COMBINING ABILITY OF CHANNEL CATFISH (*Ictalurus punctatus)* FEMALES AND BLUE CATFISH (*Ictalurus furcatus)* MALES FOR TOLERANCE OF LOW OXYGEN.

By

David Drescher

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

Auburn, Alabama
May 7, 2017

Approved by

Rex A. Dunham, Chair, Alumni Professor, School of Fisheries, Aquaculture, and Aquatic Sciences
Terrill R. Hanson Professor Extension Specialist, School of Fisheries, Aquaculture, and Aquatic Sciences

Nagaraj Chatakondi, Affiliate Professor, School of Fisheries, Aquaculture, and Aquatic Sciences

Abstract

Catfish are the backbone of the aquaculture industry in the United States accounting for the majority of finfish production. The primary species cultured was channel catfish (*Ictalurus punctatus*), but that has changed as 50-70% of production is now the channel catfish female X blue catfish (*I. furactus*) male hybrid catfish due to its increased growth and disease resistance. Full-sib and half-sib channel catfish female X blue catfish male hybrid families were evaluated for tolerance to low oxygen. Family variation was observed and general and specific combining abilities were calculated. Dam general combining ability had the greatest influence on tolerance to low oxygen in regards to survival and sire general combining ability had the largest effect on time to death. Strain of channel catfish female and blue catfish male had strong effects on the tolerance of low oxygen parameters for hybrid catfish fingerling. Both selection of individual channel catfish females, blue catfish males and strains of the parent species for increased tolerance of low oxygen should result in channel catfish female X blue catfish male hybrids genetically improved for tolerance of low oxygen.

Acknowledgments

I want to thank my lab mates, friends, and family for their assistance and encouragement in completing this project. A special thank you to the SRAC team members and Dr. Dunham for the endless help throughout my degree. Thank you to all of the E.W. Shell Fisheries Center staff for their support, especially Karen Veverica and Renee Beam.

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Introduction

The order Siluriformes is one of the most diverse families on Earth containing 34 families and just under 3,000 species (Lévêque et al. 2008). This diversity and hardiness has resulted in several catfish species cultured for food across the globe. In North America the species raised in aquaculture belong to the family Ictaluridae. The production of channel catfish (*Ictalurus punctatus)* and the channel-blue hybrid catfish (*Ictalurus punctatus* ♀ *x Ictalurus furcatus* ♂) plays an important economic role in the aquaculture industry in the United States. Alabama, Arkansas, Mississippi, and Texas are the top producers of catfish with production accounting for approximately 68% of the total domestic freshwater production in 2015 (NOAA, 2015). However, domestic catfish production shrank by more than 50% since 2003 (Hanson and Sites, 2015). There are several factors that caused the decline of the U.S. catfish industry including competition from Asia, increased feed and labor costs, and the control of fish diseases (Wagner et al. 2002; FAO, 2011).

Perhaps the largest reason for the decline of the domestic catfish industry is due to the imported *Pangasianodon* catfish. Which are incredibly suited to intensive aquaculture as they are capable of withstanding extremely low dissolved oxygen conditions and can be stocked at a much higher density than North American catfish species. Imported *Pangasianodon* has increased from 17.4 million kilograms in 2007 to 131.2 million kilograms in 2016 (NOAA, 2015). The reason for this increase is that *Pangasianodon* reach market size very quickly which creates an advantage to farmers as it allows them to produce much larger crops at a cheaper price (Phan et al. 2009). This has led to many U.S. catfish farmers leaving the industry as they were unable to turn a profit competing against foreign imports. Pond acreage from Mississippi, Arkansas, and Alabama were down 11% in 2014(Hanson and Sites 2015). One mechanism for U.S. farmers to become more competitive, profitable, and sustainable is to utilize the advances genetic enhancement could provide.

Genetic enhancement through artificial selection has been occurring in agriculture for centuries as farmers have been able to select parents with desirable traits and breed them in a controlled setting (Dunham 2004). In aquaculture, culturists have long selected the largest and hardiest fish as broodstock to sire future generations in an effort to increase production. In the United States, the production of channel catfish has historically dominated the industry due to its superior growth to market size, tolerance to handling stress, high ammonia and nitrite resilience, early sexual maturity, resistance to columnaris, *Flavobacterium columnare*, and the parasite *Ichthyophthirius multifiliis* when compared to other ictalurid species (Dunham et al. 1993; Dunham and Argue 2000). Several ictalurid species have preferable culture traits such as the blue catfish (*Ictalurus furcatus*), which displays superiority in reduced growth variation, increased resistance to channel catfish virus (CCV) and *Edwardsiella ictaluri* (Enteric Septicemia of Catfish, ESC), higher dress-out percentage, and increased harvestability (Dunham et al. 1993; Dunham and Argue 2000). Overall, the channel catfish was selected as the primary culture species due to exhibiting the most characteristics suitable to large-scale commercial production.

Since channel catfish were the main culture species they have received the majority of attention and funding in the past with genetic research beginning in the 1970's to improve their culture traits (Dunham 2004). A multitude of techniques have been employed in the past to increase desirable production characteristics with focus on mass selection for faster growth to market size (Dunham et al. 1987;Dunham and Brummett 1999; Dunham et al. 1999, Rezk et al. 2003), intraspecific breeding of channel catfish strains to improve various characteristics (Dunham et al. 1983; Dunham et al. 1987), the creation of a sterile, triploid channel catfish (Lilyestrom et al. 1999), and interspecific hybridization with other ictalurid species (Dunham et al. 1987; Dunham and Brummett 1999; Dunham et al. 1999; Argue et al. 2003). These methods have resulted in significantly improved culture traits with the exception being triploid channel catfish (Lilyestrom et al. 1999). Mass selection for size has been one of the most powerful methods for improvements in growth of channel catfish with an 11-18% body weight increase after one generation and a 50% increase is possible after four generations. (Bondari 1983; Dunham and Smitherman 1983; Dunham et al. 1987; Dunham and Smitherman 1987; Dunham and Brummett 1999; Dunham et al. 1999). The boost in growth also had positive pleiotropic effects of higher survival, better feed conversion ratios (FCR), and increased disease resistance (Dunham and Smitherman 1983). Overall, the most effective of these breeding programs has been the interspecific cross between a female channel catfish and a male blue catfish. The process of crossing two different species is known as hybridization and in aquaculture the goal is to create a hybrid that possesses multiple characteristics that are superior to that of the parents (positive heterosis) and therefore are more profitable to the producer (Masser and Dunham 2012). It is important again to note that the CXB hybrid is made from a female channel catfish and male blue catfish. The reciprocal between a channel male and blue female does not produce a superior hybrid (Masser and Dunham 2012).

When comparing two channel catfish lines selected for faster growth for two generations to the interspecific cross of channel catfish female X blue catfish male hybrid(CB hybrid) the CB hybrid exhibited faster growth than either of the two select lines (Dunham and Brummet 1999). This hybrid cross of the blue catfish male and channel catfish female has shown significant improvements in a variety of traits including; enhancement in growth uniformity (Dunham et al. 1982; Smitherman et al.1983; Argue et al. 2003), accelerated grow out (Dunham and Smitherman1981; Dunham et al. 1987; Dunham et al. 1990; Dunham and

Brummett1999**),** greater tolerance to lower dissolved oxygen concentrations (Dunham et al. 1983), greater resistance to disease in particular *Edwardsiella ictalurid* (ESC) (, Dunham et al.1990;Wolters et al. 1996), higher dress-out percentage (Smitherman et al. 1983; Argue et al.2003), higher harvestability, (Tave et al. 1981; Dunham et al. 1982;Smitherman et al. 1983; Dunham et al. 1986), better feed conversion ratios (FCR) (Li et al. 2004), and lower mortality rates (Dunham et al. 1987). Hybrids exhibit increased body weight yields of 18-100% over channel catfish (Smitherman et al. 1983; Dunham et al.1987; Dunham et al. 1990; Dunham and Brummett 1999). One reason C x B hybrids grow faster is because they start feeding earlier in the spring (Dunham and Masser 2012). C x B hybrid catfish also perform better in intensive, densely stocked systems such as the in pond raceway (Brown 2010). Due to these factors hybrid catfish have constituted an increasing proportion of total catfish processed in the USA, increasing from less than 5% in 2002 to 40% in 2014 (Mischke et al. 2017) and currently estimated at 50-70% (Avery, USDA, Stoneville, MS).

The hybrid is not without some drawbacks. The smaller head size means that traditional seining and grading techniques may increase stress in the fish as they tend to get caught in the net. These harvesting issues have the potential to affect yield as C x B hybrids can quickly become oversized for processors due to their fast growth rate. These larger fish will continue to consume large quantities of feed. Not only do these larger fish reduce yield when they finally go to the processor, the producer is penalized with a lower price for the oversized fish. Spawning to produce CXB hybrids has been a problematic in the past. Pen spawning is not very effective having only a 15% success rate (Masser and Dunham 2012). Currently, artificial fertilization is the most effective method of producing hybrid embryos with a 67-100% success rate when the female channel catfish were injected with hormones and hand stripped followed by the male blue catfish being sacrificed for their sperm (Dunham and Masser 2012), but is labor intensive. Xenogenesis is currently being researched to reduce the skill and labor needed to produce the CXB hybrid. Xenogenesis is a method of reproduction where the offspring differ from their parents. When creating the CxB hybrid, either primordial germ cells (PGCs) or spermatogonial stem cells are taken from the male blue catfish and transplanted into a triploid channel catfish male. Triploid fish are used because they lack endogenous germ cells so the foreign cells can propagate once introduced (Perera et al. 2016). When the injected triploid male channel catfish mates with a normal female catfish the result is CxB hybrid progeny. Xenogenesis still has yet to be perfected for ictalurid catfish and, thus cannot yet be used for commercial production.

In aquaculture, one must maintain adequate dissolved oxygen levels for the species being cultured**.** During the summer, this is particularly important as this is when fish have the highest metabolic rate and grow the most. This high and more frequent rate of feeding results in higher nutrient levels in the water. The combination of increased sunlight and nutrients stimulate a rapid rise in phytoplankton levels. These phytoplankton blooms lead to an upsurge in photosynthesis during the day and dissolved oxygen levels can become supersaturated (Smith and Piedrahita 1988). However, during the night there is no oxygen production due to lack of sunlight so photosynthesis cannot occur. The phytoplankton begin to respire taking up large amounts of oxygen which can quickly deplete the dissolved oxygen in the water. If nighttime DO levels are not monitored and treated via emergency aeration, oxygen levels can decrease to <1 mg/L, causing the fish to stress which may lead to disease or death. If phytoplankton numbers are too large it may also result in a die-off, which can impair water quality and decrease dissolved oxygen levels. The effects of low dissolved oxygen levels are well documented for nearly every cultured fish species and in hybrid catfish, hypoxia has been linked to increased susceptibility to a variety of pathogens including *Edwardsiella ictaluri, Aeromonas hydrophila* and *Edwardsiella tarda* (Welker et al. 2007). In 2010 it was reported that 28.1% of U.S. catfish producer's loss fish due to low oxygen event. 22.8% of the losses due to low oxygen were classified as severe meaning that greater than 2,000 lbs was lost in a single event (USDA 2010). In addition to being a contributing factor for a myriad of diseases, hypoxic conditions result in reduced feed consumption and metabolic rate. This negatively impacts farmers as their ultimate goal is growing the fish to market size as quickly as possible (Torrans 2008). Studies have shown the CXB hybrid consumes significantly more feed when compared to channel catfish in low oxygen saturation conditions (Green and Rawles 2011). The CB hybrid is capable of consuming 12% more and having an 11% greater net yield when compared to channel catfish at 25% oxygen saturation. Further studies suggest that oxygen saturation levels must be ≥48% to maximize net yield (Green et al. 2012). Due to feeding being the largest expense when raising catfish accounting for up to 50% of total costs it is important to have as much feed as possible consumed and efficiently converted into biomass. While it is often not possible to achieve optimal conditions all of the time due to environmental changes, levels must be satisfactory to the cultured organism (Giachelli et al. 1982, Burel et al. 1996).

Due to the great variation in oxygen levels in the aquatic environment, fish have evolved the ability to withstand a variety of stressors including varying degrees of tolerance to hypoxic conditions. To deal with hypoxic conditions some species such as the *Pangasianodon* catfish have evolved mechanisms such as a modified gas bladder that functions as an air breathing organ or the Crucian carp (*Carrassius carassius)* whose blood has an affinity for oxygen allowing it to maintain oxygen consumption at a levels as low as 5-10%

air saturation (Graham 1997; Sollid et al 2003). At the genomic level, being exposed to hypoxic conditions can place selective pressure on hypoxia tolerant genotypes.

Genetic variation in low oxygen tolerance exists in teleosts. In Atlantic salmon (*Salmo salar*), individuals within a Tasmanian population have been found to have the ability to better regulate their metabolic rate during periods of low dissolved oxygen, therefore having a higher degree of tolerance to hypoxic conditions (Barnes et al. 2011). This increased dissolved oxygen tolerance may be due to a population bottleneck of the source stock due to poor survival. These inadvertent changes in allele frequency and the rise of new alleles due to mutation in the Australian population may have generated individuals with increased potential for hypoxia tolerance (Innes and Elliott 2006). A study conducted on Canadian Atlantic salmon families demonstrated that hypoxia tolerance is heritable. When exposed to hypoxic conditions full-siblings within families were more similar in resistance or susceptibility to one another than when compared to other families (Anttila et al. 2012). Therefore, this high level of phenotypic variation in Atlantic salmon results in the potential to respond to artificial selection for tolerance to low dissolved oxygen. Tolerance to hypoxia in headwater stream fishes revealed no intraspecific differences on tolerance to low dissolved oxygen between fish from the same drainage.

In the case of largemouth bass (*Micropterus salmoides*) from different drainages, significant differences in tolerance to hypoxia were found (Smale and Rabeni 1995). Golden carp (*Carassius carpio*) and common carp (*Cyprinus carpio*) backcross triploid interspecific hybrids and gynogenic 7th generation hybrids were found to be significantly more tolerant to low dissolved oxygen when compared to their parents. The backcross triploid hybrids by golden carp were also found to have the highest heritability when compared to gynogenetic 7th generation hybrid carp and backcross triploid hybrid carp in regards to tolerance to low oxygen (Balashov and Recoubratsky 2011). However, CxB hybrid diploids were found to be significantly more tolerant to low oxygen compared to triploids (Lilyestrom et al. 1999).

While hybrids already have superior tolerance over their parent species to low dissolved oxygen, further genetic enhancement of the hybrid for this trait would reduce losses further. The level of heterosis observed in the CB hybrids can potentially be improved by recurrent or reciprocal selection based on combining ability (Bosworth and Waldbieser 2014). Selection occurs at all stages of the lifecycle and in nature is the force that causes adaptive evolutionary change (Frankham et al. 2011). Understanding combining ability and selection can potentially lead to identifying superior parent strains and individuals which could lead to the production of superior hybrids. The general combining ability (GCA) of a dam or sire is the average performance value of offspring from a dam or sire when crossed to all other sires or dams. While GCA is expressed as a deviation from the mean of all (dam by sire) crosses, the specific combing ability (SCA) of a cross represents the deviation of the performance of this cross from the expected performance based on the GCA of the dam and the sire involved in the cross. SCA is also a measure of the dam by sire interaction effect(s) or dominance genetic effects (Bosworth and Waldbieser 2014). Factorial and diallel cross mating designs have been used to estimate additive and dominance genetic effects in plants (Machado et al. 2002; Salem and Ali 2012). Many studies have used the factorial mating designs (North Carolina Design II) provide estimates of the relative importance of additive genetic effects for both sires and dams (GCA), dominance effects (SCA), and maternal effects (Comstock and Robinson 1952; Busack and Knudsen 2007). Similar approaches could be used to estimate the relative magnitude of additive (GCA) and dominance (SCA) genetic effects for traits of hybrid catfish.

Initial analysis of hybrid catfish produced from the matings of different strains would be informative in determining the genetic basis for trait variation for tolerance to low oxygen**.** This study investigates the potential combining ability of hybrid catfish to tolerate hypoxic conditions which will hopefully lead to greater improvements in the performance of the CXB hybrid. Calculating both general and specific combining abilities is important for identifying the potential value of channel and blue catfish lines as the GCA and SCA variances provide an estimation for additive and dominant gene action (Falconer 1989).

In this study, CxB hybrids were compared in an attempt to determine their tolerance to hypoxic conditions. The objectives of this study were to determine general and specific combining ability of channel catfish females and blue catfish males for tolerance to low oxygen, and to identify which channel catfish females and blue catfish males produce the best performing CXB hybrid. Cumulative mortality, total time to death in minutes, mean dissolved oxygen (mg/l), and body size (g) were all measured in an attempt to understand combining ability and tolerance to low dissolved oxygen. This will enable the potential effectiveness of reciprocal recurrent selection for tolerance to low oxygen. This selection may lead to an even greater increase in hybrid tolerance of low dissolved oxygen.

Methods and Materials

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

Experimental Fish

Brood stock used for these experiments were raised at the Genetics Research Unit, E.W. Shell Research Center, Auburn University. A 20 X 12 factorial mating design was used. A large number of families did not hatch, thus, the actual design became an unbalanced factorial with 40 hybrid families from 20 channel catfish females and 12 blue catfish males. These fish were mated using a half diallel design with 40 different families hatching and surviving for the experiment.

The twenty channel catfish females (dams) were from nine strains. The strains used included Kansas Random which originated from Ninnescah River in 1911, and is the oldest domestic strain of channel catfish (Dunham and Smitherman 1984). This strain was randomly bred and has the traits increased resistance to disease, rapid growth, and late sexual maturity. Kansas Select, a line derived from Kansas random that has been selected for body weight for eight generations. AR line which was derived from the crossbreeding of Auburn and Rio Grande strains followed by 6 generations of mass selection for body weight. Traits of this line include spawning late in the season. ARMK, was initiated by producing a 4-way crossbreed (Auburn X Rio Grande) X (Marion X Kansas) followed by 6 generations of mass selection, these fish are also late spawners. Thompson strain originated from the Yazoo River, MS, and on-farm selection was conducted for several traits including body size, disease resistance, and early spawning. Tishomingo strain originated from the Tishomingo federal hatchery in Oklahoma. Kansas X Thompson was a F1 crossbreed between Kansas random females and Thompson males. Rio

Grande a single surviving wild female of a collection of 50 fish from the Rio Grande River, Texas. Traits of individuals of this strain collected 40 years ago included high dress out percentage, susceptibility to disease, slow growth, large sexual dimorphism for body weight, high fecundity, small egg size, and maturity at two years of age. Mix strain was initiated by the mixing of multiple strains.

The twelve blue catfish males (sires) were from 4 strains. These included: Tombigbee (TBB), which originated from the Tombigbee River. Rio Grande was from the Rio Grande River in Texas, and has distinctive spots on the entire body. D&B originating from D&B Fish Farms in Crockett, TX and, were selected for small head size (Dunham and Smitherman 1984). DxR was an F1 crossbreed between D&B females and Rio Grande males.

Spawning and incubation

In June 2015, brood stock were not fed for two days and then harvested from earthen ponds for spawning. Fish were loaded into a fish hauler for transportation, and three parts per million (ppm) sodium chloride (NaCl) was added and dissolved oxygen levels in the hauler were maintained at >5mg/l. Female channel catfish were placed into labeled mesh bags and placed into tanks with adequate water flow and oxygen (>5mg/L) The females were then artificially spawned via an intraperitoneal implant of slow release luteinizing hormone releasing hormone analog (LHRHa) at a dosage of 90 µg/kg. Ovulation for the majority of fish was between 1,040- and 1,170 degree hours post-injection of the LHRHa. Degree hours were calculated by multiplying the temperature (Celsius) by the time in hours. After 36 hours, females were checked for eggs and subsequently every 4 hours. If no eggs were seen on bags after 100 hours and the female was still gravid they were re-injected with LHRHa.

Once eggs were visible on the bag and ovulation confirmed, females were anesthetized in a buffered 100ppm tricaine methane sulfonate (MS-222) solution, rinsed with fresh water, dried, and eggs hand stripped into pie pans. Crisco was applied to both the female's genital opening and the pie pans to avoid sticking and clumping of the eggs. All blood was removed from the eggs by rinsing with 0.9% saline solution. Males were euthanized for removal of their testes in preparation for sperm extraction. Testes were rinsed with a 0.9% saline solution to remove any blood and then dried and weighed. The cleaned testes were then mashed in a 0.9% saline solution at a ratio of 10ml saline to one gram of testes.

Eggs and sperm were mixed in pan with water for five minutes to allow fertilization. After fertilization, the pans were transferred to a hatching trough with supplemental calcium chloride ($CaCl_2$) drip to keep water hardness above 30ppm where they were allowed to water harden for approximately one hour. After hardening, the eggs were transferred to baskets with fry catchers in flow-through hatching troughs with paddlewheels to properly aerate and incubate the eggs. Water flow was around 20l/minute and $CaCl_2$ was dripped into the troughs to increase water hardness (>30ppm) and embryo survival. Dissolved oxygen and temperature was checked daily in troughs containing eggs. Formalin at a rate of 100ppm once per day was used to prevent the growth of fungus. If eggs were found to have fungus it was removed by hand and egg masses were treated with a 32ppm of copper sulfate ($CuSO_4$) for one hour. All chemical treatments were ceased about 12 hours before expected hatch.

Fish culture

Once hatched, the fry were moved to the hatching trough and 210 fry were retained in their respective fry catchers until the yolk sac was absorbed. Once the fry in the catchers reached the swim-up stage, they were enumerated and moved into separate aquaria by family. Each family was separated into three replicates and randomly assigned an aquaria. All tanks were checked twice a day to ensure adequate DO levels (>5mg/l) and water flow. Dead fish were removed daily and water quality was monitored twice a week to ensure that the fish were within established water quality parameters (Table 1). Fry were initially fed the Purina® AquaMax® powdered starter diet containing 50% protein until they reached 2.5cm in size. From 2.5-3.8 cm the fish were fed Purina® AquaMax® 100 diet containing 50% protein, from 3.8-5 cm they were fed Purina® AquaMax® 200 containing 50% protein, and from 5-7.6 cm Purina® AquaMax® 300 containing 50% protein. Fish were fed to satiation daily. Fish were PIT tagged after 9 months of growth to identify individuals after mixing families. Retention rate was 95% and any fish that did not have a detectable PIT tag were re-tagged later.

All aquaria were flow-through for 323 days with the water coming from a reservoir pond. After 323 days the system was switched to a recirculation system. Water quality parameters were measured every 2-3 days. If ammonia approached semi-lethal levels a partial water exchange was conducted to alleviate these issues. If nitrite was high, NaCl was applied at a ratio of 10ppm NaCl to 1ppm nitrite to prevent brown blood disease

Low dissolved oxygen challenge

Two separate oxygen challenges were conducted using a total of 40 different families (Appendix; Tables 6, 7). All fish were re-checked for PIT tags and weighed prior to the challenge and then transported to the challenge tank. Families were then split evenly into two groups and placed into a 670 liter communal tank at a density of 512 fish per tank to eliminate environmental variation. One tank was used as a control and the other served as the challenge tank. City water was dechlorinated with sodium thiosulfate and used for the challenge. Water quality was tested prior to the experiment. The water in the challenge and control tank was 27C, the same as the recirculating system.

DO levels were lowered via removal of aeration and the fishes respiration. Individuals were collected and their PIT tag scanned when deemed moribund. Moribund was defined as loss of equilibrium with slight opercular movement. A recovery tank with an average DO content of 7 ppm was used to recover the moribund fish. DO levels in both the challenge and recovery tanks were recorded every 5 minutes. The differences in body weight among families were significant (p= 2.2e-16) ranging from 10 to 235.5 grams with an average of 58.1 grams. The average DO level through challenge number 1 was 0.85 mg/l and 0.58 mg/l in challenge number 2. Initial and final dissolved oxygen were 7 mg/l and 0.54 mg/l, respectively, for challenge one (Fig. 1). Initial and final dissolved oxygen were 5.23 mg/l and 0.52 mg/l respectively, for challenge (Fig. 2).

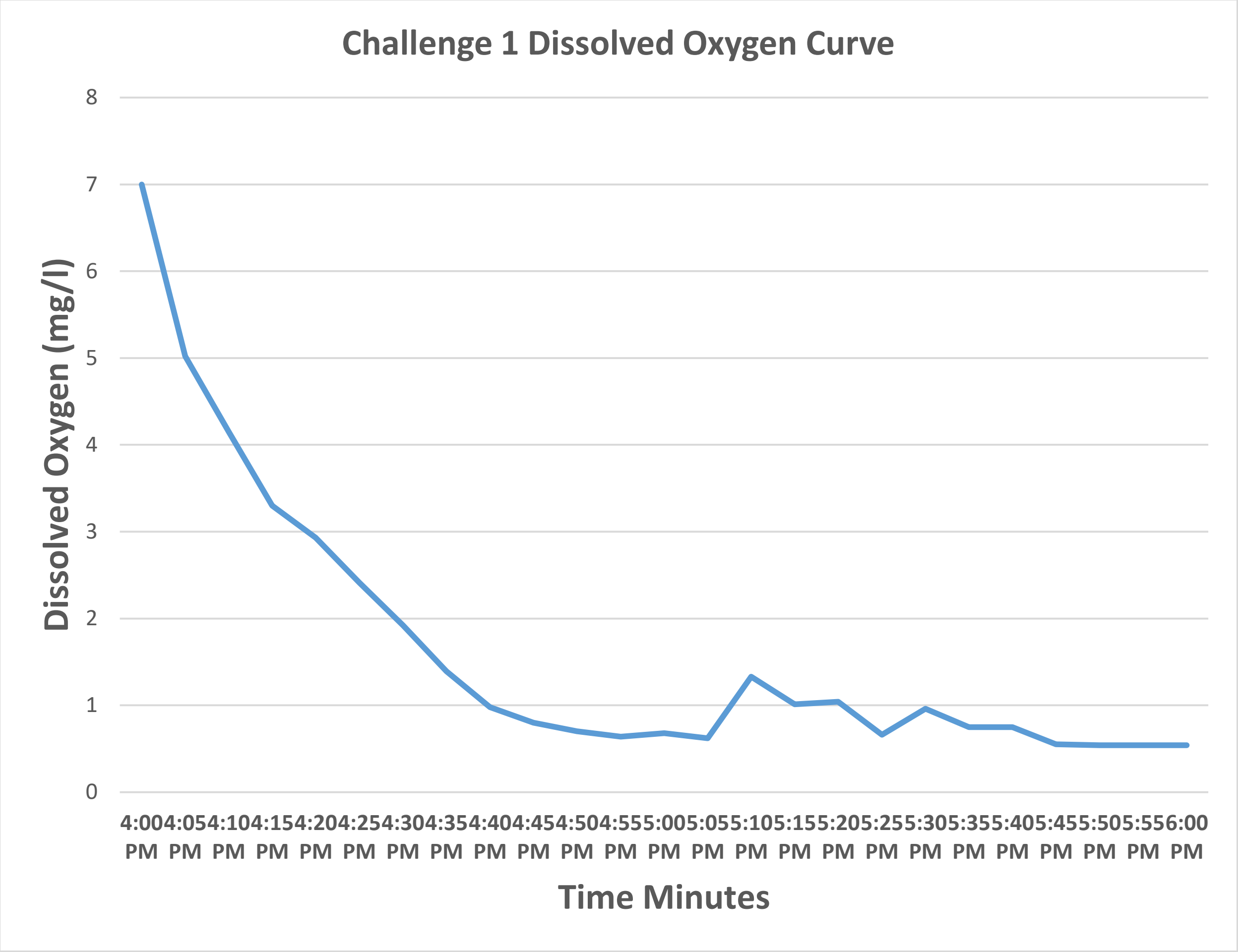

Figure 1. Dissolved oxygen (mg/l) levels at five minute intervals for dissolved oxygen challenge one for female channel catfish (*Ictalurus punctatus*) X male blue catfish (*I. furcatus*). The challenge was conducted in a communal 670 liter tank at 27°C. F1 hybrid catfish ranging from 10 to 235.5 grams were deemed moribund when fish lost their equilibrium and opercula movement ceased.

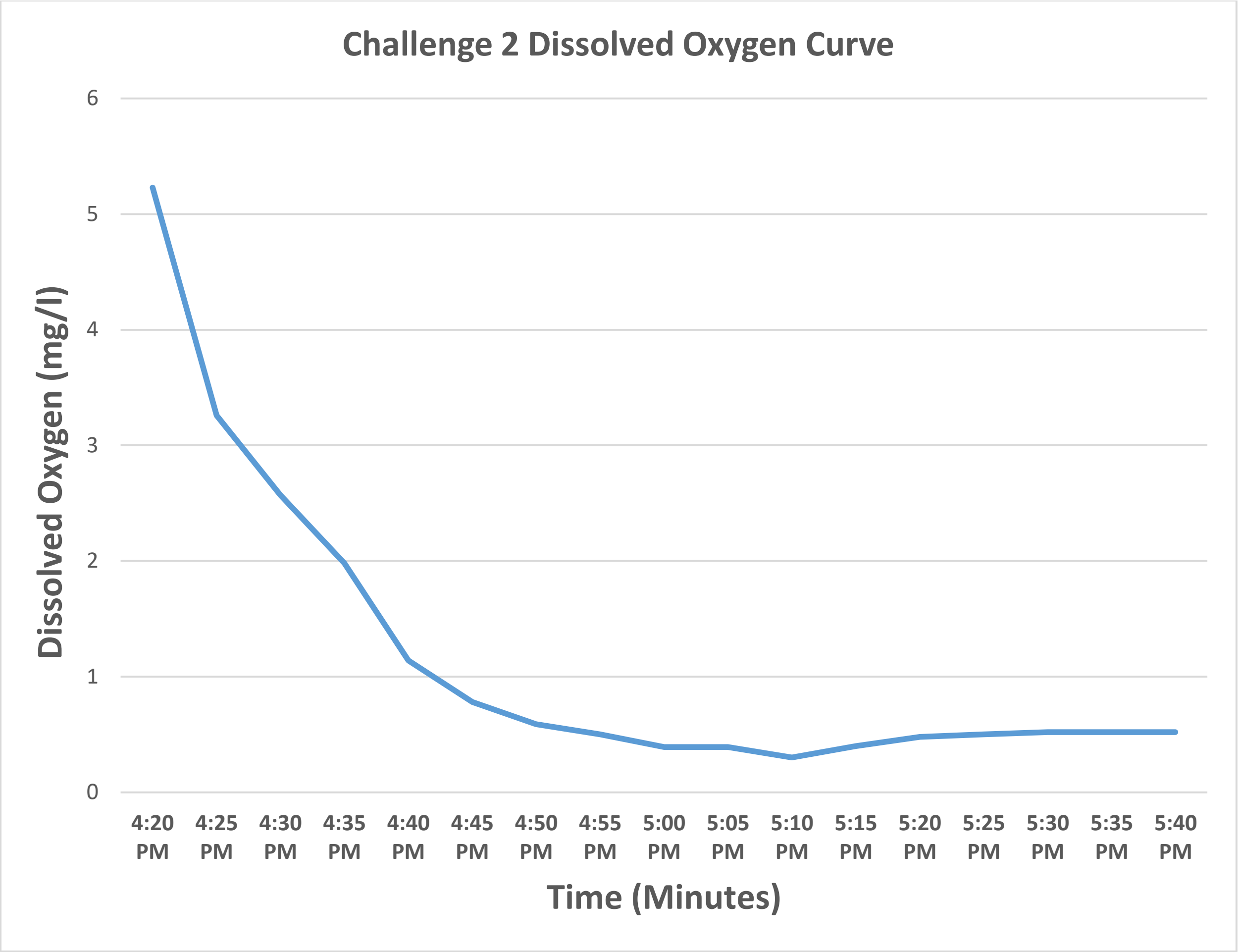

Figure 2. Dissolved oxygen (mg/l) levels at five minute intervals for dissolved oxygen challenge two for female channel catfish (*Ictalurus punctatus*) X male blue catfish (*I. furcatus*). The challenge was conducted in a communal 670 liter tank at 27°C. F1 hybrid catfish ranging from 13 to 162.5 grams were deemed moribund when fish lost their equilibrium and opercula movement ceased.

Table 1: Water quality parameters for dissolved oxygen challenge 1 and 2 of channel female catfish (*Ictalurus punctatus*) X blue male catfish (*I. furcatus*) hybrids. Each experiment was conducted in a communal 670 liter tank at 27°C. F1 hybrid catfish ranging from 10 to 235.5 grams were deemed moribund when fish lost their equilibrium and opercula movement ceased.

	Challenge 1	Challenge 2
Ammonia (NH_3)	0 ppm	0 ppm
Nitrite (NO_2)	0 ppm	0 ppm
Nitrate (NO_3)	0 ppm	0 ppm
Alkalinity	28 ppm	28 ppm
Hardness	32 ppm	28 ppm
Chlorine	0 ppm	0 ppm
pH	6.8	6.8

Data Analysis

Statistical analysis was conducted using the program R, Microsoft Excel 2013, and SAS. Moribund fish were collected, time of death and DO level at death were recorded. Cumulative mortality was calculated as number of deaths divided by total number of fish per family. Means, standard deviations, and coefficient of variation (CV) were all calculated in Microsoft Excel 2013. A proc mixed with restricted estimation of maximum likelihood (REML) program in SAS (version 9.4, SAS, Cary, NC) was used to determine combining ability. For this study, general combining abilities for dams and sire are equivalent to the dam and sire variances, respectively, and specific combining ability is equivalent to the sire × dam variance (Cotterill et al., 1986). REML was used due to the unbalanced data. One-way analysis of variance (ANOVA) was used to compare families and cumulative mortality An ANOVA was also conducted to compare channel catfish females (dams) for dissolved oxygen (mg/l) upon mortality. An ANOVA followed by a Tukey post-hoc test were conducted to compare blue catfish males (sires) to DO level upon mortality.

An unpaired t-test was used to determine any differences in mean weights among families. Correlation between body weight (g) and survival/ time to death was calculated using the CORREL function and regression using the data analysis ToolPak in Microsoft Excel 2013. Significance was tested at α=0.05.

Results

Fish behavior

The majority of the fish were able to reach the surface and pipe in an attempt to utilize atmospheric oxygen, however, a few fish could not reach the surface due to a lack of space. Among the initial mortalities, approximately, 50% of the hybrid catfish that lost equilibrium swam down to the tank bottom after gasping, and then lost equilibrium while the other half stayed at the surface and lost equilibrium. After initial mortality there was enough room for all fish to pipe and mortality subsequently slowed. When the challenges were concluded the surviving hybrid catfish were displaying phenotypic stress and piping at the surface but did not become moribund due to low dissolved oxygen.

Combining ability

The genetic aspect of variation for cumulative mortality due to low dissolved oxygen was mostly associated with the GCA of the dam (25%) while variance component estimates for sire were the lowest (0%) (Table 2, Fig. 3). The variance estimate for SCA was low (3%), suggesting dominant gene interactions were not prevalent, and selection for specific female x male parental combinations would not enhance hybrid tolerance for low oxygen (Table 2 Fig. 3). For tolerance to low oxygen in regards to cumulative mortality, one Kansas Random female (Fig. 4, Table 7) had significant general combining at (p =0.0312).

Variation for total time to death was only associated with GCA with sires contributing the most (15%) while the GCA estimates for dams was low (5%). The SCA estimate was 0%, suggesting dominant gene interactions had no effect on mean time to death (Fig. 5). No individual dams or sires had significant GCA for total time to death (Fig. 6, Appendix Table 1).

Variation for DO level (mg/l) at death was low and associated with GCA estimate with dams contributing the most (2%), SCA contributed 1%, and GCA estimate for sire was 0% (Fig. 7). While the estimates are low there was one Tishomingo female that had significant negative general combining for DO level (mg/l) at death and while not significant the Kansas Select female performed the best in regards to DO level (mg/l) at death (Fig. 8).

Table 2. Estimates for variance of female channel catfish (*Ictalurus punctatus*) dam general combining ability (σ^2 GCA dam), male blue catfish (*I. furcatus*) sire combining ability (σ^2 GCA sire (±SE)), channel catfish female x blue catfish male specific combining ability (σ^2 SCA cross (±SE)), and error variance (σ^2 error) for cumulative mortality (%) and total time to death (minutes), and DO (mg/l) level at death due to low dissolved oxygen. The experiment was conducted in a communal 670 liter tank at 27C. F1 hybrid catfish ranging from 10 to 235.5 grams were deemed moribund when fish lost their equilibrium. Estimates were calculated using a proc mixed with default restricted estimation of maximum likelihood (REML), using SAS. Samples were limited to only fish that became moribund.

Genetic parameter	Cumulative Mortality (%)		Total Time to Death (Minutes)		DO(mg/l) Level at Death	
	Ratio	Estimate (Variance)	Ratio	Estimate (Variance)	Ratio	Estimate (Variance)
σ^2 GCA dam (±SE)	0.25	98.76 (83.15)	0.05	5.52 (11.46)	0.02	0.002 (0.002)
σ^2 GCA sire (±SE)	1.43E-36	5.73 E-34	0.15	15.20 (17.54)	0	0
σ^2 SCA cross (±SE)	0.03	14.06 (83.52)	0	0	0.01	0
σ^2 error (±SE)	0.72	399.28 (91.6)	0.8	101.03 (19.37)	0.97	0.08 (0.005)

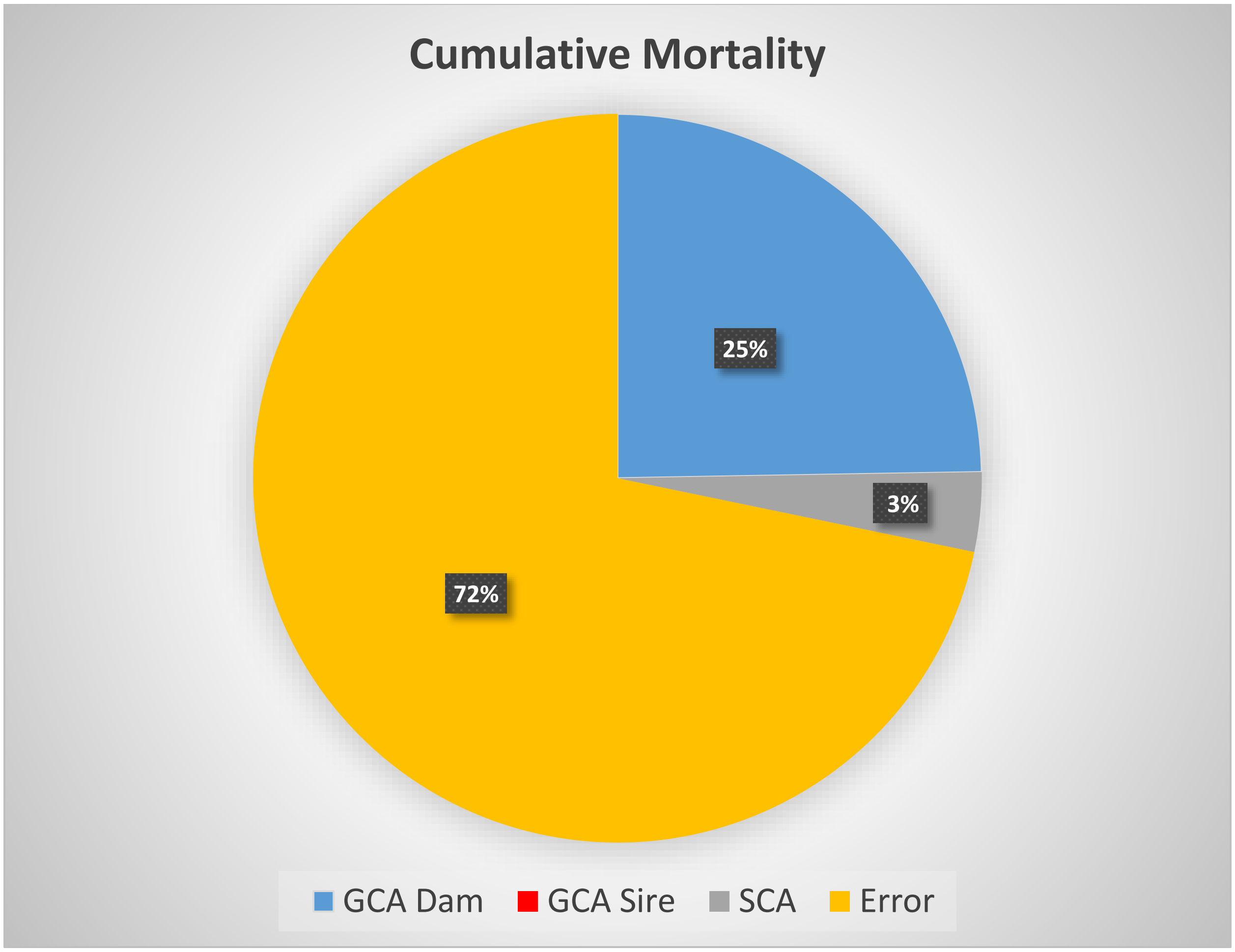

Figure 3: Pie chart displaying the percentage of the general combining ability (GCA for) channel catfish female (*Ictalurus punctatus*) dams and blue male catfish (*I. furcatus*) sires, channel x blue specific combining ability (SCA), and error for cumulative mortality for dissolved oxygen challenge experiment one and two in tanks.

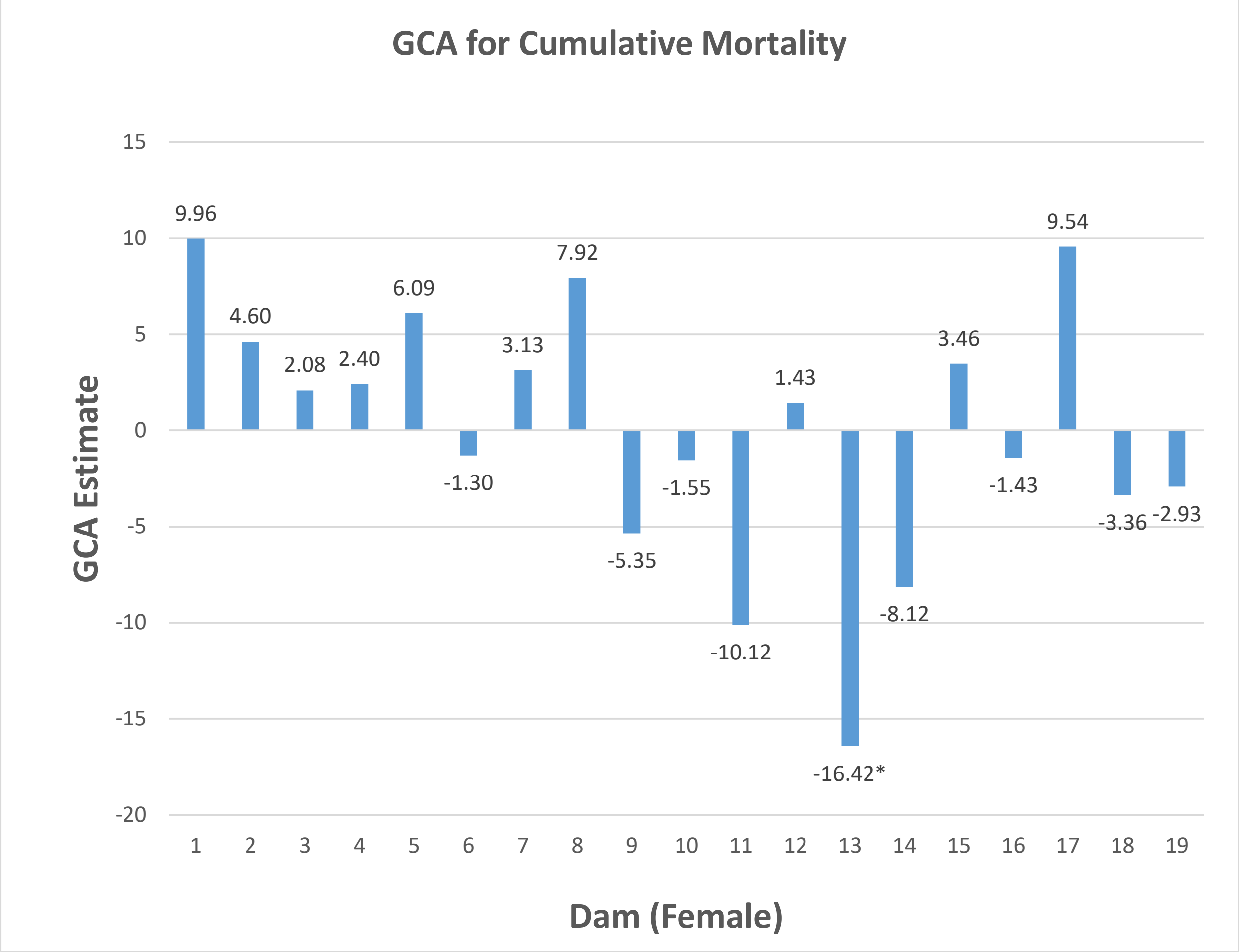

Figure 4: General combining ability (GCA) estimates for female channel catfish (*Ictalurus punctatus*) dams based on cumulative mortality. Asterisks indicates significant difference for that dams resistance to low dissolved oxygen (p value <0.05). Negative values indicate dam produced progeny with less mortality for challenge experiment one and two in tanks. Dams 1, 9, 10, 12 are mix channel catfish strain. Dam 2 is ARMK channel catfish strain dams 3, 4, 5, 6, and 7 is Tishomingo channel catfish strain. Dam 8 is a Kansas X Thompson channel catfish strain. Dam 11 is a Rio Grande channel catfish strain. Dams 13, 14, 15, 16 and 17 is Kansas Random channel catfish strain. Dam 18 is a Kansas Select channel catfish strain. Dam 19 is an AR channel catfish strain.

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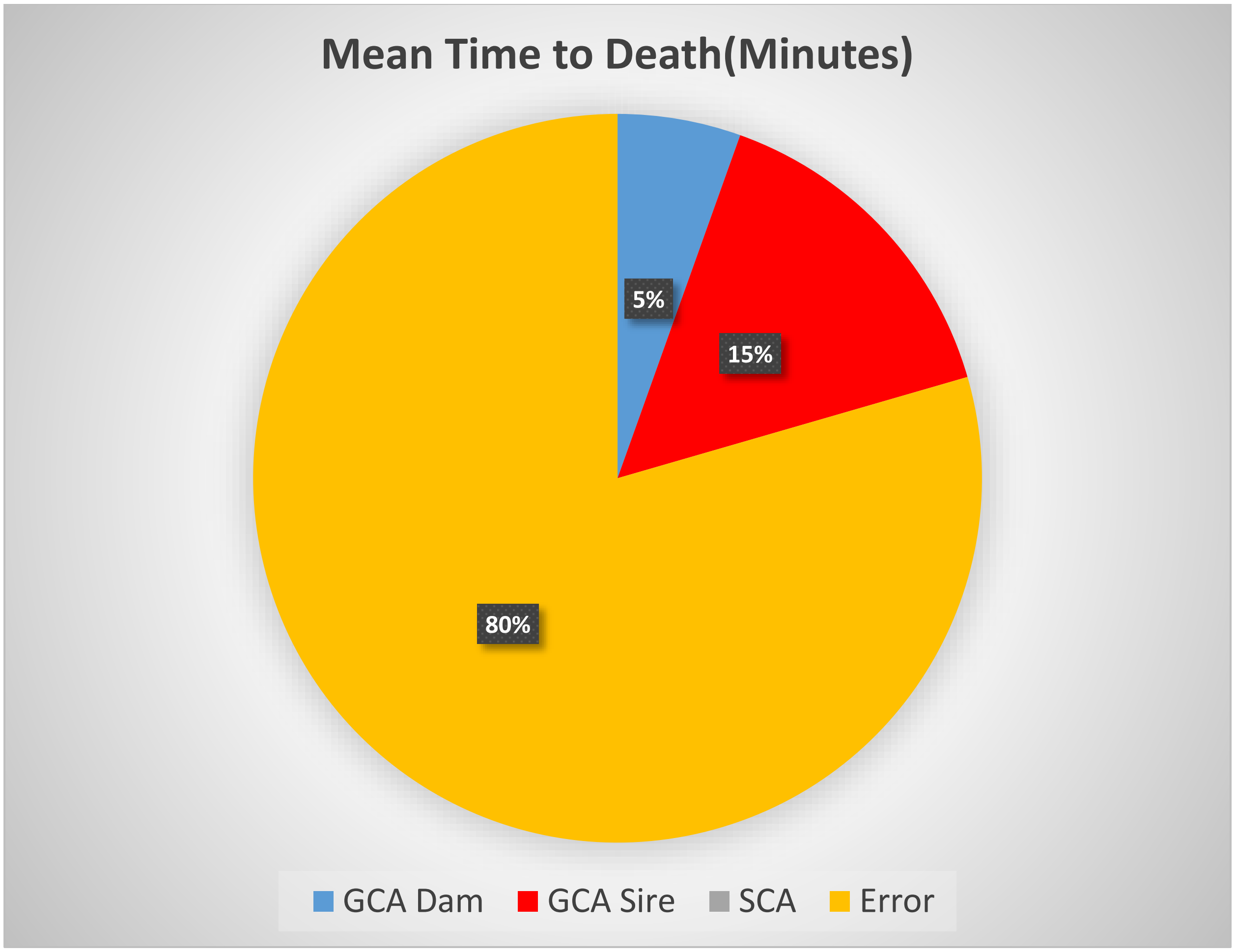

Figure 5: Pie chart displaying the percentage of the general combining ability (GCA) for female channel catfish (*Ictalurus punctatus*) dams and male blue catfish (*I. furcatus*) sires, channel x blue specific combining ability (SCA), and error for time to death in minutes for dissolved oxygen challenge experiment one and two in tanks.

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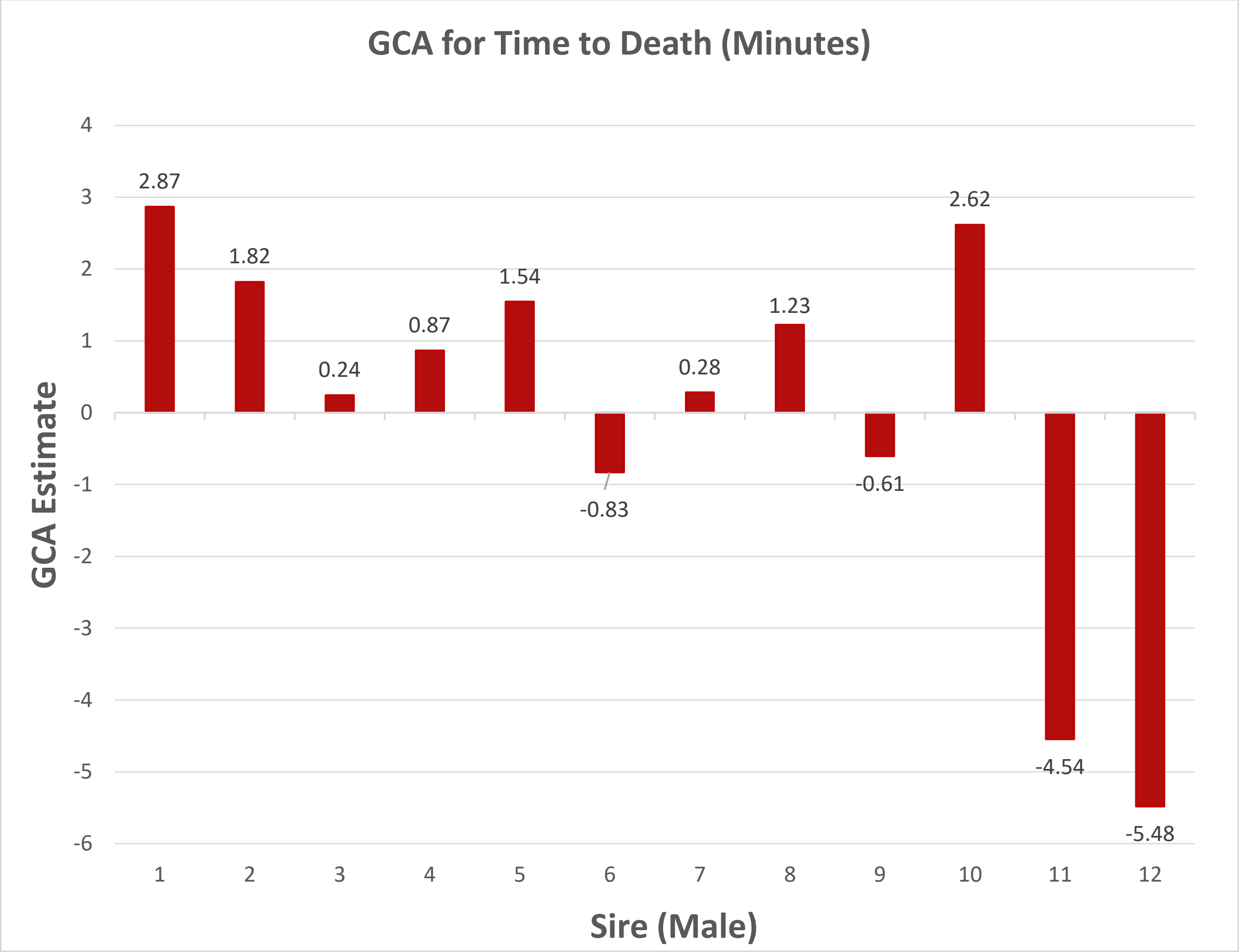

Figure 6: General combining ability (GCA) estimates for male blue catfish (*Ictalurus furcatus*) sires based on mortality in time to death in minutes for dissolved oxygen challenge experiment one and two in tanks. Sire 1 and 2 are DxR blue catfish strain. Sires 5, 7, 8, 9, 10, 11, 12 are D&B blue catfish strain. Sire 6 is a TBB blue catfish strain. Sires 3 and 4 are Rio Grande blue catfish strain.

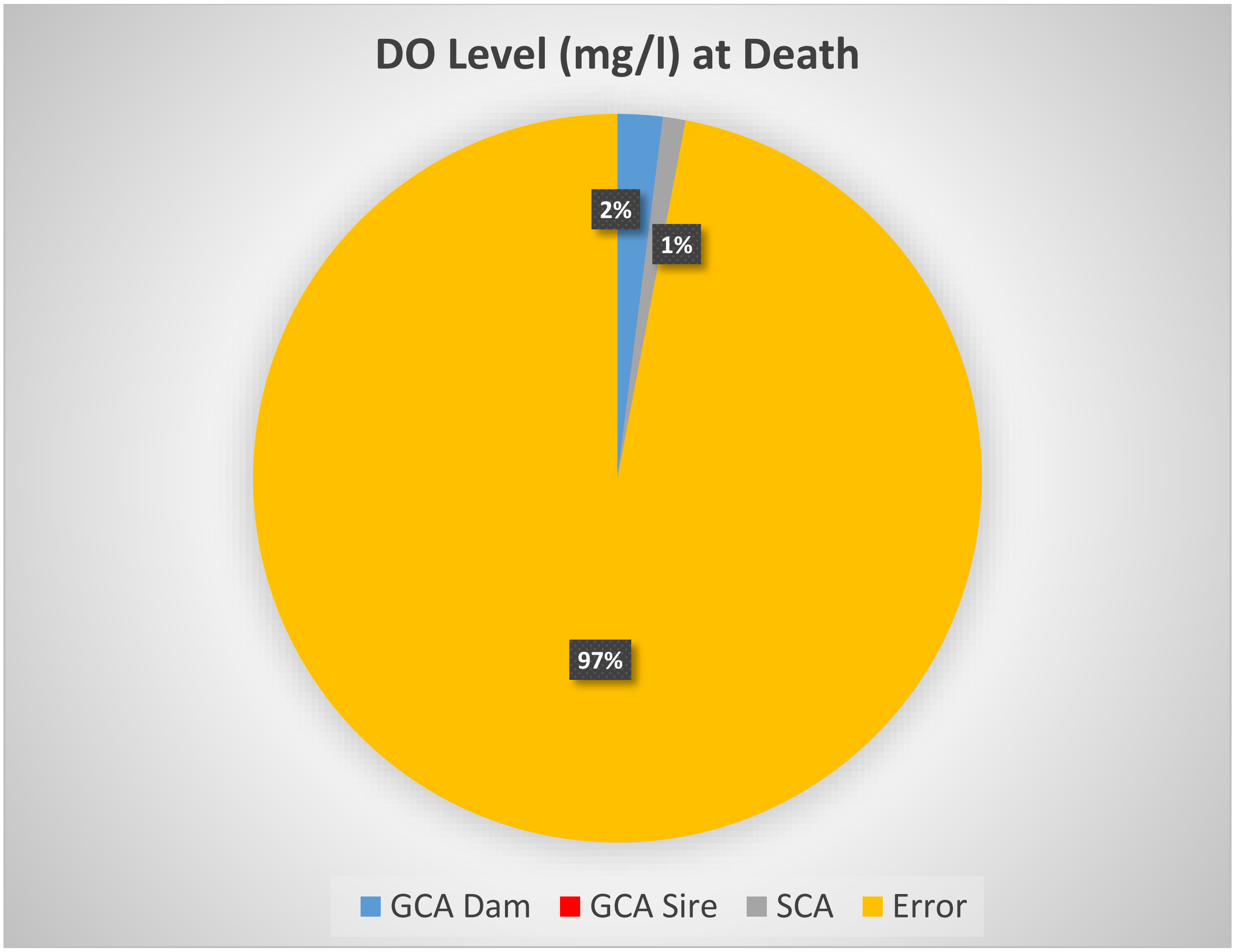

Figure 7: Pie chart displaying the percentage of the general combining ability (GCA) for female channel catfish (*Ictalurus punctatus*) dams and male blue catfish (*I. furcatus*) sires, channel x blue specific combining ability (SCA), and error for DO level (mg/l) at death for dissolved oxygen challenge experiment one and two in tanks.

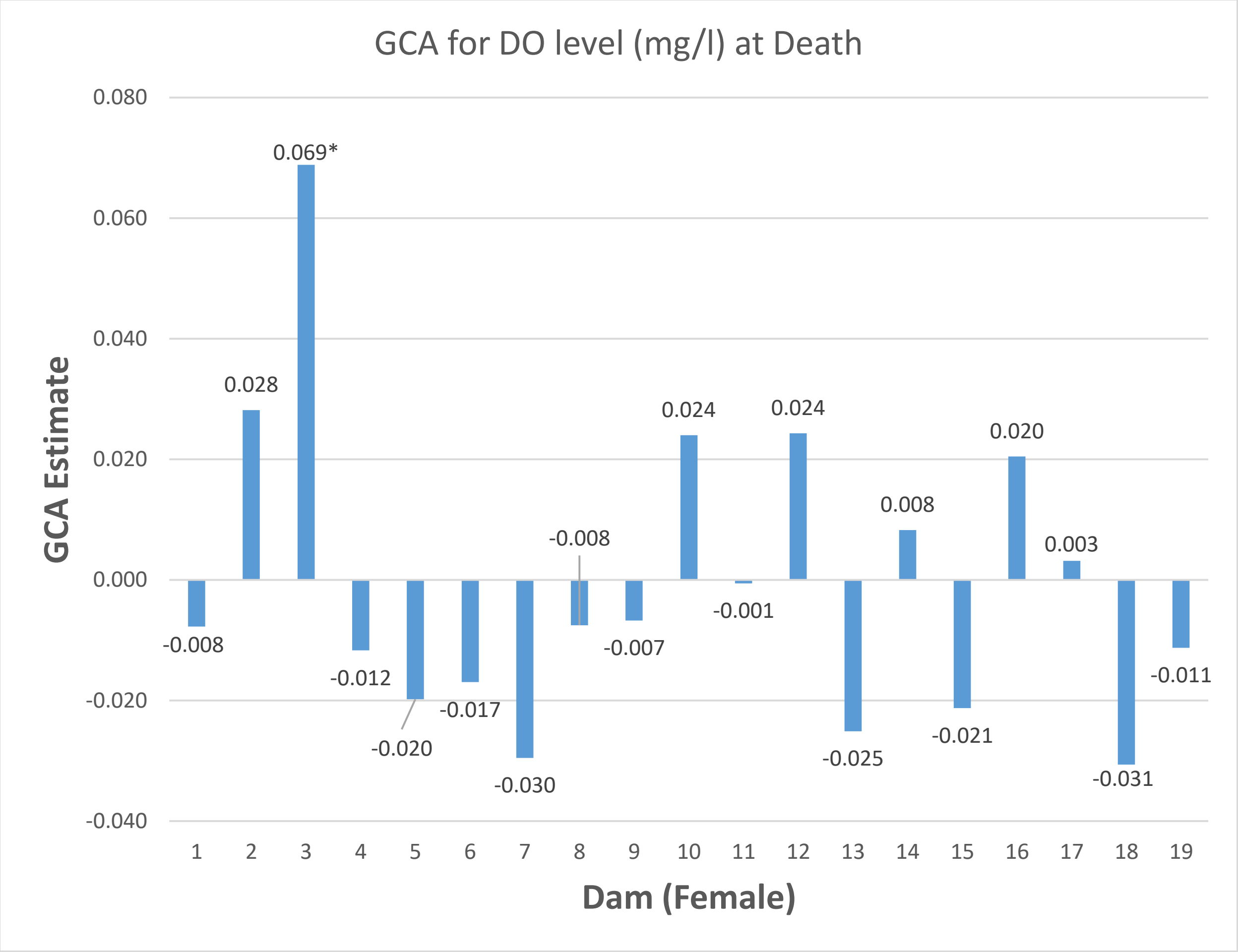

Figure 8: General combining ability (GCA) estimates for male blue catfish (*Ictalurus furcatus*) sires based on DO level (mg/l) at death for dissolved oxygen challenge experiment one and two in tanks. Dams 1, 10, 11, 13 are mix channel catfish strain. Dam 2 is ARMK channel catfish strain dams 3, 4, 5, 6, and 7 is Tishomingo channel catfish strain. Dam 8 is a Thompson channel catfish strain. Dam 12 is a Rio Grande channel catfish strain. Dams 14, 15, 16 and 17 is Kansas Random channel catfish strain. Dam 18 is a Kansas Select channel catfish strain. Dam 19 is an AR channel catfish strain.

Regression analysis

Body weight among families was significantly different, p= 2.2e-16. The results of the regression and correlation analysis are reported in table three. Changes in body weight were found to not be associated with time to death, $p>0.05$. There was no linear relationship between body weight and mortality, but was significant when combining challenge one and two. There was no linear relationship between body weight and time to death. The low R^2 value indicates that the dependent variable (time to death) cannot be predicted from the independent (body weight) variable. The low beta value for both challenges shows how weakly body weight influences time to death (minutes). The correlation values show a very weak relationship between body weight and time to death. The p value was >0.05 and not significant for survival and body weight in challenge one. For the second challenge and both challenges together the p value was significant at 0.039 and 0.002 respectively. The correlation values for all challenges show a weak correlation between body weight (g) and survival. Since the correlation between body weight and survival was minimal, though significant, the survival the data was not corrected for body weight.

Table 3: Regression and correlation analysis of channel catfish (*Ictalurus punctatus)* females *X* blue catfish (*I. furcatus*) males between time to death (minutes) and body weight (g) and survival and body weight (g) for dissolved oxygen challenge 1 and 2. The experiment was conducted in a communal 670 liter tank at 27C. F1 hybrid catfish ranging from 10 to 235.5 grams were deemed moribund when fish lost their equilibrium and opercula movement.

	Time to death and body weight-challenge one	Survival and body weight-challenge one	Time to death and body weight-challenge two	Survival and body weight-challenge two	Time to death-challenge one and two	Survival and body weight-challenge one and two
R^2-value	0.006	0.008	0.004	0.008	0.003	0.009
b- value	-0.092	0.002	0.029	-0.001	-0.025	-.002
p-value	0.094	0.091	0.145	0.039*	0.19	0.002**
Correlation	-0.076	0.045	0.065	0.076	-0.046	-0.095

Analysis of strain of dams.

Differences in mortality were observed among hybrids with dams from different strains was found to be significant at p= 0.058 with Kanas random (51%), Kansas select (49%), and Rio Grande (53%) dams having progeny with the lowest mortality when exposed to low DO (Fig. 5). Overall, hybrids from Thompson (75%) and Kansas X Thompson (79%) dams had the heaviest mortality (Fig. 9). There was a no significant correlation between cumulative mortality and mean time to death upon death between these two strains. DO levels at time of death among other strains were highly variable. The differences in time to death were not significant, but the observed means for both Kansas random and Kansas select were 56 and 58 minutes prior to death respectively. Surprisingly, Thompson hybrid progeny has the highest mean minutes until at, 59 minutes, although they also had one of the highest mortalities. There was not a large range, 49-59 minutes, among dam hybrid genotypes for time to death (Fig. 10). The median time to death, however, was significant different p value= 0.004 (Fig. 11). Kansas select had the highest observed median time to death at 61 minutes upon death. Mix and AR X MK had the lowest observed median time to death at 51 minutes (Fig. 11). Using a Tukey's multiple comparison test between channel female catfish strains for median time to death a significance difference was found (Table 4).

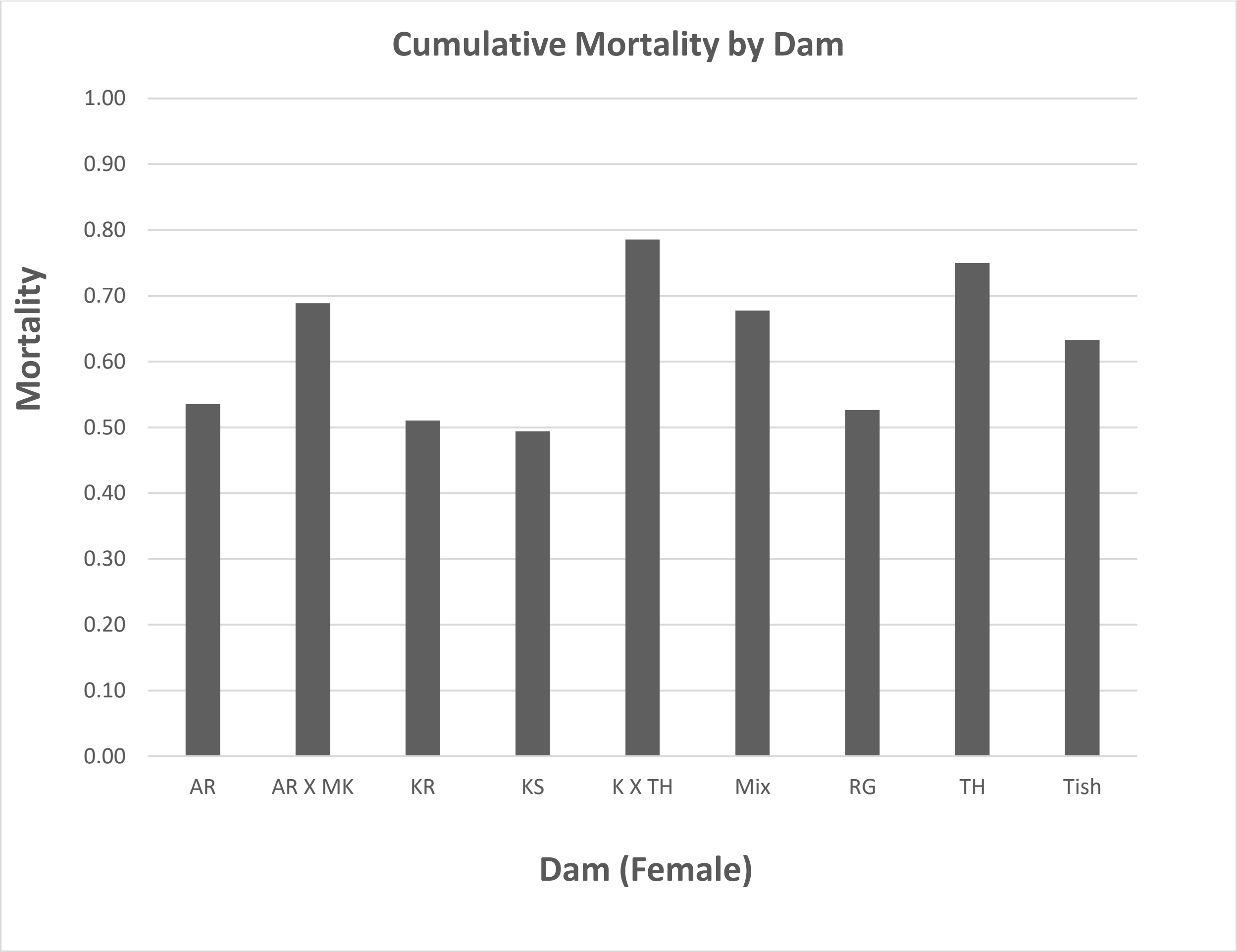

Figure 9: Bar graph comparing cumulative mortality based on female channel catfish (*Ictalurus punctatus*) dam strain for dissolved oxygen challenge experiment one and two in tanks. A total of nine dam strains were used, AR, AMK, Kansas Random, Kansas Select, Kansas X Thompson, Mix, Rio Grande, Thompson, and Tishomingo. Differences in mortality were observed among hybrids with dams from different strains was found to be significant at p= 0.058 using ANOVA.

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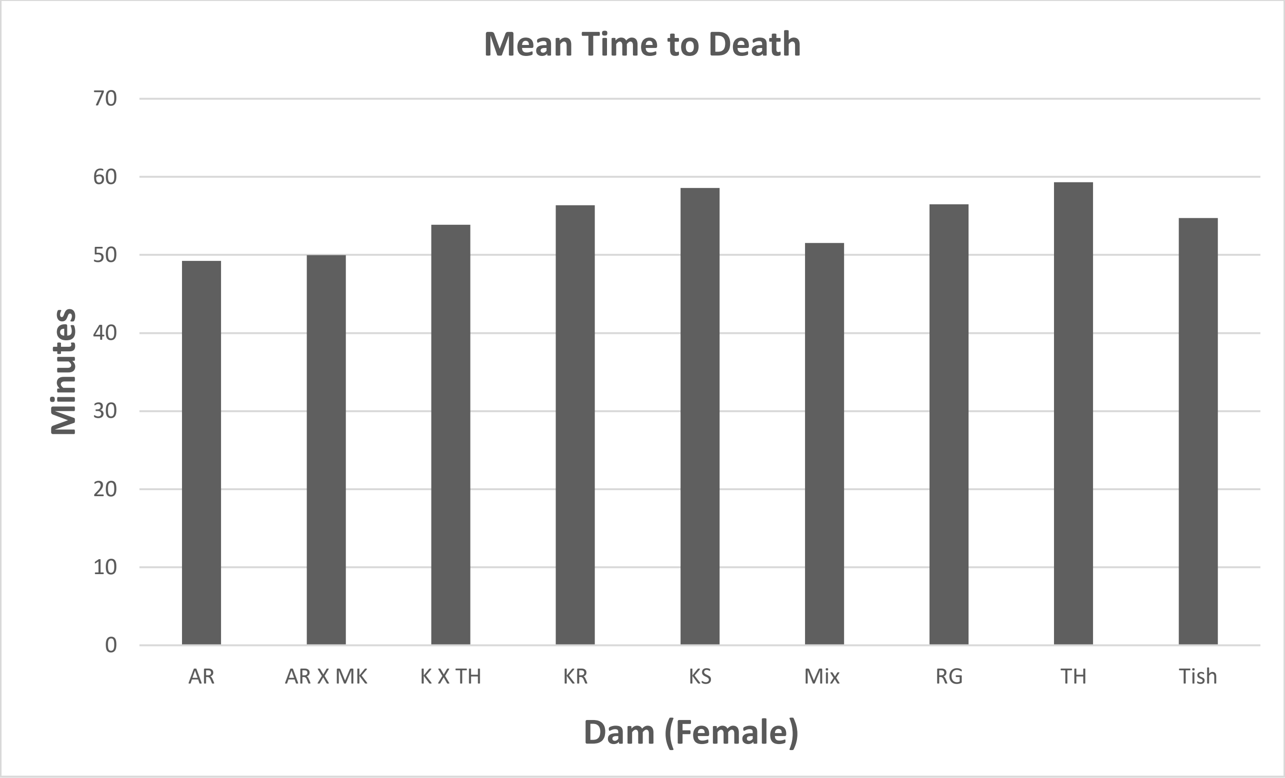

Figure 10: Bar graph displaying the mean time to death in minutes for each female channel catfish (*Ictalurus punctatus*) dam strain for dissolved oxygen challenge experiment one and two in tanks. A total of nine dam strains were used, AR, AMK, Kansas Random, Kansas Select, Kansas X Thompson, Mix, Rio Grande, Thompson, and Tishomingo.

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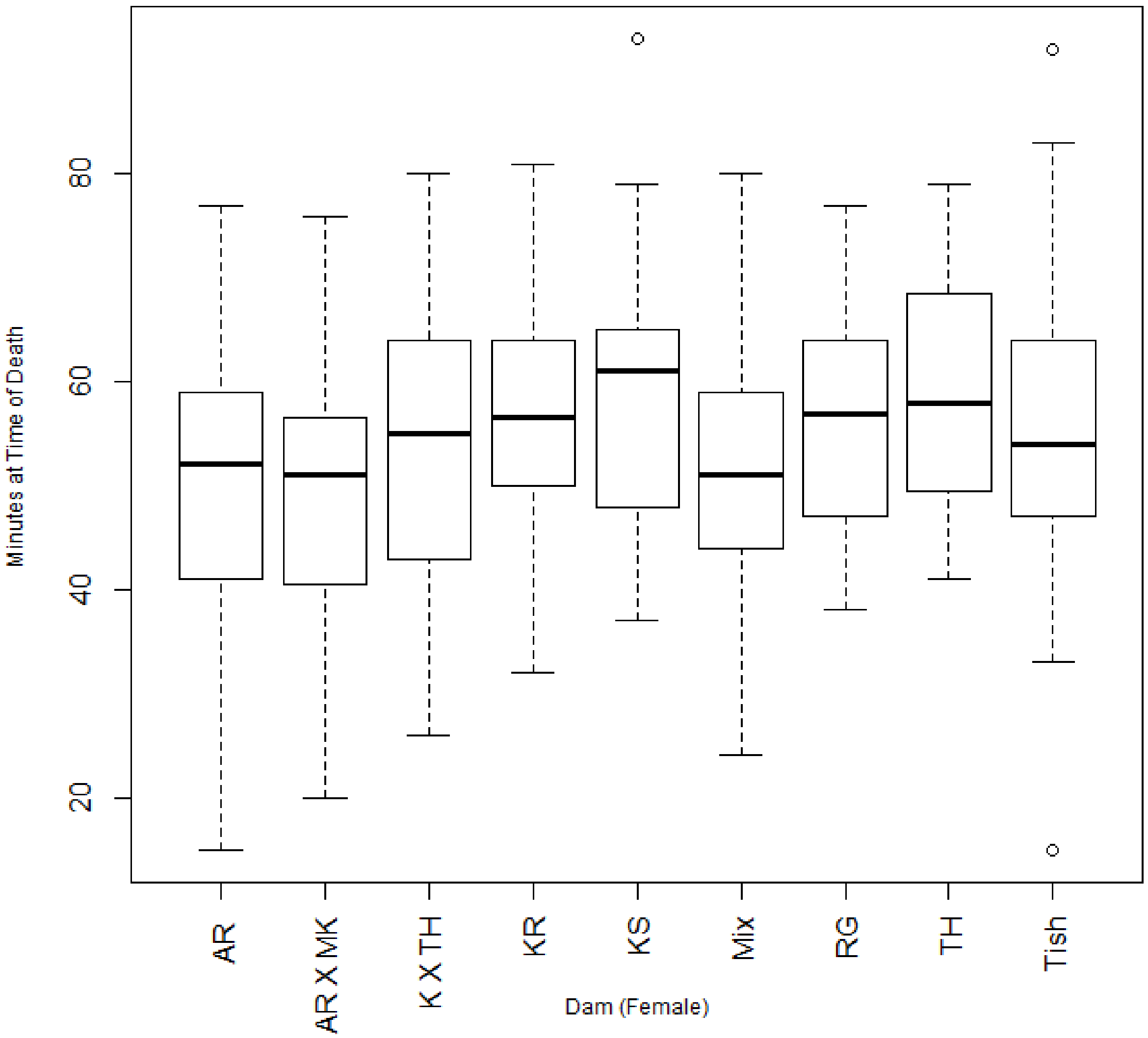

Figure 11. Box and whisker plot of minutes at time of death between female channel catfish (*Ictalurus punctatus*) dam strains for dissolved oxygen challenge experiment one and two in tanks. A total of nine dam strains were used, AR, AMK, Kansas Random, Kansas Select, Kansas X Thompson, Mix, Rio Grande, Thompson, and Tishomingo. Significant differences in DO level were observed between strains p=0.004 using ANOVA. Bars indicate the median dissolved oxygen levels at death.

Table 4: Tukey Analysis comparing all female channel catfish (*Ictalurus punctatus*) dam strains used to make F1 hybrids and the median time to death in minutes for dissolved oxygen challenge experiment one and two in tanks. Statistics were ran using the program R.

Strain Comparison	Median Time to Death (Minutes)
AR	52^{c}
AR x MK	51^{b}
K x TH	55^{abc}
KR	56.5^{ab}
KS	61^{a}
Mix	51^{c}
RG	57^{abc}
TH	59.3^{abc}
Tish	54^{abc}

[1] A total of nine dam strains were used, AR, ARMK, Kansas Random, Kansas Select, Kansas X Thompson, Mix, Rio Grande, Thompson, and Tishomingo. Values with the same superscript are not significantly different at 0.05 probability level using Tukey multiple comparison test.

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Analysis of strain of sire

Strain influenced cumulative mortality with DB having 54% mortality and RG having 73% mortality ($p=0.063$) (Figure 12). The mean time to death in minutes was also affected by strain of sire ($p= 2.36e-05$) with DR having the highest mean, 56 minutes, but the lowest mean was only 53 minutes (Fig. 13). The DO level upon mortality was different among hybrids from different strains of sire ($p= 0.051$) with the observed median DO level of RG the lower at 0.52 mg/l than DB hybrids at 0.62 mg/l when mortality occurred (Fig. 14). Using a Tukey's multiple comparison test between strains for median DO level there was a significance difference ($p<0.05$) between RG and DB hybrids (Table 5).

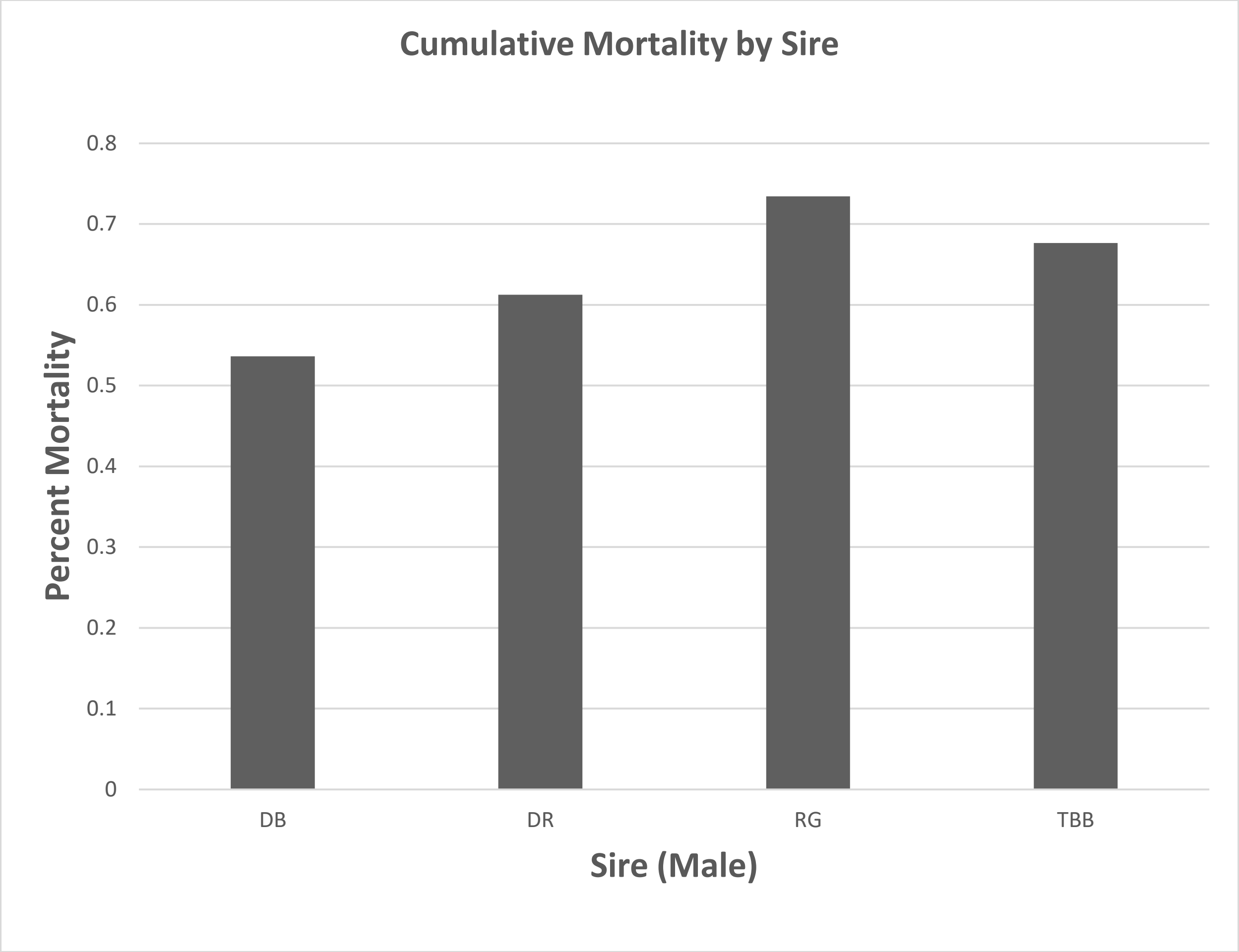

Figure 12: Comparison of cumulative mortality based on male blue catfish (*Ictalurus furcatus*) sire strain for dissolved oxygen challenge experiment one and two in tanks. A total of four different sire strains were used including, DB, DB X Rio Grande (DR), Rio Grande (RG), and Tombigbee (TBB). Differences in mortality were observed among hybrids with dams from different strains was found to be not significant at p= 0.063 using ANOVA.

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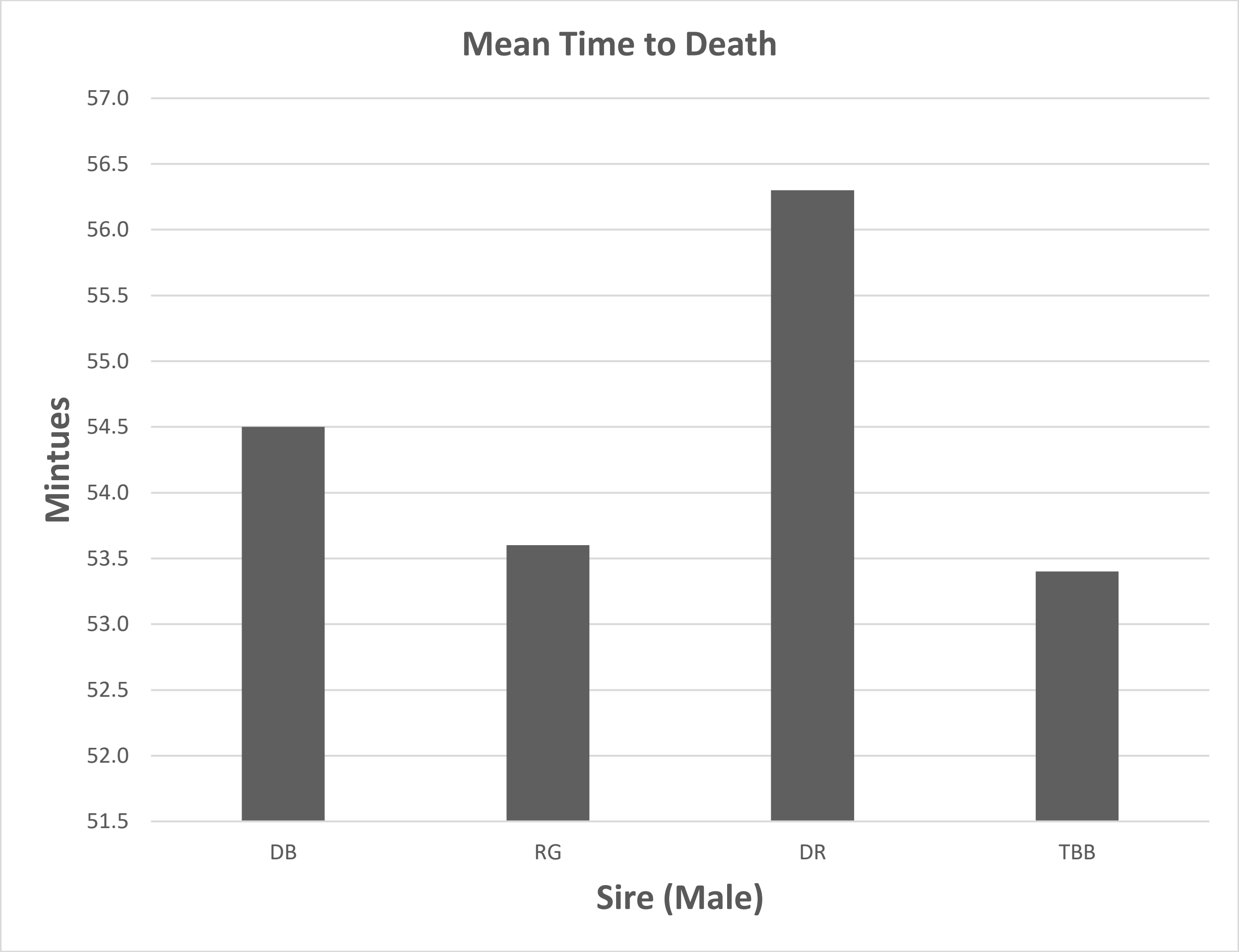

Figure 13: Mean time to death in minutes for each male blue catfish (*Ictalurus furcatus*) sire strain for dissolved oxygen challenge experiment one and two in tanks. A total of four different sire strains were used including, DB, DB X Rio Grande (DR), Rio Grande (RG), and Tombigbee (TBB).

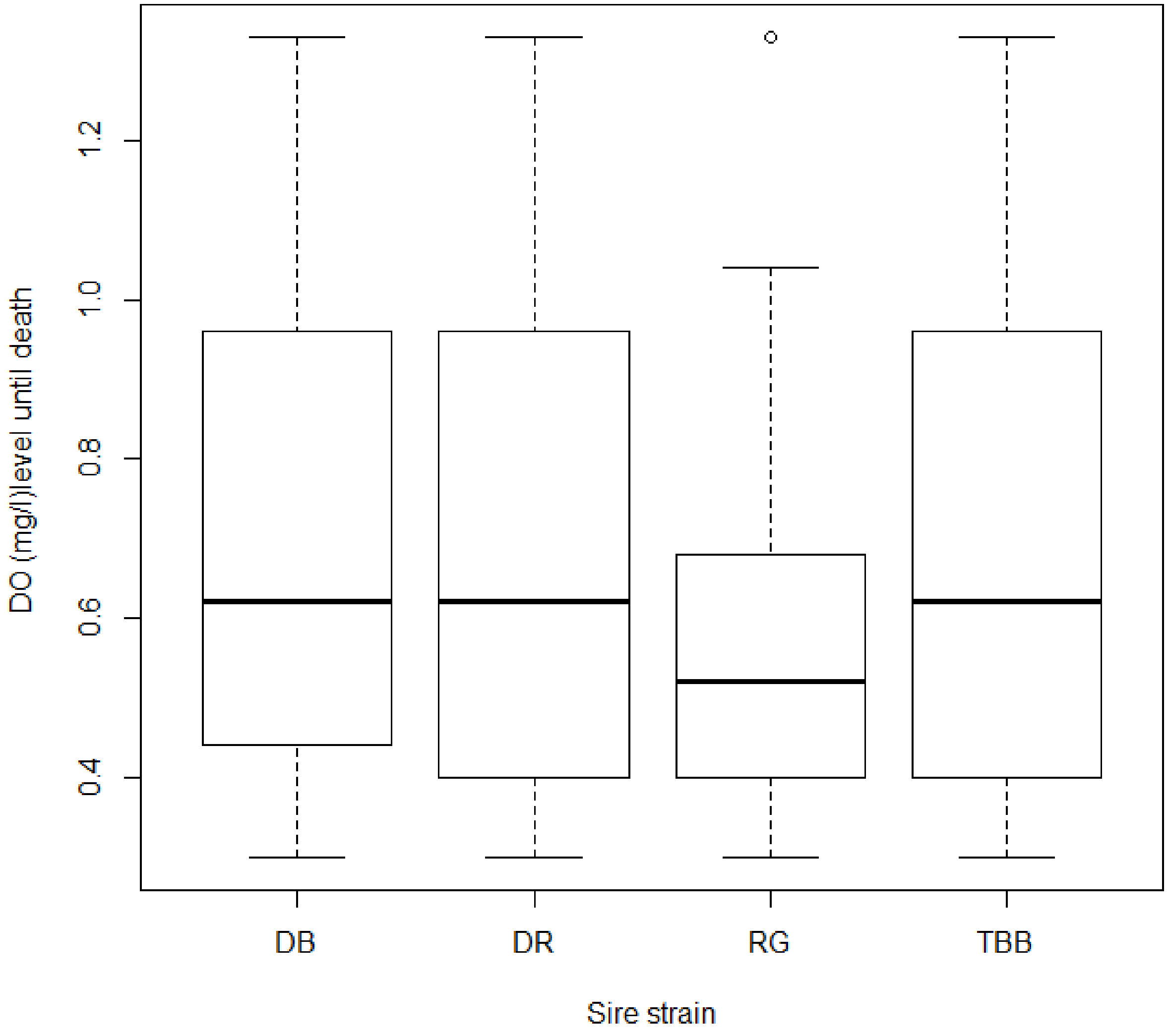

Figure 14: Median dissolved oxygen (mg/l) levels at time of death for each male blue catfish (*Ictalurus furcatus*) sire strain for dissolved oxygen challenge experiment one and two in tanks. A total of four different sire strains were used including, DB, DB X Rio Grande (DR), Rio Grande (RG), and Tombigbee (TBB). Median DO level was 0.62, 0.62, 0.52, and 0.62 respectively.

Table 5: Tukey Analysis comparing all male blue catfish (*Ictalurus furcatus*) sire strains used to make F1 hybrids and the median DO (mg/l) level at time of death for dissolved oxygen challenge experiment one and two in tanks. Statistics were ran using the program R.

Strain Comparison[1]	Mean DO level (mg/l)
RG	0.6^{a}
DR	0.65^{ab}
TBB	0.66^{ab}
DB	0.69^{b}

[1] A total of four different sire strains were used including, D&B, D&B X Rio Grande (DR), Rio Grande (RG), and Tombigbee (TBB). Values with the same superscript are not significantly different at 0.05 probability level using Tukey multiple comparison test.

Discussion

Maintaining adequate dissolved oxygen on production farms is crucial to fish health and for obtaining efficient FCRs. While catfish farmers aerate their ponds to prevent death from low dissolved oxygen, mechanical or human errors can lead to significant mortality events. Genetic enhancement is a tool to reduce this mortality or to give culturists a larger window of time to discover and correct oxygen problems. Families of channel catfish X blue catfish hybrids were evaluated for tolerance of low oxygen. Body weight was negatively correlated with survival under conditions of low oxygen but the correlation, -0.10, was of low biological significance. Dam GCA for cumulative mortality and sire GCA for time to death were higher than the corresponding GCAs and SCA was near zero. The strain of the channel catfish dam affected mortality, and there were near significant trends in time to death and level of DO that caused death. Strain of blue catfish sire had a more distinctive effect on tolerance of low oxygen. Mortality, time of death and lethal level of DO varied among hybrids for strains of blue catfish sire.

Generally, the expectation is that larger fish have lower tolerance of low oxygen, and this could be a confounding factor in low oxygen challenges. Both a positive and negative correlations have been found between body weight and tolerance of low oxygen in fish (Sloman et al. 2006; University of Florida 2013; Wang 2017). Larger Oscars (*Astronotus ocellatus*) were more efficient in regulating their oxygen consumption via adjustments to respiration (Sloman et al. 2006) than smaller Oscars (*A. ocellatus*). In the current study, as hybrid fingerlings became larger, their low oxygen tolerance decreased (r= -0.10), however, this is of small biological significance. Thus, the data was not adjusted for body weight. These findings on hybrid catfish fingerlings contradict those found for channel catfish, which had a positive correlation between fish size and tolerance to low oxygen for stocker size fish averaging 180g (Wang 2017), and were more similar to results with food-size channel catfish selected for increased body weight that had decreased low oxygen tolerance compared to controls (Rezk 1993, Padi 1995). This may indicate that genetic relationships for low oxygen tolerance can change in ictalurid catfish depending upon their size, genetic correlations can change over generations or the dynamics and genetic relationships in hybrid and channel catfish are different for low oxygen tolerance.

The variance components for cumulative mortality for tolerance to hypoxic conditions and corresponding estimates of combining abilities was largest for dams, intermediate for dam x sire interactions, and non-existent for the sire interactions. The small σSCA (sire × dam interaction) relative to the larger σGCAD (GCA dam) indicates that the majority of phenotypic variation pertaining to cumulative mortality of CxB hybrid catfish was due to additive genetic effects attributable to the dam. The variance components for total time to death (minutes) was largest for sire, intermediate for dams, and non-existent for dam x sire interactions, indicating in contrast to the cumulative mortality, that the additive genetic effects were more attributable to the sire than the dam CxB hybrid catfish. The variance components for mean dissolved oxygen level at time of death was small for dams and dam x sire interactions. The GCA variance estimate was for sires was zero.

In the current study, GCA had more influence than SCA. Similarly, analysis of combining ability analysis of other fish species, hybrid striped bass (Wang et al. 2006) and carp (Vandeputte et al 2004), also revealed larger additive variance relative to dominance variance. Bosworth and Waldbieser (2014) also found GCA, particularly that of the dam, much more important for determining growth and carcass yield of food size hybrid catfish compared to SCA. The current results indicate that there is potential for improvement in CxB hybrids in regards to tolerance to low oxygen by selecting for dams or sires for various measures of low oxygen tolerance. Future experiments should include hypoxia trials in ponds in order to compare genotype-environment interactions.

Selecting the appropriate strain of dam or strain of sire also is a mechanism to increase tolerance of low dissolved oxygen measures in hybrid catfish as significant strain effects on hybrid performance occurred. In general, hybrids that had dams with Kansas ancestry followed by those that had dams with Rio Grande ancestry had the lowest mortalities and those with Thompson ancestry had the highest mortalities.

Warmer water holds less dissolve oxygen so the RG channel catfish strain may naturally be more tolerant to periods of low DO. Supporting this premise, southern channel catfish strains were more tolerant to higher temperatures when compared to northern strains (Stewart and Allen 2014), and the relationship of heat and low oxygen tolerance should be explored. Using and selecting parent strains that come from environmental conditions that naturally have low dissolved oxygen may also allow production of more low oxygen tolerant hybrids. Tolerance to low dissolved oxygen is a complex trait, and how it is defined can affect data interpretation and conclusions (Dunham et al. 2002). Growth hormone transgenic common carp, Cyprinus carpio, had lower survival than controls when exposed to low oxygen levels, however, they had the longest mean survival time (Dunham et al. 2002). Obviously, high survival is important, but long survival times before death are also important to give the farmer a longer period of time to discover and correct a low oxygen event. Thompson had a high mortality, but contradictorily had the longest observed survival time. The lethal level of oxygen is another important factor in the equation for defining low oxygen tolerance. Although, there were no significant differences, the median level of oxygen resulting in death was, in general, correlated with cumulative mortality as the hybrids with the Kansas ancestry had median levels of oxygen at death of 0.5 and those from Thompson dams 0.75 ppm.

Strain of blue catfish sire also had effects on tolerance of low oxygen in fingerling hybrid catfish. For mortality, hybrids from DB males had the lowest mortality and those from RG sires the highest. Time to death was longest for hybrids from DR males, and again, shortest for those from RG sires, however, RG hybrids had the lowest mean and median lethal level of oxygen compared to the other hybrids, and particularly, compared to DB hybrids.

These results in some ways contradict those of Dunham et al. (2014), and in other ways support the results of this previous study. Dunham et al. (2014) found the RG hybrids were more tolerant to low dissolved oxygen than hybrids from other sires at 26˚ C, and especially so at cold temperatures, 17˚C and 8˚ C. However, there were several differences compared to the current study, including the dam strains being different, no DB hybrids were evaluated, the other sires were all derived from Alabama strains and the hybrids were food size fish. The temperature related data appears to contradict that of Stewart and Allen (2014) as one might expect Rio Grande hybrids to be inferior rather than superior for any trait at low temperature. Another key difference in these experiments is the evaluation of either fingerlings or food-sized fish. Future experiments should address the effect of this variable on the outcome of low oxygen challenge outcomes.

The low oxygen tolerance of CB hybrid catfish could likely be improved by mass or family selection for channel catfish females and for blue catfish males for the low oxygen tolerance parameters. Additionally, strain combining ability, selection for strains of channel catfish and strains of blue catfish with the best low oxygen tolerance and crossing these will result in hybrids with increased tolerance of low oxygen. Time to death and lethal level of oxygen had relatively small variation among families of hybrids and different strain types of hybrids, thus improvements in these low oxygen parameters may not be dramatic. However, the range of cumulative mortalities was much larger compared to the other traits, thus, the potential for improvement may be much more for this low oxygen trait of higher importance.

The current study, and those of Dunham et al. (2014) and Wang et al. (2017) all indicate that genetic variation exists for tolerance of low oxygen in ictalurid catfish, and various strategies would likely improve low oxygen tolerance in CB hybrid catfish and channel catfish. Variables such as age and size of fish, temperature, experimental protocol and genotype-environmental interactions could have contributed to differences within and between these experiments. The possible role of these factors should be studied in future experiments of low oxygen tolerance in ictalurid catfish.

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Appendix

Table 6: Mean weight (g), standard deviation (SD), coefficient of variation (CV), and minimum and maximum weights (g), and cumulative mortality for 40 families of female channel catfish (*Ictalurus punctatus*) X male blue catfish (*I. furcatus*) hybrids for dissolved oxygen challenge 1.

Cross	**Mean Weight (g)**	**SD**	**CV**	**MIN(g)**	**MAX(g)**	**Cumulative Mortality**
?AC-1X14TBB	40.714	12.247	0.301	27	53	0.93
?AC-1XRG3	47.929	19.132	0.399	10	55	0.93
1R?-1X14TBB	43.357	15.394	0.355	37	74.5	0.64
1R?-1X16DB	50.433	14.402	0.286	27.5	76	0.47
2L-1X12DR	44.577	16.429	0.369	31	67.5	0.38
2L-1X19DB	36.750	9.737	0.265	46	83.5	0.67
2L-2X12DR	49.056	13.789	0.281	32	73.5	0.67
2L-2X18DB	45.536	13.708	0.301	16	62.5	0.29
2L-3X12DR	44.233	14.268	0.323	36.5	88	0.67
2L-3X21DR	44.000	12.406	0.282	23	71	1.00
2L-4X13DB	51.735	47.156	0.911	36	68.5	0.65
2L-4XRG1	45.464	13.958	0.307	35.5	123	0.79
2L-5X14TBB	46.731	23.835	0.510	20.5	46	0.62
2L-5XRG3	43.042	13.192	0.306	22	63	0.83
2R-1X13DB	50.462	18.220	0.361	21.5	59.5	0.54
2R-1X17DB	53.577	15.983	0.298	32.5	68.5	0.62
2R-1X21DR	45.600	11.656	0.256	32	73.5	0.40
5R-1X15DB	40.192	14.086	0.350	32	52	0.69
5R-1X18DB	46.538	18.851	0.405	26.5	54	0.69
6L-1X14TBB	50.444	11.824	0.234	27	54	0.70
6L-1X16DB	53.227	27.314	0.513	13.5	67.5	0.82
71L-1X21DR	43.053	10.889	0.253	29	75.5	0.60
71L-1X14TBB	46.200	16.723	0.362	43.5	59.5	0.80
71L-1X18DB	45.133	15.348	0.340	20.5	52	0.80
71L-1XRG1	45.286	14.412	0.318	16.5	53.5	0.36
75L-1X19DB	46.500	11.336	0.244	35	53	0.86
75L-1X21DR	42.512	13.320	0.313	17	79.5	0.72
75L-2X12DR	43.900	16.064	0.366	27.5	54.5	0.80
75L-2XRG3	54.600	12.619	0.231	31.5	57	0.80
75L-3X17DB	39.962	7.350	0.184	19.5	68	0.85
75L-4X17DB	35.455	7.350	0.207	27	63	1.00

75L-4X18DB	41.938	11.356	0.271	23.5	40	0.38
7LX15DB	45.750	18.223	0.398	32	70	0.75
K-THX12DR	35.688	7.915	0.222	22.5	235.5	0.94
UNK-1X12DR	47.500	13.407	0.282	34.5	77.5	0.50
UNK-1X20DB	52.400	8.593	0.164	37.5	68	0.90
UNK-2XRG1	32.923	10.894	0.331	34.5	65	0.62
UNK-2X20DB	46.306	12.999	0.281	28	66	0.68
UNK-3X13DB	53.750	1.250	0.023	66	71	0.50
UNK-3X14TBB	41.643	7.671	0.184	33	65	0.71

Table 7: Mean weight (g), standard deviation (SD), coefficient of variation (CV), and minimum and maximum weights (g), and cumulative mortality for 40 families of female channel catfish (*Ictalurus punctatus*) X male blue catfish (*I. furcatus*) hybrids for dissolved oxygen challenge 2.

Cross	**Mean Weight (g)**	**SD**	**CV**	**Min(g)**	**Max(g)**	**Cumulative Mortality**
?AC-1X14TBB	64.036	25.148	0.393	38.5	93.5	0.79
?AC-1XRG3	60.077	19.537	0.325	25	91	0.62
1R?-1X14TBB	69.600	16.051	0.231	56.5	111.5	0.40
1R?-1X16DB	70.500	23.579	0.334	13.5	119	0.08
2L-1X12DR	61.719	29.792	0.483	43	148	0.18
2L-1X19DB	69.167	14.203	0.205	71	76	0.00
2L-2X12DR	61.318	16.178	0.264	43	117	0.40
2L-2X18DB	81.600	20.002	0.245	41.5	109.5	0.40
2L-3X12DR	75.222	22.455	0.299	52	102	0.75
2L-3X21DR	77.000	17.743	0.230	54	113	0.30
2L-4X13DB	65.750	28.930	0.440	55	118	0.08
2L-4XRG1	67.433	26.153	0.388	32	127	0.80
2L-5X14TBB	72.167	23.632	0.327	23.5	89	0.85
2L-5XRG3	101.346	141.042	1.392	49	137.5	0.93
2R-1X13DB	63.344	14.736	0.233	34	97	0.38
2R-1X17DB	131.313	171.850	1.309	42	86	0.33
2R-1X21DR	64.684	16.528	0.256	63	132	0.63
5R-1X15DB	63.250	23.972	0.379	29	87	0.27
5R-1X18DB	71.714	33.427	0.466	46.5	88	0.53
6L-1X14TBB	70.353	21.546	0.306	24	96	0.38
6L-1X16DB	64.045	17.894	0.279	28	128	0.91
71L-1X14TBB	65.400	14.385	0.220	49.5	95.5	0.73
71L-1X18DB	64.875	7.569	0.117	37	128.5	0.80
71L-1X21DR	79.333	22.281	0.281	45	109	0.36
71L-1XRG1	80.767	31.330	0.388	23	69	0.67

75L-1X19DB	72.813	21.565	0.296	35	103.5	0.56
75L-1X21DR	77.342	24.696	0.319	23	152	0.49
75L-2X12DR	81.800	16.188	0.198	44	89	0.80
75L-2XRG3	67.000	12.026	0.179	37	55	0.75
75L-3X17DB	80.571	17.725	0.220	29	106.5	0.29
75L-4X17DB	67.567	21.868	0.324	43	81	0.60
75L-4X18DB	65.714	21.366	0.325	29	96.5	0.71
7LX15DB	50.333	11.789	0.234	31	162	0.78
K-THX12DR	66.889	23.668	0.354	36	110.5	0.58
UNK-1X12DR	71.125	25.076	0.353	57	128	0.00
UNK-1X20DB	49.938	12.009	0.240	33.5	106	0.50
UNK-2XRG1	49.786	13.527	0.272	45	113	0.50
UNK-3X13DB	68.000	28.476	0.419	48	94	0.50
UNK-3X14TBB	62.042	30.742	0.496	47	113.5	0.83

Table 8- Estimates for variance of female channel catfish (*Ictalurus punctatus*) dam general combining ability, male blue catfish (*I. furcatus*) sire general combining ability (sire), and specific combining ability of female channel catfish (*I. punctatus*) x male blue catfish (*I. furcatus*) (cross) for cumulative mortality.

Covariance Parameter Estimates					
Cov Parm	**Ratio**	**Estimate**	**Standard Error**	**Z Value**	**Pr > Z**
dam	0.2473	98.7558	83.1471	1.19	0.1175
Sire	1.43E-36	5.73E-34	.	.	.
Cross	0.03520	14.0551	83.5237	0.17	0.4332
Residual	1.0000	399.28	91.6009	4.36	<.0001

Table 9: Calculated general combining ability for cumulative mortality for each female channel catfish (*Ictalurus punctatus*) dam. Bold text indicates significant tolerance to low dissolved oxygen.

Estimates				
Label	**Estimate**	**Standard Error**	**t Value**	**Pr > |t|**
DAM 1	9.9593	7.339	1.36	0.1828
DAM 2	4.5989	7.339	0.63	0.5346
DAM 3	2.0796	6.2694	0.33	0.7419
DAM 4	2.4014	7.339	0.33	0.7453
DAM 5	6.0929	8.285	0.74	0.4666
DAM 6	-1.3047	8.285	-0.16	0.8757
DAM 7	3.1267	7.339	0.43	0.6725
DAM 8	7.915	8.285	0.96	0.3454
DAM 9	-5.351	7.339	-0.73	0.4704
DAM 10	-1.5528	7.339	-0.21	0.8336
DAM 11	-10.1158	7.339	-1.38	0.1762
DAM 12	1.4252	7.339	0.19	0.8471
DAM 13	**-16.4248**	**7.339**	**-2.24**	**0.0312**
DAM 14	-8.1236	7.339	-1.11	0.2753
DAM 15	3.4558	7.339	0.47	0.6404
DAM 16	-1.4315	7.339	-0.2	0.8464
DAM 17	9.5414	7.339	1.3	0.2014
DAM 18	-3.3585	6.2694	-0.54	0.5953
DAM 19	-2.9337	7.339	-0.4	0.6916

Table 10: Calculated specific combining ability for each female channel catfish (*Ictalurus punctatus*) dam X male blue catfish (*I. furcatus*) sire cross for cumulative mortality.

Estimates				
Label	**Estimate**	**Standard Error**	**t Value**	**Pr > |t|**
CROSS 1	-0.7295	3.6559	-0.2	0.8429
CROSS 2	1.3841	3.6559	0.38	0.7071

CROSS 3	-0.3254	3.6451	-0.09	0.9293
CROSS 4	-0.7638	3.6451	-0.21	0.8351
CROSS 5	0.254	3.6451	0.07	0.9448
CROSS 6	1.1311	3.6451	0.31	0.758
CROSS 7	-0.1749	3.6559	-0.05	0.9621
CROSS 8	0.5167	3.6559	0.14	0.8884
CROSS 9	0.8672	3.6668	0.24	0.8143
CROSS 10	-0.1857	3.6668	-0.05	0.9599
CROSS 11	1.0622	3.6559	0.29	0.773
CROSS 12	-0.6172	3.6559	-0.17	0.8668
CROSS 13	1.1265	3.6668	0.31	0.7604
CROSS 14	-0.07938	3.6559	-0.02	0.9828
CROSS 15	-0.6822	3.6559	-0.19	0.853
CROSS 16	0.002628	3.6559	0	0.9994
CROSS 17	-0.2236	3.6559	-0.06	0.9515
CROSS 18	0.4287	3.6559	0.12	0.9073
CROSS 19	0.9887	3.6559	0.27	0.7883
CROSS 20	-0.799	3.6559	-0.22	0.8282
CROSS 21	1.0018	3.6559	0.27	0.7855
CROSS 22	0.1012	3.6559	0.03	0.9781
CROSS 23	-1.5408	3.6559	-0.42	0.6758
CROSS 24	-1.0683	3.6559	-0.29	0.7717
CROSS 25	-1.2693	3.6559	-0.35	0.7304
CROSS 26	0.04839	3.6559	0.01	0.9895
CROSS 27	-1.2046	3.6559	-0.33	0.7436
CROSS 28	0.4378	3.6559	0.12	0.9053
CROSS 29	0.05403	3.6559	0.01	0.9883
CROSS 30	1.315	3.6559	0.36	0.7211
CROSS 31	-1.5188	3.6559	-0.42	0.6802
CROSS 32	1.1728	3.6559	0.32	0.7501
CROSS 33	0.1852	3.6559	0.05	0.9599
CROSS 34	-0.3804	3.6451	-0.1	0.9174
CROSS 35	1.3244	3.6451	0.36	0.7184
CROSS 36	-0.7688	3.6451	-0.21	0.8341
CROSS 37	-0.6531	3.6451	-0.18	0.8588
CROSS 38	-0.6471	3.6559	-0.18	0.8604
CROSS 39	0.2296	3.6559	0.06	0.9503
CROSS 40	0	.	.	.

Table 11: Estimates for variance of female channel catfish (*Ictalurus punctatus*) dam general combining ability (dam), male blue catfish (*I. furcatus*) sire general combining ability (sire), and specific combining ability of female channel catfish (*I. punctatus*) x male blue catfish (*I. furcatus*) (cross) for mean time to death (minutes) due to low dissolved oxygen.

Covariance Parameter Estimates

Cov Parm	Ratio	Estimate	Standard Error	Z Value	Pr > Z
Dam	0.05467	5.5230	11.4551	0.48	0.3149
Sire	0.1505	15.2042	17.5396	0.87	0.1930
Cross	0	0	.	.	.
Residual	1.0000	101.03	19.3672	5.22	<.0001

Table 12: Calculated general combining ability for female channel catfish (*Ictalurus punctatus*) dam and male blue catfish (*I. furcatus*) sires to total time to death (minutes) due to low dissolved oxygen.

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
DAM 1	0.4578	2.1634	38	0.21	0.8335
DAM 2	-1.2112	2.1672	38	-0.56	0.5795
DAM 3	1.0915	2.0411	38	0.53	0.5959
DAM 4	-0.3234	2.1673	38	-0.15	0.8822
DAM 5	-0.2705	2.2449	38	-0.12	0.9047

Estimates					
Label	Estimate	Standard Error	DF	t Value	Pr > |t|
DAM 6	-0.5006	2.2516	38	-0.22	0.8252
DAM 7	0.08857	2.1652	38	0.04	0.9676
DAM 8	-0.2902	2.2449	38	-0.13	0.8978
DAM 9	-2.1134	2.1649	38	-0.98	0.3351
DAM 10	0.1021	2.1722	38	0.05	0.9627
DAM 11	0.8504	2.1672	38	0.39	0.6969
DAM 12	-0.9209	2.1630	38	-0.43	0.6727
DAM 13	-1.0248	2.1649	38	-0.47	0.6387
DAM 14	0.8862	2.1584	38	0.41	0.6837
DAM 15	0.9832	2.1566	38	0.46	0.6511
DAM 16	0.3159	2.1683	38	0.15	0.8849
DAM 17	-0.05808	2.1634	38	-0.03	0.9787
DAM 18	1.8732	2.0293	38	0.92	0.3618
DAM 19	0.06413	2.1750	38	0.03	0.9766
SIRE 1	2.8683	2.6159	38	1.10	0.2798
SIRE 2	1.8210	2.7088	38	0.67	0.5055
SIRE 3	0.2433	2.9760	38	0.08	0.9353
SIRE 4	0.8675	2.9772	38	0.29	0.7723
SIRE 5	1.5444	2.9761	38	0.52	0.6068
SIRE 6	-0.8317	2.7049	38	-0.31	0.7601
SIRE 7	0.2811	3.4733	38	0.08	0.9359
SIRE 8	1.2258	3.1898	38	0.38	0.7029
SIRE 9	-0.6063	2.9807	38	-0.20	0.8399

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
SIRE 10	2.6175	2.8207	38	0.93	0.3593
SIRE 11	-4.5474	3.1894	38	-1.43	0.1621
SIRE 12	-5.4834	3.1889	38	-1.72	0.0936

R code

One way ANOVA

```
my_data<-read.csv(file.choose())

my_data

oneway.test
```

Tukey test with boxplot

```
my_data<-read.csv(file.choose())

my_data

data<-aov(my_data)

TukeyHSD()

Boxplot( )
```