(K68 X K3) X (B1)	(K68 X K3) X (B3)	(K214/209 X K81/78)	(K68 X K3) X (N/A)	(K89 X K5) X (B6)	(K117 X K55)
(K121 X K47) X (B7)	0.6537											
(K194 X K71) X (B1)	0.7206	0.8471										
(K62 X K1) X (B1)	0.1541	0.8924	0.5736									
(K62 X K1) X (B3)	0.7469	0.3347	0.3895	0.0024								
(K62 X K1) X (K121 X K47)	0.0362	0.0684	0.0328	0.0001	0.0741							
(K68 X K3) X (B1)	0.9297	0.7036	0.5741	0.0021	0.9334	0.0153						
(K68 X K3) X (B3)	0.5765	0.3376	0.3908	0.0046	0.9059	0.0465	0.9961					
(K68 X K3) X (K214/209 X K81/78)	0.6390	0.7228	0.8743	0.1947	0.1705	0.0043	0.1832	0.3657				
(K68 X K3) X (N/A)	0.0001	0.0003	0.0001	0.0000	0.0001	0.0013	0.0000	0.0003	0.0000			
(K89 X K5) X (B6)	0.0630	0.0611	0.0527	0.0003	0.0794	0.7672	0.0826	0.1934	0.0280	0.0153		
(K89 X K5) X (K117 X K55)	0.9698	0.4651	0.6239	0.6393	0.5914	0.2918	0.9735	0.5905	0.8740	0.0026	0.1676	
(K89 X K5) X (KS64 X M2)	0.2449	0.1226	0.1354	0.0010	0.6632	0.4776	0.2965	0.3948	0.0766	0.0025	0.5777	0.3401

There was a significant difference in survival time observed between the two genetic types (p = 0.015, log-rank test). Hybrid catfish exhibited slightly better than channel catfish throughout the challenge period.

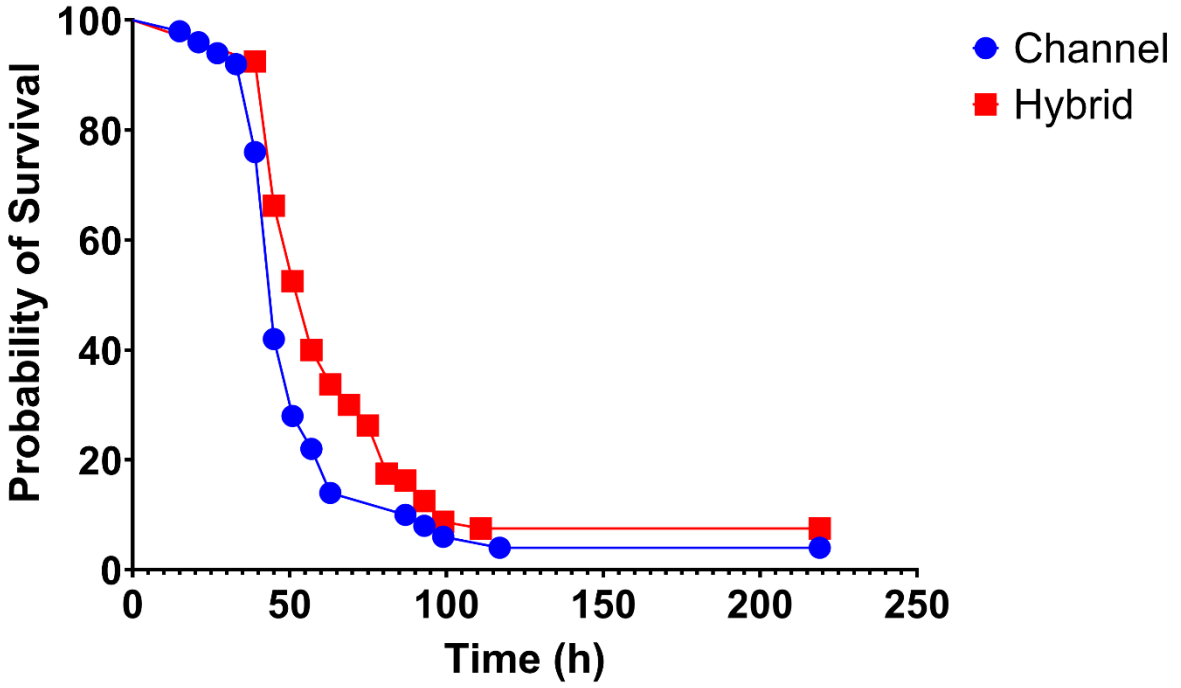

Figure 1.3. Kaplan-Meier analysis comparing survival time of channel catfish (*Ictalurus punctatus*) and hybrid catfish (channel catfish ♀ x blue catfish (*I. furcatus*) ♂) challenged with channel catfish virus. A significant difference in survival was observed (p = 0.015).

3.4 Sire and dam contribution to survival outcomes

The log-rank test by dam (Fig. 1.5) indicated no significant difference in survival among offspring of different dams (p = 0.19). However, progeny of (KMIX 68 X KMIX 3) and (KMIX 62 X KMIX 1) appeared to perform better with higher mean and median survival times. The log-rank test by sire (Fig. 1.6) showed differences (p < 0.0001) among the sires as hybrid catfish sired by blue catfish 1 and blue catfish 7 had among the highest survival means with progeny from some channel catfish sires exhibiting more variable results. The survival outcomes for progeny of KMIX 214 X KMIX 81 or KMIX 209 X KMIX 78 were high, while progeny from B3 exhibited lower survival.

Pairwise log-rank comparisons were performed to explore survival differences among families sharing the same dam or sire. The analysis of dam effects revealed no statistically significant differences between all pairings with all p-values exceeding 0.05. In contrast, sires showed high significance differences between groups ($p < 0.0001$) with sharper separations between curves compared to the dam analysis. Among the nine sires represented in the challenge, four were blue catfish males (B1, 3, 6, and 7) and five were channel catfish males. B1 contributed to three genotypes, B3 and B7 to two each, whereas every remaining sire was represented in a single family.

Bonferroni-adjusted pair-wise log-rank tests (matrix excerpt above) revealed that the N/A channel catfish sire produced offspring with significantly lower survival than progeny of every other sire except Z4 KS 64 × M 2 (13) (a $p \leq 0.05$). Notably, significant differences also emerged within the blue catfish group. Survival of B1 families differed from that of B6 families ($p = 0.038$), and B1 differed from the KMIX 121 X KMIX 47 sire ($p = 0.0076$). In contrast, B3 and B7 did not differ from B1 or one another once the Bonferroni correction was applied. The remaining channel sires (KMIX 117 X KMIX 55, KMIX 214 X KMIX 81 / KMIX 209 X KMIX 78, KMIX 121 X KMIX 47) formed a homogeneous cluster with no significant pair-wise differences after adjustment.

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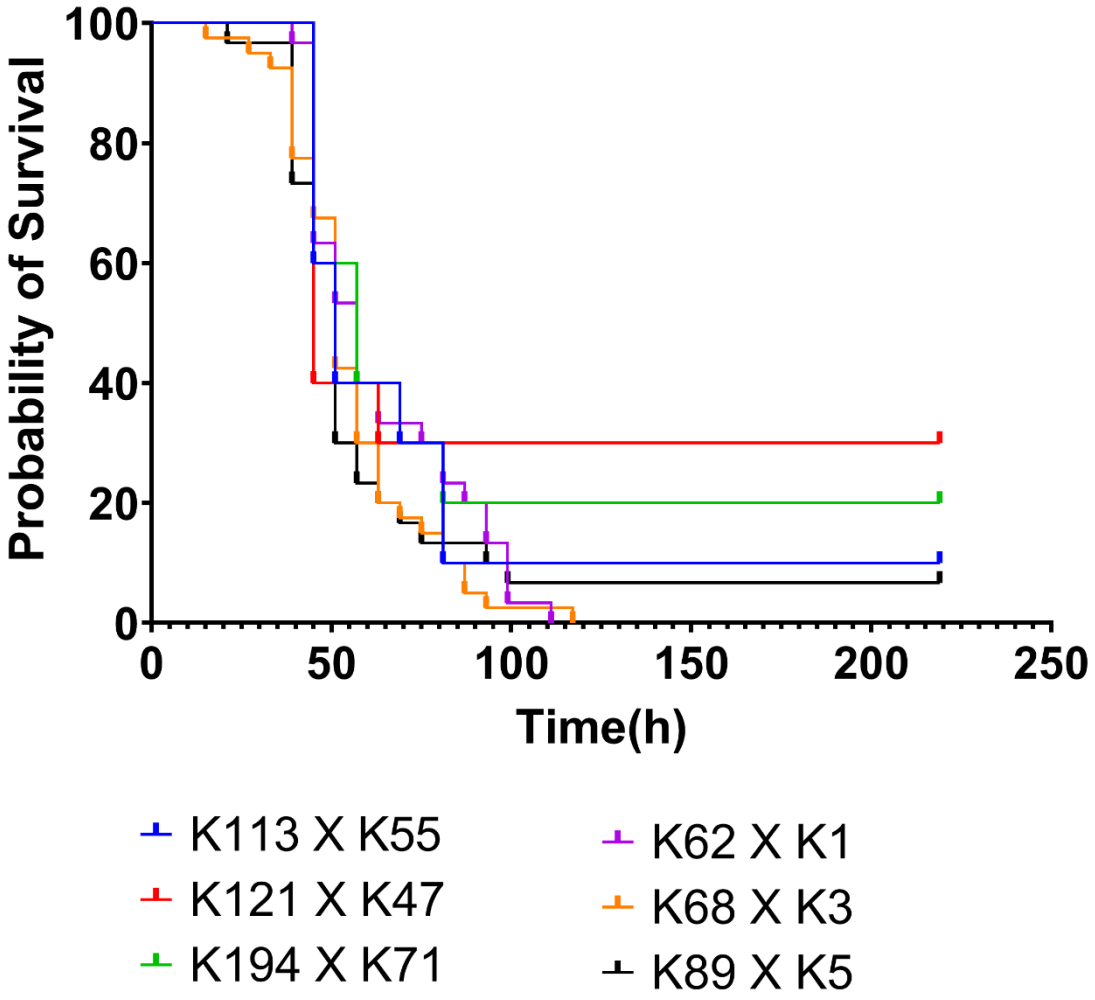

Figure 1.4. Kaplan-Meier survival analysis of channel catfish dams (*Ictalurus punctatus* challenged with channel catfish virus. Based on dam identity revealed no significant differences in survival probabilities among offspring groups derived from different dams (p = 0.19), (K for channel catfish)

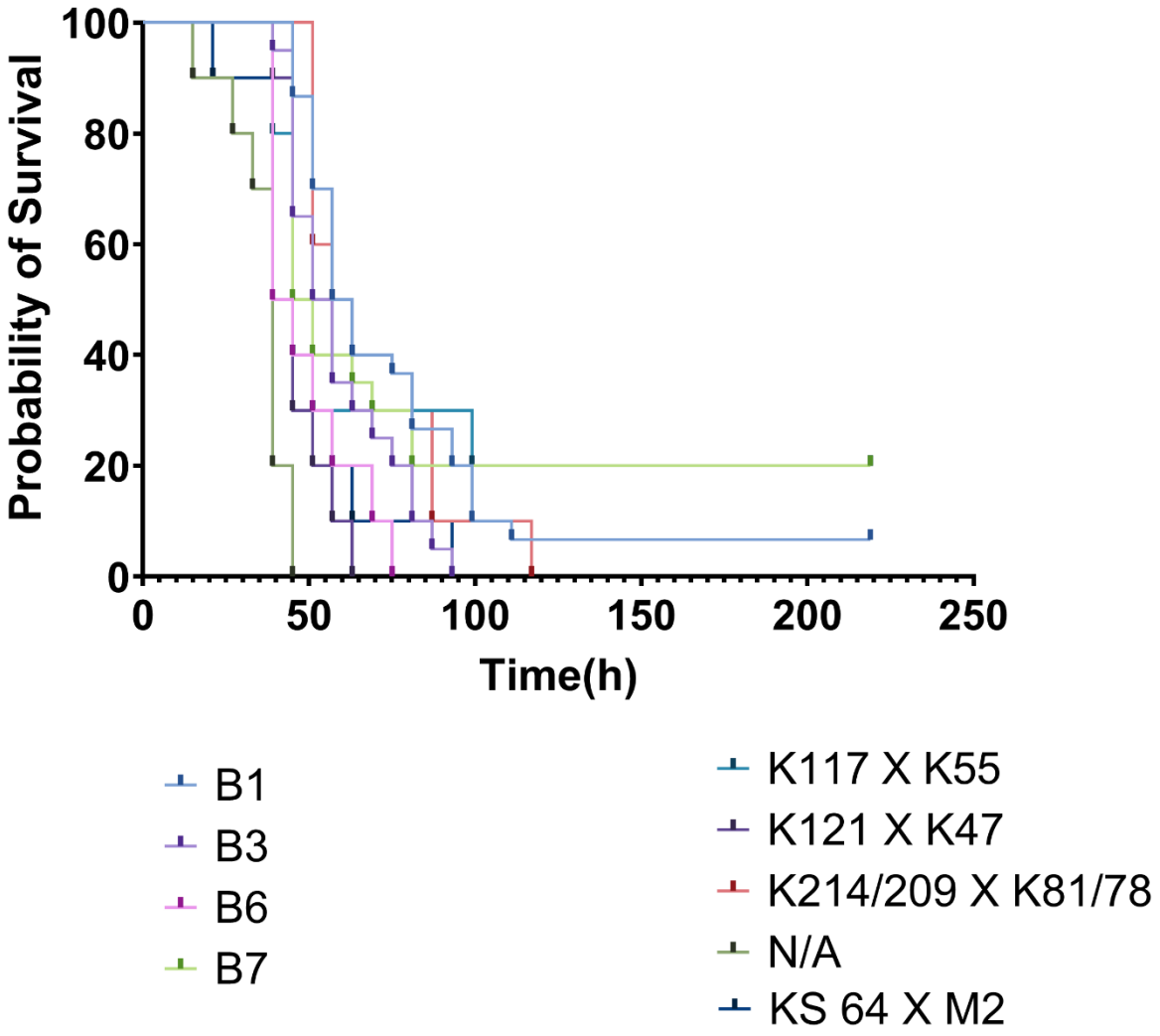

Figure 1.5. Kaplan-Meier survival curves based on sire of progeny, channel catfish (K) and blue catfish (B) challenged with channel catfish virus.

4.0 Discussion

The combined Kaplan-Meier curve shows that CCV-induced mortality was concentrated within the first ~50 h after immersion, indicating under the current experimental conditions the bulk of deaths occurred in the initial 2–3 days post-exposure. This steep early decline indicates a critical window in which viral replication and host-pathology coincide. Fish that survive beyond that point appear to enter a recovery phase with greatly increased chance

of survival. The restricted-mean survival time of ~60 h further underscores CCV's acute rather than chronic impact under the dose used here. These results demonstrate that differences in survival were driven by certain specific genotype comparisons rather than being broadly distributed across all genotypes. Hybrid catfish demonstrated a more consistent survival pattern across families. Sires, especially blue catfish, had a stronger effect on CCV resistance than dams.

The genotype (KMIX 68 × KMIX 3) × (N/A) exhibited significantly lower survival than nearly every other family, while (KMIX 121 × KMIX 47) × (B7) had the highest median (93 h) and mean survival times (~100 h). Such family-based differences are consistent with earlier reports that CCV resistance has a genetic basis and can vary several-fold among catfish families selected under identical rearing conditions (Plumb et al., 1975). Hybrid catfish maintained higher survival throughout the trial, which is in contrast to other studies. Plumb and Chappell (1978) showed that there was no difference between hybrid catfish and channel catfish when injected with CCV, and, Silverstein et al.(2008) found that hybrid catfish also had the same or less resistance than channel catfish to CCV depending upon the strain of channel catfish that they were tested against. These different results may be due to strain or family effects, strain of CCV, epigenetics, maternal effects, mode of challenge or a variety of environmental and genotype-environment interactions.

Dam effects neared but were not significant. This suggests that any maternal contribution had largely dissipated by the 6-week fingerling stage evaluated here, a finding concordant with early CCV work showing stronger resistance in fry than in fingerlings. (Hanson et al., 2004; Plumb et al., 1975). An earlier study indicated that maternal effects significantly influence resistance immediately post-hatch (Plumb et al., 1975) but tend to dissipate as fingerlings mature to 2-4 months of age, consistent with the results observed in the current study. When maternal effects are environmental, they typically dissipate with time (Dunham 2023). Given that the fish in the current experiment were challenged at an older developmental stage, maternal effects might have diminished significantly, aligning with the observed lack of substantial maternal impact in the analysis. Dunham et al.(unpublished) found that heritability for CCV resistance in Auburn strain of channel catfish was 0.0, but maternal effects were highly significant in contrast to the maternal effects appearing to have mostly dissipated in the current study, In the case of growth rate, maternal effects last for different

periods of time within channel catfish and within other fish (Dunham, 2023), which is not unexpected as environmental influence would likely vary among studies.

In contrast, sires exerted a clear influence on survival. The unidentified channel catfish sire (N/A) produced the poorest-surviving progeny and differed significantly from nearly every other male tested. Even among blue-catfish males, survival of the B1 progeny exceeded that of B6. This indicates that a follow-up is needed to determine the extent and form of combining ability, which would allow improvement of CCV resistance in hybrid progeny via recurrent selection. Given that only sires, not dams, displayed significant heterogeneity, paternal genetics or sire-specific epigenetic factors appear more important than maternal effects at this developmental stage.

5.0 Conclusion

Under the experimental conditions used here, the window for effective intervention against CCV was extremely short with virtually all deaths occurring within the first 50 hours post-challenge. Hybridization (channel catfish ♀ × blue catfish ♂) offered a survival advantage. Parental effects were evident, with sire (paternal) influence proving more critical than dam (maternal) influence in these two-month-old fingerlings. Thus, further research is needed to determine combining abilities as they appear to be significant and need to be defined to allow precise development of genetic enhancement programs for CCV resistance. The current results vary from two previous experiments. CCV resistance appears complicated from both a genetic and environmental perspective, and further experimentation is needed to clarify these effects. Future research should examine different doses, strain of CCV, strains of parents, method of virus delivery and other factors that could further explain the results and the importance of both genetic effects as well as genotype-environment interactions.

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Chapter III: Genetic Variability in Resistance of Channel Catfish (*Ictalurus punctatus*) and Hybrid Catfish (Channel Catfish ♀ x Blue catfish, *I. furcatus* ♂) to *Flavobacterium covae* Under Different Bacterial Challenge Doses

ABSTRACT

Two sequential immersion challenges were conducted to quantify genetic resistance to Flavobacterium covae in genetically improved channel catfish (Ictalurus punctatus) and hybrid catfish (channel catfish ♀ × blue catfish, I. furcatus ♂). In each trial, 11 family genotypes (five channel catfish, six hybrid catfish) were evaluated. Survival was recorded every 6 hours during the high-dose challenge and every 4 hours during the half-dose challenge for maximum durations of 80 hours and 192 hours, respectively. Survival data were analyzed using Kaplan–Meier curves and pairwise log-rank tests.

Significant differences in survival outcomes were observed among families under the high-dose exposure at $p < 0.0001$, and at $p = 0.0791$ under the half-dose exposure. Under high-dose conditions, survival times were considerably lower, with the most resistant genotypes such as *(*K62 × K1) × (K121 × K47) and (K68 × K3) × (B1) demonstrating delayed mortality compared to the most susceptible family, (K194 × K71) × (B1). In contrast, the low-dose trial exhibited uniformly high survival with mean survival times ranging from 108.4 to 176 hours and median survival clustering near 176 hours for most genotypes.

In previous studies, hybrid catfish displayed greater resistance to columnaris disease than channel catfish. The unexpected, contradictory results in the current study may be partially attributed to environmental stressors as hybrid catfish may have experienced greater stress under the small, confined mesh cage conditions, as they exhibit fast swimming nervous behavior is small culture unit potentially influencing their disease susceptibility. These findings emphasize the importance of evaluating genetic resistance under varying environmental conditions and pathogen doses.

Key words: Keywords: Channel catfish (*Ictalurus punctatus*), Hybrid catfish, Selective breeding, Genetic improvement, Crossbreeding, *Flavobacterium columnare*, Disease resistance

1.0 Introduction

Columnaris disease, caused by *Flavobacterium covae,* poses a significant threat to freshwater aquaculture, particularly impacting channel catfish populations. This Gram-negative, filamentous bacterium primarily infects fish gills, skin, and fins, leading to severe lesions and high mortality rates, especially in warmer water temperatures above 25°C that favor bacterial growth (Davis 1922; Anderson & Conroy 1969; Wolke, 1975). The bacterium can form biofilms, enhancing its resistance to antibiotics and its persistence in aquaculture environments (Austin & Austin, 1993; Wakabayashi, 1993). Environmental stressors such as poor water quality, high stocking densities, and fluctuating temperatures exacerbate disease outbreaks, significantly contributing to economic losses, particularly in channel catfish, *Ictalurus punctatus*, farming where columnaris disease ranks second only to *Edwardsiella ictaluri* in economic impact (Wagner et al., 2002).

The genetic diversity of *F. covae* complicates disease management efforts, as specific genomovars exhibit varying levels of virulence. Genomovar II isolates, for instance, are significantly more virulent compared to genomovar I, causing up to 100% mortality in challenged channel catfish fry (Shoemaker et al., 2008). The adaptability of *F. covae*, evidenced by its expanding host range, including a documented outbreak in black carp (*Mylopharyngodon piceus*) in Northern Vietnam resulting in substantial mortality, highlights its ongoing threat to diverse aquaculture species (Hoai et al., 2025). Consequently, selective breeding programs focused on enhancing disease resistance in channel catfish must navigate these genetic complexities, advocating tailored breeding approaches to effectively counteract pathogen variability.

Resistance to columnaris has been demonstrated to be a heritable trait in aquaculture species. In Nile tilapia (*Oreochromis niloticus*), quantitative genetic analyses using full-sib families exposed to controlled *columnarischallenges* revealed genetic variation in survival with disease resistance evaluated as both days to death and binary survival outcomes. These traits were analyzed using established linear animal and sire-dam models, providing direct evidence that resistance is influenced by additive genetic factors and can be enhanced through selective breeding efforts (Wonmongkol et al., 2018).

More broadly, reviews of selective breeding in aquaculture confirm that traits related to disease resistance are generally heritable and respond well to selection, reinforcing the

feasibility of breeding programs aimed at improving survival against bacterial diseases such as columnaris (Gjedrem & Rye, 2018). In channel catfish, studies have investigated genetic and immunological variation among catfish families. Arias et al. (2012) compared channel catfish and blue catfish, *I. furcatus*, with hybrid catfish (channel catfish female × blue catfish male), showing that hybrid catfish experienced significantly lower mortality rates after exposure to highly virulent *F. covae* strains, suggesting heterobeltiosis for columnaris resistance.

Genomic approaches have been applied to further dissect the genetic basis of columnaris resistance. A genome-wide association study (GWAS) in backcross hybrid catfish identified markers associated with resistance traits, though the specific outcomes of this work have not been made fully available in detailed publications (Geng, 2016). In parallel, studies have explored immune mechanisms underlying resistance, including differences in mucosal immune polarization between susceptible and resistant channel catfish (Peatman et al., 2013).

Despite these advances, there is a lack of direct comparative studies evaluating the efficacy of mass versus family-based selection for improving survival against *F.covae*. General reviews of selective breeding in aquaculture support the use of family-based methods, particularly when combined with genomic technologies, for achieving sustainable gains in disease resistance while controlling inbreeding (Gjedrem & Rye, 2018).

The primary objective was to examine family variation for survival in channel catfish and hybrid catfish when challenged with different doses of *Flavobacterium covae* and determine any genotype-environment interactions. Another objective was to compare the columnaris resistance of channel catfish and hybrid catfish families and determine dam and sire effects.

2.0 Materials and Methods

All investigations and experimental studies on animals were conducted according to the Institutional Animal Care and Use Committee (IACUC) and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) protocols and guidelines for Auburn University Institutional Animal Care and Use Committee (AU-IACUC # 2023-5149).

2.1 Broodstock management

Broodstock were housed at the Auburn University E.W. Shell Fisheries Center in Auburn, Alabama from 2019 to 2024. Channel catfish and blue catfish were cultured in earthen ponds and fed five days a week with 32% protein pelleted feed during the summer and three days per week during the winter. Leading up to the spawning season, feed was shifted to a 36% protein broodstock feed provided five days per week. In June, during peak spawning season, mature (7- -year-old) channel catfish females (N = 14, mean body weight = 2.95 kg), channel catfish males (N = 18, mean body weight = 2.12 kg) and blue catfish males (N = 7, mean body weight = 3.06 kg) were harvested from the earthen ponds by seining, using a 3.8 cm mesh net.

2.2 Experimental fish

Channel catfish were KMIX line derived from the Kansas strain,which is the oldest domestic strain of channel catfish collected from the Ninnescah River, Kansas by the Kansas Fish and Game in 1911 Dunham and Smitherman, 1984). The Kansas strain was obtained by Auburn University in 1970 from the first catfish farm, Krebiel Farm, Kansas. It later proved to be one of better growing and disease-resistant strains of channel catfish, but it has a late maturation. After 6-7 generations of mass selection (Dunham & Smitherman, 1983; Dunham & Brummett, 2019; Rezk et al., 2003; Padi, 1995; Dunham 2007), these fish showed severe inbreeding depression and significantly reduced reproduction. They were backcrossed with Kansas random strain, to reinvigorate their reproduction and selection for body weight begun again for another 3 generations and renamed KMIX.

Parents for the experimental fish were produced by a series of full-sib and half-sib matings (Johnson, 2021). Twelve of these channel catfish females were mated with 20 channel catfish and blue catfish males to produce a series of full-sib and half-sib families. These spawns had poor hatch, and for the current experiment 11 families, 5 channel catfish and 6 hybrid families, representing 5 dams and 7 sires were used in the challenges (Table 2.1).

2.3 In vitro fertilization procedures

Upon harvest, gravid channel catfish females were administered luteinizing hormone releasing hormone analogue (LHRHa) at 90 μg/kg body weight via intraperitoneal implantation. Following implantation, the females were placed into individual mesh bags (38 cm × 56 cm) and held ~30 cm apart in 670 to 750 L flow through (30 L/min) pond water holding tanks. Temperature in the tanks ranged from 26 to 28ºC. At 36 h post-implantation,

bags were checked every 4 h for eggs attached to the spawning bag, which indicates that the female is ovulating. Once eggs were detected, the females were anesthetized with 100 mg/L tricaine methanesulfonate (MS-222, Ferndale, WA) and 100 ppm NaHCO3 solution. Once anesthetized, the female fish were rinsed with pond water and dried thoroughly. Crisco® vegetable shortening was carefully rubbed on the underside of the females and eggs were hand-stripped into Crisco® coated metal pans (~25 g of eggs/pan). Blue catfish and channel catfish males were euthanized and their sperm were collected for *in vitro* fertilization (IVF) following protocols by Dunham and Masser (2012) and Hettiarachchi et al. (2022). After euthanasia, testes were removed from the body cavity with a sterile scalpel and forceps. Testes were rinsed with 0.9% saline solution to remove any blood. After rinsing, the testes were gently dried and then minced with a scalpel blade. Following mincing, the testes were filtered with a 100 μm mesh. Thereafter, 10 mL of 0.9% saline was added for each 1 g of testes (w/v). Following filtration, the sperm solution was ready for fertilization.

All *in vitro* fertilization IVF was conducted at 27 to 28^{o}C. Temperature in the hatching trough ranged from 26 to 28^{o}C and the flow rate was held at 3.79 L/min. After 1 h, eggs were moved into hanging mesh baskets (7.0 m × 0.4 m × 0.2 m), which were suspended in a flow-through pond water hatching trough with paddlewheel agitation and compressed aeration. Temperature remained between 26 to 28^{o}C and the flow rate was 15 L/min in the hatching trough.

2.4 Fry rearing

Following hatchery and yolk sac absorption, fry from different families were reared separately in two rearing systems: a flow-through system and a recirculating aquaculture system (RAS). The flow-through tanks measured 0.35 m × 0.65 m × 0.25 m (depth), while the dimensions for the RAS tanks 0.30 m × 0.60 m × 0.20 m (depth). Each family was stocked into a separate tank, and stocking densities were 1.8 fry/L and 2.0 fry/L in RAS and flow-through systems, respectively.

Fry were initially fed ad-libitum with a powdered 50% Protein fish feed. Once they reached the appropriate size (0.5-1 grams), fry were transitioned to a 300 mm pelleted feed. Feed was administered multiple 4-5 times daily to ensure uniform growth and minimal competition. At two months of age, fry reached an average weight of approximately 2.4 grams.

Water quality was monitored daily in both systems. In the RAS, water temperature was maintained between 28–30°C, dissolved oxygen (DO) was kept above 5 mg/L, and salinity was maintained at 2–3 ppt. Other monitored parameters included ammonia, pH, and salinity, with nitrite, nitrate, GH, and KH also checked as needed which remained within narrow, fish-safe limits, pH ranged from 7.0 to 8.0, salinity held at 2–3 ppt, nitrite stayed below 0.25 mg L^{-1} and nitrate below 5 mg L^{-1}, while general hardness (GH) and carbonate hardness (KH) each remained between 71.6 and 89.5 mg L^{-1} as $CaCO_3$.

2.5 Columnaris disease challenge

Two sequential *Flavobacterium covae* immersion-challenge trials were conducted in the same rectangular flow-through tank (1.20 m × 0.34 m × 0.10 m) supplied with carbon-filtered, heated municipal water (28–29 °C, 2 L min^{-1}) and continuously aerated. After the first (high-dose) trial the challenge tank, cages, pumps, and all associated equipment were drained, rinsed, and soaked for 24 h in a 1 % Virkon™ solution (10 g L^{-1}). The system was then rinsed with de-chlorinated water before being refilled and used for the second half-dose challenge.

A separate, identically plumbed tank located in the same room served as the mock-control system throughout the study, receiving only sterile modified Shieh broth without the pathogen. Within the challenge tank, twelve mesh cages of identical (10 cm width X10cm length X 20 cm height) were suspended along the walls, each holding ten fingerlings of a single genotype. The experimental set in both trials included five channel catfish families and six channel catfish female × blue catfish male hybrid families.

Immediately before each immersion, flow to the challenge tank ceased and the volume reduced to 100 L. For the first trial, a suspension of *Flavobacterium covae* ARS-00-530 (4.25×10^8 CFU mL^{-1} stock) was added at 550 mL, yielding an exposure concentration of approximately 2.32×10^6 CFU mL^{-1}. Mortalities were recorded every six hours for 80 h. After tank disinfection, the second trial was run under identical conditions but with the bacterial inoculum diluted 1 : 1, producing a half-dose bath of roughly 1.16×10^6 CFU mL^{-1}. Mortalities were collected every four hours for 200 h. In both experiments, fish were immersed for one hour with vigorous aeration and water circulated, after which flow was restored to 2 L min^{-1} and the tank was refilled to 330 L. Circulating pumps were used to mix the water constantly to

ensure that all families were equally exposed to the same water quality and bacterial titer in the tank. Dead fish were removed at each inspection, and survival time (hours post-exposure) recorded as an event status (dead = 1, alive = 0).

2.6 Statistical analyses

All statistical analyses were performed using R software (version [4.4.2]). Survival analysis was conducted using the Kaplan-Meier estimator to evaluate differences in survival probability among families, dams, sires, and family types (channel catfish vs. hybrid catfish) under the two pathogen doses. The survival and survminer packages were used to generate survival curves and conduct log-rank tests. A global log-rank test was applied to assess overall survival differences across groups, followed by pairwise log-rank tests with Bonferroni correction to identify specific group comparisons showing significant differences.

Mean and median survival times and mean survival were calculated for each family, and families were ranked accordingly. Additional stratified analyses were performed to assess the influence of shared parentage (dam or sire) on survival outcomes. Graphical representations of survival curves were generated using ggsurvplot, with confidence intervals included or excluded as needed. Data visualization and summary statistics were also performed using packages from the tidyverse suite.

Additionally, all survival statistics were independently verified in GraphPad Prism (version 10.4.2, Windows 10). The raw data were entered into a Prism survival table containing three columns—time (h), event status (1 = death, 0 = censored), and the grouping variable (family, sire, dam, or treatment). Within Prism, Kaplan–Meier survival curves were generated for each analysis stratum, overall log-rank (Mantel–Cox) tests were calculated, and pair-wise log-rank comparisons were performed using the “compare all pairs of groups” option with Bonferroni adjustment of p-values. The resulting curves and accompanying risk-tables were exported as high-resolution figures and used directly in the manuscript; all numerical p-values from Prism were cross-checked against the R output to ensure consistency.

2.7 Data stratification for survival analyses

For every challenge overall survival was analyzed first, pooling all 110 fingerlings in first high does challenge and all 110 fingerlings in the second low dose challenge into a single Kaplan–Meier curve. The dataset was then stratified into progressively finer groupings.

Family genotype (n = 11 cages per trial;) – Each mesh cage contained ten full-sib fingerlings, giving 110 observations distributed across five channel catfish families and six channel × blue hybrid catfish families. Kaplan–Meier curves and pair-wise log-rank tests were generated for these 11 strata to identify the most and least resistant crosses.

Sires and dams – After the overall and family-genotype evaluations in the high dose and low-dose experiment, fish were regrouped by parent line to test for maternal and paternal effects on columnaris resistance. five dams and seven sires were represented (10 fingerlings per parental line). Kaplan–Meier curves were generated for each group with the survfit() function, and global differences were assessed with log-rank tests (survdiff). Where the global test was significant, pairwise log-rank comparisons (pairwise_survdiff, no p-value adjustment) were performed; resulting p-value matrices were exported to Excel for interpretation.

Treatment class (n = 2) – All pure-channel catfish families were combined (50 fish per trial) and contrasted with all hybrid catfish families (60 fish) to test the broad genetic hypothesis of channel catfish versus hybrid catfish resistance.

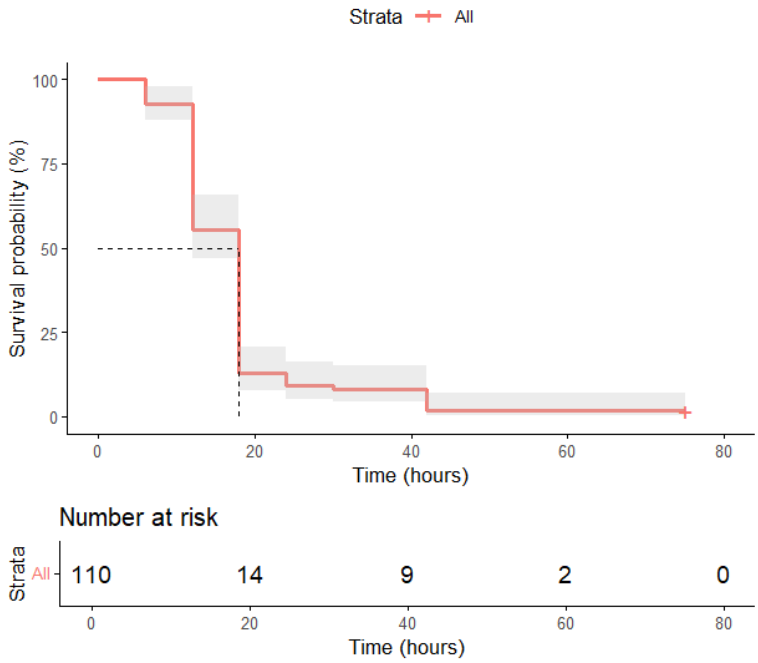
3.0 Results

3.1 Survival

In the high dose *Flavobacterium covae* challenge (Fig. 2.1), the overall survival pattern indicated a rapid and severe response to the bacterial exposure. Within the first 24 hours post-challenge, survival dropped dramatically with only 16 individuals remaining alive out of the original 110 by the 24-hour mark. The steep decline continued, and by 72 hours post-challenge, complete mortality was observed in 9 families while two families had 10% survival. This rapid progression of mortality suggests that the bacterial dose and/or exposure conditions were extreme. The Kaplan-Meier survival curve reflects this trend with a sharp descent and a low

median survival time, and the number-at-risk table supports this with a near-complete drop-off in survivors within a very short time frame.

In contrast, the low-dose challenge (Fig. 2.1), which was performed using the same bacterial dose but half exposure time, exhibited a moderate rate of mortality trend. The survival probability rate was 76.03 % at the end of the trial period of 192 hours (8 days), with only gradual declines observed over time. Of the 110 individuals initially challenged, 95 were still alive at the final observation point.



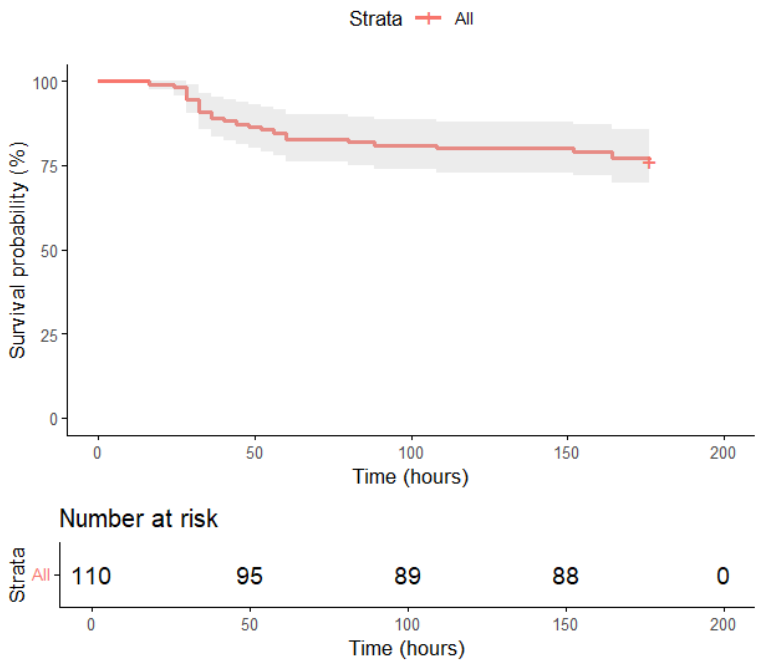


Figure 2.1. Kaplan-Meier survival curves for families of channel catfish (*Ictalurus punctatus*) and hybrid catfish (channel catfish ♀ x blue catfish (*I. furcatus*) ♂) challenged with *Flavobacterium covae*. Above: high-dose challenge, displaying rapid and extensive mortality within the first 72 hours post-challenge. Below: low-dose challenge, showing a more gradual decline in survival over the 192-hour observation period. Shaded areas represent the 95% confidence intervals, and the tables below indicate the number of fish at risk at each time point.

3.2 Survival analysis by family

Survival probability differed between families under high-dose challenge conditions at $p < 0.0001$), and at $p = 0.0791$ under low-dose conditions (Fig 2.2 and 2.3). In the high-dose experiment, family-level variation in survival was pronounced, with survival curves separating early and steeply after challenge, whereas in the low-dose experiment, families maintained high survival probabilities with minor separations across time.

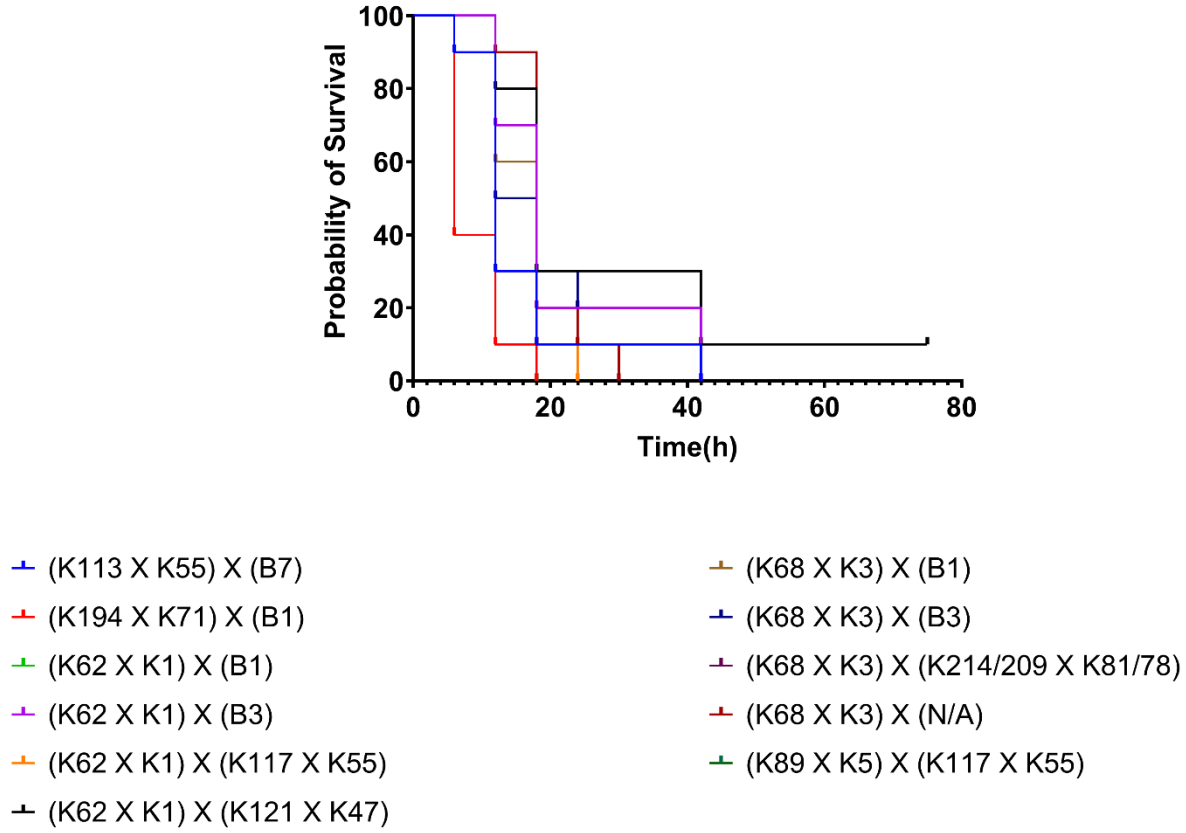
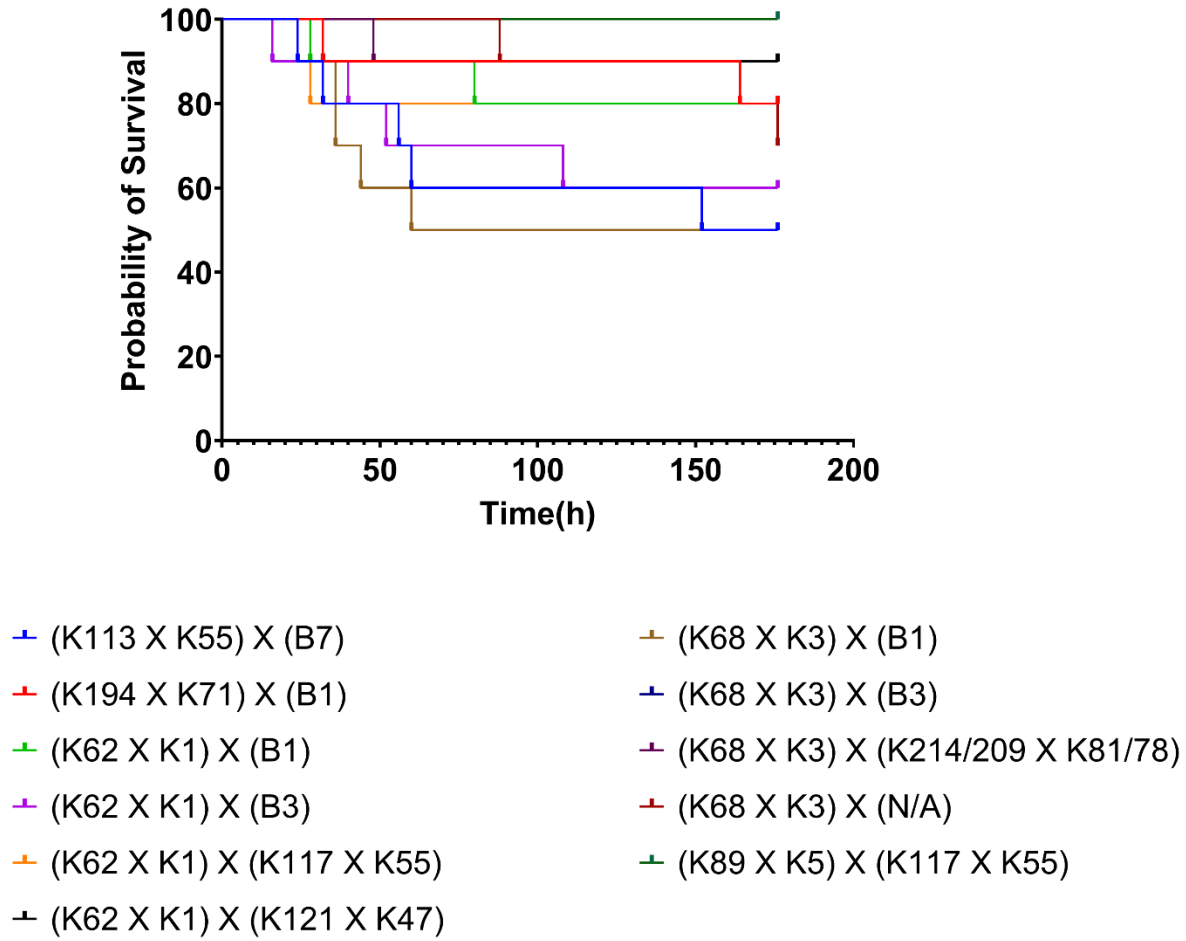


Figure 2.2. Kaplan–Meier survival curve for families of channel catfish (*Ictalurus punctatus*) and hybrid catfish (channel catfish ♀ x blue catfish (*I. furcatus*) ♂) showing family-level survival probabilities following high-dose *Flavobacterium covae* challenge. Significant separation was observed among families ($p < 0.0001$) with rapid declines in survival beginning within the first 20 hours post-challenge.



2.3. Kaplan–Meier survival curve for families of channel catfish (*Ictalurus punctatus*) and hybrid catfish (channel catfish ♀ x blue catfish (*I. furcatus*) ♂) showing family-level survival probabilities following low-dose *Flavobacterium covae* challenge. Survival differences were observed at $p = 0.0791$.

To further identify specific family-level differences, pairwise comparisons were performed using the log-rank test. Bonferroni correction was applied to adjust for multiple testing. Since 11 families were included after removing the control group, a total of 55 pairwise comparisons were possible. Therefore, the Bonferroni-adjusted significance threshold was calculated as $0.05/55 = 0.00091$.

Under high-dose conditions, pairwise comparisons revealed significant differences only between (K194 X K71) X (B1) with both (K62 X K1) X (K121 X K47) and (K194 X K71) X (B1) and (K68 X K3) X (N/A). No other significant pairwise differences were observed among families in the high-dose challenge after Bonferroni correction. In contrast, under low-

dose conditions, no significant pairwise differences were detected between any of the families based on the adjusted threshold.

Family-level mean survival percentages, mean survival times (hours), and medians were calculated and are summarized in Fig 2.4 & Table 2.1. In the high-dose challenge, survival rates varied from 0% to 10%, whereas in the low-dose challenge, survival rates were substantially higher, ranging from 50% to 100%.

Under high-dose conditions, families such as (K62 X K1) X (K121 X K47) and (K62 X K1) X (B3) exhibited relatively higher mean survival percentages (10%). Under low-dose conditions, multiple families achieved 90% or higher mean survival with (K89 X K5) X (K117 X K55) having 100% survival. Median survival times in the low-dose experiment were uniformly high across families (176 hours), whereas in the high-dose experiment, median survival times varied, ranging from 6 to 18 hours depending on the genotype.

Table 2.1: Mean survival percentage, mean survival time (hours), and median survival time for families of channel catfish (*Ictalurus punctatus*) and hybrid catfish (channel catfish ♀ x blue catfish (*I. furcatus*) ♂) under high-dose and low-dose Flavobacterium covae challenges. Statistically significant differences in survival were observed among certain families using Log-rank (Mantel-Cox) test. Descriptive statistics presented in the table—including mean survival percentage, mean survival time, and median survival time—were calculated using standard arithmetic functions in R mean, median, and survivor proportion.

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Genotype	High dose			Low dose		
	Mean survival (%)	Mean(h)	Median	Mean survival (%)	Mean (h)	Median (h)
(K62 X K1) X (K117 X K55)	0	16.8	18	80	146.4	176
(K113 X K55) X (B7)	0	15.6	12	50	120.4	152
(K68 X K3) X (B1)	10	21.3	18	50	108.4	60
(K62 X K1) X (B1)	0	13.2	12	80	151.6	176
(K62 X K1) X (K121 X K47)	10	27.3	18	90	161.6	176
(K62 X K1) X (B3)	0	21	18	60	127.2	176
(K194 X K71) X (B1)	0	9	6	80	160.4	176
(K68 X K3) X (N/A)	0	19.2	18	70	166	176
(K68 X K3) X (B3)	0	20.4	15	90	161.6	176
(K89 X K5) X (K117 X K55)	0	15.6	18	100	176	176
(K68 X K3) X (K214/209 X K81/78)	0	16.2	18	90	163.2	176

Table 2.2: Pairwise comparison matrix for survival differences under high-dose Flavobacterium covae challenge conditions for families of channel catfish (Ictalurus punctatus) and hybrid catfish (channel catfish ♀ x blue catfish (I. furcatus) ♂) family. Raw p-values from pairwise log-rank tests are presented. Bonferroni correction was applied, with a significance threshold set at 0.00091. Significant pairwise differences were observed primarily between (K194 × K71) × (B1) and two other families, but only those two comparisons remained significant after adjustment.

Genotype	(K113 X K55) X (B7)	(K194 X K71) X (B1)	(K62 X K1) X (B1)	(K62 X K1) X (B3)	(K62 X K1) X (K117 X K55)	(K62 X K1) X (K121 X K47)	(K68 X K3) X (B1)	(K68 X K3) X (B3)	(K68 X K3) X (K214/209 X K81/78)	(K68 X K3) X (N/A)
(K194 X K71) X (B1)	0.0362									
(K62 X K1) X (B1)	0.7080	0.0439								
(K62 X K1) X (B3)	0.1295	0.0015	0.0380							
(K62 X K1) X (K117 X K55)	0.3585	0.0017	0.0521	0.4049						
(K62 X K1) X (K121 X K47)	0.0402	0.0006	0.0114	0.3965	0.1302					
(K68 X K3) X (B1)	0.1866	0.0034	0.1098	0.8787	0.9555	0.4030				
(K68 X K3) X (B3)	0.2984	0.0037	0.0855	0.7487	0.5760	0.2816	0.8797			
(K68 X K3) X (K214 X K81 X K209 X K78)	0.5246	0.0034	0.1098	0.2775	0.7421	0.0849	0.7215	0.4304		
(K68 X K3) X (N/A)	0.1702	0.0002	0.0054	0.7738	0.2074	0.2937	0.5475	0.9590	0.1324	
(K89 X K5) X (K117 X K55)	0.4029	0.0035	0.1495	0.2684	0.4239	0.0820	0.6844	0.4094	0.6844	0.0547

3.3 Comparison of survival between channel catfish and hybrid catfish families

In the high-dose experiment, channel catfish families demonstrated higher survival probability compared to hybrid catfish families ($p = 0.0383$; Fig 2.4). Mortality in hybrid families occurred earlier and more rapidly, whereas channel catfish families displayed delayed and more gradual mortality over time.

In the low-dose experiment, channel catfish families also exhibited superior survival compared to hybrid catfish families ($p = 0.0282$; Fig 2.5). Although mortality was much lower overall in this trial, the separation between the channel catfish and hybrid catfish curves was still evident with hybrid catfish consistently displaying lower survival probability throughout the experiment.

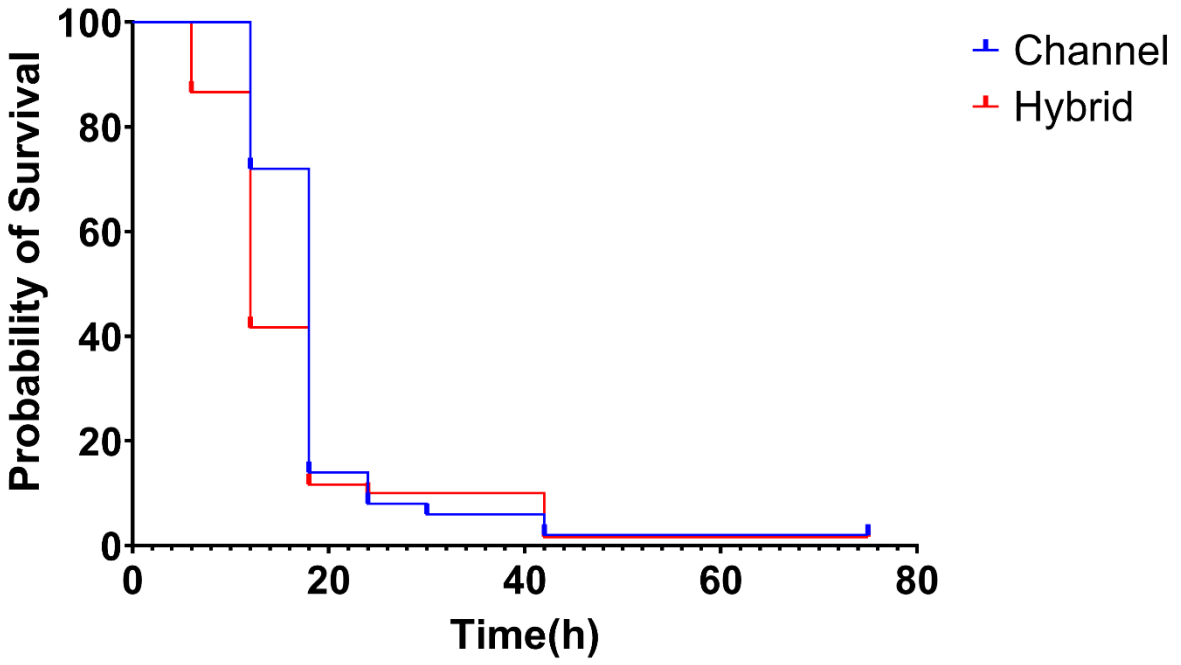

Figure 2.4. Survival probability under high-dose *Flavobacterium covae* challenge conditions for families of channel catfish (*Ictalurus punctatus*) and hybrid catfish (channel catfish ♀ x blue catfish (*I. furcatus*) ♂). Channel catfish had higher survival probabilities compared to hybrid catfish ($p = 0.0383$).

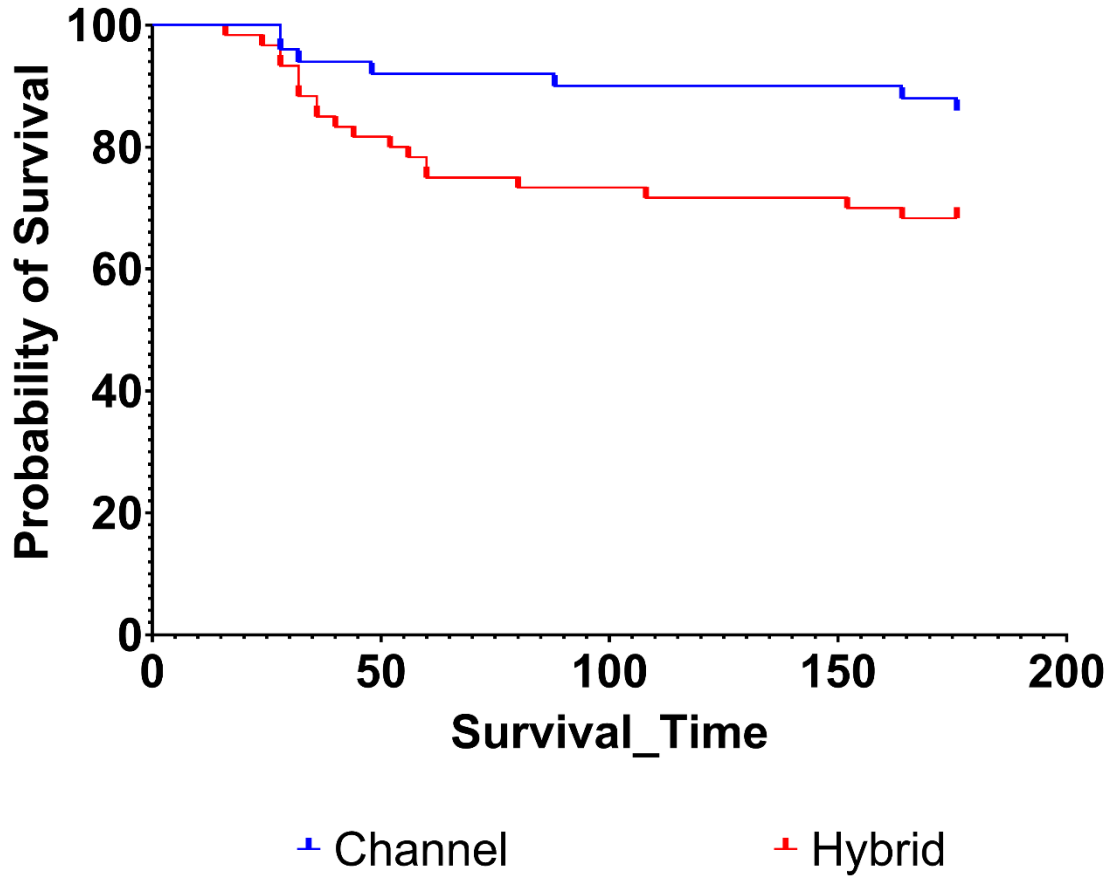

Figure 2.5. Survival probability for survival differences under low-dose *Flavobacterium covae* challenge conditions for families of channel catfish (*Ictalurus punctatus*) and hybrid catfish (channel catfish ♀ x blue catfish (*I. furcatus*) ♂). Channel catfish had higher survival relative to hybrid catfish (p = 0.0282).

3.4 Sire and dam contribution to survival outcomes

In the high-dose challenge experiment, significant differences in survival were detected among dams ($p < 0.0001$) but not among sires ($p = 0.0103$) after applying the Bonferroni adjustment. Bonferroni correction was calculated based on 5 dams (for 11 families) and 7 sires (for 11 families), resulting in adjusted significance thresholds of 0.005 and 0.0071, respectively. Pairwise analysis identified that the dam K194 × K71 was significantly different from three other dams, K62 × K1 ($p >0.001$), K68 × K3 ($p >0.001$) and K89 × K5 ($p = 0.0035$).

While the overall log-rank test for sires in the high-dose challenge suggested statistical significance ($p = 0.0103$), no specific pairwise comparisons between individual sires remained significant after Bonferroni adjustment. Thus, no sire combination showed statistically meaningful differences in survival under the high-dose condition.

In the low-dose challenge, no significant differences were observed either among dams ($p = 0.18$) or sires ($p = 0.24$). Pairwise comparisons confirmed the absence of significant variation between any specific dams or sires at the lower pathogen exposure level.

4.0 Discussion

The results of the two *Flavobacterium columnare* (FC) challenge experiments indicated a low level of genotype environment interactions as a result of pathogen dose. In both the low and high dose *Flavobacterium covae* challenges, channel catfish had better resistance to the pathogen compared to channel X blue hybrid catfish. This is the opposite of the results obtained by Ella (1984) and Arias et al. (2012), that indicated channel catfish × blue catfish hybrid were more resistant than channel catfish. One potential explanation is that an improved channel catfish line (KMIX strain) that has undergone several generations of selection for general performance traits, has reached a high level of resistance to columnaris or this line does not combine well with blue catfish for this disease resistance trait. Additionally, genetics of the pathogen could be important. The strain of *F. covae* utilized could result in genotype-environment interactions. Our findings agree with the family-level analysis of LaFrentz et al. (2012), who reported substantial variation in columnaris resistance among channel catfish families and concluded that selective breeding within this species could deliver genetic gain.

These findings highlight the genotype-specific nature of disease resistance, and the potential of certain family combinations, especially those involving KMIX 62 and KMIX 121, as strong candidates for further selection in breeding programs aimed at enhancing columnaris resistance**.** Thus, under acute exposure, channel families were markedly more resistant to *F. columnare* than their hybrid counterparts.

Taken together, these trials indicate that channel families consistently outperformed hybrid catfish, but the advantage is most evident under severe infection pressure. Once the challenge was moderated, the performance of hybrid catfish approached that of channel fish. The stronger divergence between genetic groups at the high dose but more convergence at the low dose points to a potential threshold effect. Such potential dose-dependent interactions indicate caution against drawing breeding conclusions from a single challenge intensity.

Biosecurity impedes pond trials for disease resistance research. There is a need to gather additional data regarding potential genotype-environment interactions of tank pond and trials. Hybrid catfish exhibit nervous behavior in small, confined environments (Wang et al. 2022, Dunham 2023), which could impact results of disease challenges and increase the incidence of genotype-environment interactions. A single virulent isolate may not capture the full ecological complexity of pond outbreaks. Larger pond experiments, multiple genomovars, and evaluation of different genetic types of channel catfish X blue catfish hybrid are needed.

In addition to genetic differences, environmental and behavioral factors may have influenced the observed survival patterns. Hybrid catfish, in particular, may experience greater stress under confined conditions such as small mesh cages. Hybrid catfish often exhibit more active and nervous behavior compared to channel catfish, which could translate into elevated physiological stress when held in restricted environments. Increased stress can compromise immune function, potentially explaining the reduced survival of hybrid families observed across both experiments.

Thus, part of the difference between channel catfish and hybrid catfish performance may not solely reflect innate genetic resistance to *F. covae* but also differences in stress tolerance and behavior under confinement. This highlights an important consideration for future experiments as evaluations of disease resistance should be conducted under a range of environmental conditions to better capture the full scope of genotype × environment interactions affecting survival. Testing resistance in both confined and more natural or larger settings could offer a more holistic understanding of genetic performance under real-world aquaculture conditions.

5.0 Conclusion

Together, these results emphasize the complex interplay between experimental conditions, environment, genetic background, and disease resistance challenge in aquaculture species. Selective breeding efforts aimed at improving disease resistance must use appropriately stressful challenge conditions to reliably differentiate superior from inferior genotypes. Future strategies should consider family, parental and maternal performance.

This study demonstrates that under high pathogen pressure, meaningful genetic variation exists within and between channel catfish and hybrid catfish families and parental lines, providing actionable opportunities to improve survival outcomes through targeted genetic enhancement programs.

6.0 References

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