COMBINING ABILITY OF CHANNEL CATFISH (*Ictalurus punctatus*) FEMALES AND BLUE CATFISH (*Ictalurus furcatus*) MALES FOR TOLERANCE OF LOW OXYGEN USING A FACTORIAL DESIGN

By

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Abstract

The channel catfish, *Ictalurus punctatus* × blue catfish, *I. furcatus* hybrid shows increased performance over the channel catfish for many traits, including tolerance of low dissolved oxygen. Performance of hybrid offspring might be further improved by selecting for desired traits in the parents based on combining ability. In addition, analysis of combining ability may aid in identifying parental strains that produce better performing hybrid offspring. Using a full factorial 6 × 6 experimental design, fry from 36 different families of hybrid catfish were produced to measure general (GCA) and specific (SCA) combining abilities for resistance to low levels of dissolved oxygen (DO).

The dam GCA was observed and accounted for 9.5% of the variance for survival when hybrid progeny were exposed to 0.6 ppm, whereas the sire GCA and SCA were zero. Thus, selecting for channel catfish females with increased tolerance of low dissolved oxygen should improve, and would be predicted to be the best mechanism to enhance, the genetic ability of the hybrid progeny to tolerate low dissolved oxygen.

Interpretation of the time to death was more complicated. If the surviving fish are included in the analysis, dam GCA accounts for 25% of the variation, sire GCA only 3% and SCA, 0%. Again, the best genetic enhancement approach will be to select for channel catfish lines resistant to low oxygen. However, if the survivors are not considered in the analysis, the genetics of hybrid tolerance of low oxygen changes. Dam GCA reduces to 8%, sire GCA reduces to 0% and SCA becomes the most important genetic factor, increasing to 17% of the total variance. How the trait is defined and analyzed has a large impact on both interpretation and recommended breeding program practices. Results were similar, but not exactly like those

obtained in another study when hybrid fingerlings averaging 58g were evaluated to determine combining ability for low DO tolerance.

The results of this study could influence genetic selection programs on a commercial scale for the selection of hybrid fry and fingerlings that have improved tolerance to low dissolved oxygen level. Using hybrids with improved tolerance to low dissolved oxygen allows for increased stocking densities and more fish available at harvest.

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Introduction

As the world's population increases, so does the demand for an affordable and environmentally conscious protein source. A continued reliance on overfishing wild fishery stocks has resulted in a reduction in species biodiversity as well as an altering of ecosystem function (Worm, et al. 2009). Aquaculture must be ready to meet potential demands. An increased demand on aquaculture must result in more efficient culture practices (Dunham 2004). Farmers must be able to produce more fish in the same amount of space than they have in the past. This will result in higher stocking rates, and a need for fish that can tolerate these conditions (Dunham, 2004). An increased risk of loss is associated with higher stocking rates. Cultured fish must be able to handle the stress and potential disease risk that is associated with increased density. Genetic enhancement techniques including selection, interspecific hybridization, polyploidy, and transgenic modification have potential to be key for increasing stocking densities and meeting future demands (Dunham 2004).

Catfish, order Siluriformes, is one of the most diverse and biogeographically ubiquitous groups of teleosts. With 37 recognized families and over 3,000 species spread across the globe, catfish represent nearly 11% of all fish and 5.5% of all vertebrates on the planet (Armbruster 2011). Catfish are on the global forefront of aquaculture due to their diversity, ability to tolerate handling while also having high tolerance to disease, high fecundity, and good feed conversion (Jin et al. 2016). Tra (*Pangasiuis hypothalamus*), African catfish (*Clarias garienpinus*), walking catfish (*Clarias batrachus*), blue catfish (*Ictalurus furcatus*), channel catfish (*Ictalurus punctatus*), and the F1 hybrid of female channel catfish × male blue catfish are the most commonly cultured catfish worldwide (Khan et al. 2009; Jin et al. 2016).

Aquaculture production in the United States has historically emphasized channel catfish. Overall, catfish production accounts for ~ 68% of all freshwater aquaculture production in the United States (NMFS, 2015). Recently, the U.S. catfish industry has seen observed declines. Catfish production in the U.S. fell from 300,278 metric tonnes in 2003 to 136,531 metric tonnes in 2014. This represented a drop in production of 54% (Hanson and Sites 2015). The main reason for the rapid decrease in production can be attributed to the growth of imported frozen catfish, primarily *Pangasius spp* (Hanson and Sites, 2015). Catfish imports have increased at a substantial rate, from 2005 to 2014, where imports of frozen catfish rose from 13,607 metric tonnes to 108,408 metric tonnes. This represented an increase of nearly 977%. Of the 108,408 metric tonnes of frozen catfish imported in 2014, a large portion (97,618 metric tonnes) were Vietnamese *Pangasius* (NOAA, 2018). In 2014, imports accounted for ~80% of all sales of frozen catfish (Hanson and Sites 2015). Frozen Vietnamese imports of *Pangasius* reached a high point in 2016 with over 131,207 metric tonnes imported into the U.S. (NOAA, 2018). Since then, frozen Vietnamese imports of *Pangasius* have shown decreases with 2017 imports totaling 96,460 metric tonnes and 2018 imports totaling 97,920 metric tonnes (2018 data available from January 2018-November 2018) (NOAA, 2018).

The major competition for and largest potential threat to U.S. catfish farmers comes in the form of Asian exports of *Pangasius*. The ability of *Pangasius* to use their swimbladders to absorb oxygen allows tolerance of dissolved oxygen levels as low as 0.05 mg/L (FAO 2019). This allows the *Pangasius* farmer to grow their crop at stocking densities observed higher than that of the U.S. catfish farmer. Harvest yields of *Pangasius* ponds can reach upwards of 300 tonnes/ha (FAO 2019). In contrast, U.S. channel catfish yields average between 5-6 metric tonnes/ha with proper aeration (Chapman, 1992). This varies greatly and depends on the

production system, management level, and skill of the farmer (Chapman, 1992). More intensive culture techniques such as the split-pond system may increase yearly yield to greater than 11 metric tonnes/ha (Tucker and Kingsbury, 2010).

Pangasius is often cheaper to import and sell in the U.S. than domestic channel catfish. The lack of a need for intensive culture practices such as nightly aeration, combined with the ability to stock *Pangasius* at high density results in high quantities of product at low price. This creates an obvious problem for the U.S. catfish industry to try to overcome. Thus, any possibility of improvement within the U.S. catfish industry, such as using genetic enhancement, needs to be thoroughly explored.

The selection of desirable traits has been constant among aquaculture practices. The first records of aquaculture practice date back more than 2000 years to the Roman Empire (Balon 1995). By breeding the largest fish in captivity the first aquaculturists were practicing genetic selection without knowing it (Dunham 2004). The act of domesticating and rearing wild-caught fish began to alter performance and gene frequencies in newly captive fish (Dunham 2004). Desirable phenotypic traits such as color, size, and body shape were the first targets for selection. The very first records of an organized breeding program date back to the 1800's with the selective breeding of koi in Japan and goldfish in China (Dunham 2004).

While many species of cyprinids had been cultured for thousands of years, the first recorded instance of channel catfish spawning in captivity occurred in 1892 (Dunham and Smitherman 1984). Many of the earliest catfish farming operations and federal hatcheries received channel catfish that were propagated from stock collected from the Red River in Oklahoma in 1949 (Dunham and Smitherman 1984). A portion of these catfish and their

progeny were distributed to some of the first commercial catfish production farms (Dunham and Smitherman 1984).

The channel catfish exhibits a considerable number of traits desirable for commercial aquaculture. These traits include fast growth rate to harvest, early age of sexual maturity, ability to handle high stress, tolerance of poor water quality, as well as naturally high resistance to columnaris (*Flavobacterium* columnare) and ich (*Ichthyophthirius multifiliis*). Because of this combination of traits, the channel catfish became the most easily and most widely produced ictalurid for commercial purposes (Dunham et al. 1993; Argue et al. 2003).

Many other desirable traits exist among different ictalurid species. The white catfish (*Ameiurus catus)* displays fast initial growth. The bullhead catfish (*Ameiurus spp)* shows increased low dissolved oxygen resistance. Blue catfish exhibit superior dress out percentage, increased harvestability, better uniformity in growth, and superior resistance to both enteric septicemia of catfish (*Edwardsiella ictaluri*) and to channel catfish virus (CVV) (Dunham et al. 1993; Argue et al. 2003). However, the channel catfish offered the best combination of desirable traits for commercial purposes and thus dominated the catfish production industry in the U.S.

Genetic enhancement research has been predominantly focused on improving desirable culture traits in channel catfish (Dunham 2004). Since the 1970's the primary focus of genetic enhancement has been on selection for increased growth rate to market size (Dunham et al. 1987). Mass selection has been extremely successful, resulting in an 8-20% increase in body weight after one generation of offspring (Dunham and Smitherman 1983, Bondari 1983).

Other genetic techniques have also been researched to increase traits desirable for production. Triploid channel catfish showed increased growth resulting from an absence of

sexual maturation. However, this characteristic was not expressed until later in development (Dunham et al 1987). Intraspecific breeding of different strains of channel catfish has been shown to improve desirable culture traits such as resistance to enteric septicemia of catfish, dress-out percentage, growth, catchability, body shape uniformity, lower overall mortality rates, better feed conversion efficiency, greater seinability, and tolerance of low dissolved oxygen (Argue et al. 2003; Dunham et al. 1987; Dunham et al. 1999; Dunham et al. 1987). The channel female × blue male hybrid is the only interspecific ictalurid hybrid resulting in culture traits superior to the channel (Dunham et al. 1987).

One of the most successful genetic enhancement programs for ictalurid catfish has been interspecific hybridization. Initial research into interspecific hybridization was attempted in 1966 with seven ictalurid species, producing 42 different interspecific ictalurid hybrids (Dunham and Masser 2012). Interspecific hybrids were attempted with assorted crosses between channel catfish, blue catfish, white catfish, black bullhead catfish, brown bullhead catfish, yellow bullhead catfish, and flathead catfish (Goudie et al. 1993, Dunham et al 1987). These interspecific hybrids created offspring with observable characteristics of each parents, but not necessarily traits desirable for industry (Goudie et al. 1993). The channel catfish female and white catfish male hybrid resulted in faster initial growth in aquaria, but slower growth to market in ponds (Dunham et al. 1987). The blue catfish female and channel catfish male hybrid resulted in offspring that showed heavy paternal predominance toward the traits of the channel catfish but exhibited few desirable traits of the blue catfish (Dunham et al. 1987).

While the channel-blue hybrid showed many obvious advantages compared to the channel catfish, the lack of efficient artificial spawning techniques kept the channel-blue hybrid from being commercially mass-produced (Liu 2011). The first channel-blue hybrid was created

in 1966, but the channel-blue hybrid catfish did not take hold in the U.S. catfish industry until the early 2000's (Dunham and Masser 2012; Mischke et al. 2017). As a result of the improved performance over the channel catfish and the development of more efficient spawning techniques, the hybrid catfish has been increasing in popularity among U.S. catfish farmers.

While the channel catfish has dominated the U.S. catfish aquaculture industry in the past, the channel-blue hybrid catfish has accounted for a growing increase in production within the last 17 years (Mischke et al. 2017). In 2002, channel × blue hybrids accounted for less than 5% of production, which increased to 40% of production in 2014 (Mischke et al. 2017) and was an estimated 75% in 2018 (Nagaraj Chatakondi, USDA, personal communication).

The channel × blue hybrid exhibits heterosis from its parents and exhibits more desirable traits for aquaculture production in comparison to the channel catfish. Due to reproductive isolation between channel catfish and blue catfish, hybrid catfish must be produced via artificial fertilization. As techniques of artificial fertilization were improved and refined, efficiency improved and the hybrid became a commercially viable fish in the catfish industry (Liu, 2011).

The threat of a low dissolved oxygen event is a constant threat for catfish farming industry. Phytoplankton die offs, power loss, and failures in aeration equipment such as blowers and paddlewheels when combined with higher stocking rates mean there is always a chance of oxygen depletion, and associated loss of fish . In 2010, 28.1% of all losses in the catfish industry were a result of low oxygen events. Almost a quarter of the losses (22.8%) were severe in nature with over 900 kg of fish killed in a single low DO event (USDA 2010).

Modern technology has helped to mitigate some of this risk with the implementation of oxygen monitoring equipment that can alert the farmer sooner to an oxygen depletion

emergency. While oxygen monitoring equipment has allowed for shorter response times to low dissolved oxygen events, culturing fish with increased performance under low dissolved oxygen will ultimately lead to less mortality and monetary loss during such an event.

Acute low dissolved oxygen is clearly a threat to kill fish on its own merit, but sub-lethal dissolved oxygen levels can act as stressors that can trigger disease. Nile tilapia (*Oreochromis* niloticus) exposed to sub-lethal levels of hypoxia were shown to have observed higher mortality rates when infected with *Streptococcus agalactiae* than Nile tilapia under non-hypoxic conditions (Evans *et al.* 2003). Channel catfish held in sub-lethal levels of dissolved oxygen showed observed higher mortality rates when exposed to high doses of *Edwardsiella ictaluri* than channel catfish exposed to the same dose of *E. ictaluri* under normal oxygen conditions (Welker et al. 2007).

Maintaining adequate dissolved oxygen levels (>5.0 ppm, Steeby and Avery, 2005) in rearing tanks and ponds is crucial to the long-term development of catfish. Exposure of channel catfish to sub-optimal levels of dissolved oxygen over a period of 6 weeks lead to reduced food consumption, growth, and development (Andrews et al. 1973). Feeding and growth rate was shown to be reduced in juvenile coho salmon (*Onchorhychus kisutch*), under various levels of sub-optimal dissolved oxygen. At dissolved oxygen levels around 2.0 mg/L, juvenile coho salmon lost weight (Herrmann et al. 1962). Growth rate and feed conversion were shown to be reduced in channel catfish when exposed to sub-lethal dissolved oxygen levels over a period of several weeks (Andrews et al. 1973). Catfish held at 100% oxygen saturation grew nearly twice as much as fish held at 36% saturation when fed the same amount and type of feed (Andrews et al. 1973).

The channel-blue hybrid catfish shows increased performance over the channel catfish for many traits, including tolerance of low dissolved oxygen. (Dunham and Smitherman 1983). Performance of hybrid offspring may be further improved by selecting for desired traits in the parents based on combining ability. In addition, analysis of combining ability may aid in identifying parental strains that produce better performing hybrid offspring (Bosworth and Waldbieser 2014). General combining ability (GCA) is defined as the average performance of a line in hybrid combinations while specific combining ability (SCA) is defined as the deviation of certain crosses from their expectation based on the average performance of the lines involved (Sprague and Tatum 1942). For the purposes of this study GCA is a measure of the performance of a specific dam or sire in comparison to the other dams and sires in the study. SCA is a measure of the deviation of expected performance of a certain cross. SCA results are based on the previously calculated GCA. GCA is used as a measure of additive gene effects and SCA is used as a measure of dominance gene effects (Bosworth and Waldbieser 2014).

The use of factorial mating designs and diallel mating designs to estimate genetic components is well-documented. Diallel mating designs have been used to estimate the GCA and SCA of different strains of common beans (Machado et al 2002) and in inbred sunflowers (Salem and Ali 2012). Models using different factorial mating designs have been shown to identify additive genetic interactions in matings of individual fish (Busack and Knudsen 2007). A factorial mating design would provide accurate measures of GCA and SCA in channel female blue male hybrid catfish.

In this study, 36 families of hybrid F_1 fry produced from a factorial mating of six female channel catfish and six male blue catfish were tested for their tolerance of acute low dissolved oxygen levels. The purpose of the study was to identify the GCA of the individual channel

catfish females and blue catfish males, as well as the SCA of each intraspecific individual cross, and to determine which channel catfish females and blue catfish males produce progeny that have better performance in acute low dissolved oxygen. The time to death (min), total death count, total survivors, dissolved oxygen levels (mg/L), and weight (g) were all measured to help determine the combining ability with regard to tolerance of low dissolved oxygen. This study will help identify which female channel catfish and male blue catfish to select for increased performance of juvenile hybrid progeny under hypoxic conditions and may serve as an effective model to help identify further strains that produce fry with increased tolerance to low dissolved oxygen.

Materials and Methods

The procedures involved with the treatment and handling of fish for this study were approved by the Auburn University Institutional Animal Care and Use committee (AU-IACUC).

Experimental Fish

All broodstock used in this study were spawned and cultured at Auburn University's E.W. Shell Fisheries Center in Auburn, Alabama. Six female channel catfish were mated with six male blue catfish in a full factorial mating design creating 36 full-sib half-sib families of channel-blue hybrid fry. The female channel catfish used were of Kansas ancestry, Kansas Random female number 280 (KR 280), Kansas Random 283 (KR 283), Kansas Random 310 (KR 310), Kansas Mix 329 (Kmix329), Kansas Mix 345 (Kmix 345), and Probable Kansas Mix 329 (PKmix332) (Dunham and Smitherman 1984). The ancestry of male blue catfish used was Auburn-Rio 1 (AR1), Auburn-Rio 2 (AR2), D&B, DR, Probable AR (P_AR), and an Unknown strain (Unk) (Dunham and Smitherman 1984).

Broodstock

Broodstock for this experiment were captured and transferred from ponds in June of 2017 at the Genetics Unit at the E.W. Shell Center. Data on each fish was recorded; including fish number, species, sex, weight, strain, and pit tag. Females were placed in wet spawning bags and clipped to the edge of a 730 L metal fish hauler and transferred to the hatchery at the Genetics Unit. Spawning bags were labeled with the number of the fish and its weight in kg. Dissolved oxygen levels in the hauler were maintained above 5 mg/L. Males were kept loose in the fish hauler and transferred to the hatchery. Upon arrival to the hatchery, male blues were placed in

837 L tanks. Female spawning bags were placed in and clipped to the edge of 670 L tanks with the fish resting at a depth of ~ half-way from the bottom of the tank (tank depth of 60 cm).

To induce ovulation, females were given injections of liquid luteinizing hormone releasing hormone analog (LHRHa). The priming dose was administered at a rate of 20 µg/kg ~36 hours prior to anticipated ovulation with a 21 gauge needle and a 3mL syringe. The resolving dose was administered at a rate of 90 µg/kg ~24 h prior to ovulation with a 21 gauge needle and a 3mL syringe (Becton Dickinson, Franklin Lakes, New Jersey, USA) (Dunham and Masser 2012). All LHRHa injections were administered intraperitoneally. Spawning bags of females were checked for eggs 24 h after the administration of the priming dose and every four h, subsequently. Once small aliquot of eggs were discovered in the spawning bag, the ovulating female was placed in a solution of ~ 100 ppm tricaine methane sulfonate (MS-222) buffered with sodium bicarbonate. Once sufficiently anesthetized, the female was rinsed in fresh water while covering the genital opening to prevent loss of eggs. The female was then dried with a towel and Crisco All-Vegetable Shortening vegetable oil applied to genital opening and the body fins around the genital opening. Eggs were then hand stripped from the female by gently stroking her anterior to posteriorly toward the genital opening. Eggs were stripped into 30.5 cm metal pie pans lined with a thin layer of Crisco All-Vegetable Shortening. Any bloody eggs were rinsed with 0.9% saline solution made by adding 34 g of Morton's pickling salt to one 3.75L of distilled water. Any clumps of blood were removed and the saline solution was decanted before fertilization (Dunham and Masser 2012).

Males used in this study were euthanized by blunt force trauma to the head and their testes extracted with scalpel and forceps. Testes were rinsed with the same 0.9% saline solution to remove blood (Dunham and Masser 2012). Once the blood was removed, testes were

macerated and the sperm strained into 50 mL vials labeled with strain information, followed by addition of 0.9% saline solution to the sperm at a rate of 10 mL per gram of testes.

Artificial fertilization was performed in the metal spawning pans by adding sperm solution to eggs at a rate of 10 mL per 100 grams of eggs. Once sperm was added and thoroughly mixed among the eggs, a small amount of hatchery water was added to the pan to activate the gametes (Dunham and Masser 2012. The pan was then let sit for ~5 min before being transferred to and submerged in a trough with a calcium chloride drip. The eggs were hardened in the calcium chloride hardening bath for ~1 h before being transferred to individual hatching baskets suspended in flow through hatching troughs. Hatching troughs had motorized paddlewheels to provide water agitation for proper incubation. Water flow in hatching troughs was maintained at ~15 L/min. Calcium chloride drips were placed at the heads of hatching troughs to maintain hardness at a minimum of 50 ppm (25 ppm was the typical harness level). Dissolved oxygen levels were maintained by diffusion of air into the tank via airstones, and the water paddling.

Eggs were monitored daily for growth of fungus. Treatment of fungus included manual removal and use of chemicals. Chemical treatment for fungus included 100 ppm formalin and 32 ppm copper sulfate baths. During chemical treatment water to the tanks was shut off for ~15 min. Chemical treatments were not administered within 12 h of anticipated hatch.

Culture and Rearing

Embryos reared at temperatures of 24-28^{O} C hatching in ~5-6 days post fertilization. Fry remained in their hatching baskets until they reached swim up stage. Number of hatched fry for each family ranged between 75 to 550 with an average of 315 fish per family. At the swim up stage, fry were transferred per family to 50 L aquaria within a recirculation system. The water

quality in the recirculation system was recorded twice per week. Water temperature fluctuated between ~23^{O} C and ~30^{O}C. In the event of ammonia or nitrite spikes within the system, partial water exchanges were performed with city water dechlorinated with sodium thiosulfate. Fry were fed Purina® AquaMax® powdered starter feed until they were large enough to eat Purina® AquaMax® 100. Both feeds contained 50% protein. All fish were fed every day to satiation. Fry remained in the aquaria until the beginning of the oxygen challenge in August 2017.

Dissolved Oxygen Challenge

The oxygen challenge was conducted with the 36 families of hybrid fingerlings spawned in the 6x6 factorial mating design, during late August, 2017. For this challenge, 30 fingerlings from each family were selected at random by removing with dip net and split into three replicate groups with ten fish per replicate. The exception to this were families 20 and 30(KR283×Unk), which did not have enough fish for replicate groups B or C. Families and replicates were placed in to 10 cm × 10 cm × 20 cm wire mesh baskets. Families were assigned a number to make identification easy. Baskets were ordered by replicate group and then by basket number. Baskets were labeled with the replicate group and family number (A1, B2, C3, etc.). Replicate group A was placed near the standpipe, replicate group B was centered in the tank, and replicate group C was placed near the rear. In total there were 106 baskets.

The challenge was conducted in a 345 L hatching trough that measured 507 cm × 60 cm × 19.5 cm. The challenge began at 5 pm with the removal of airstones from the tank and terminating water flow. Water quality parameters (pH, nitrite, nitrate, alkalinity and hardness), were measured at the beginning of the challenge using Tetra (Blacksburg, Virginia) EasyStrips™

6-in1 Aquarium Test Strips. Dissolved oxygen levels were measured at the beginning of the challenge and every 5 minutes after until the completion of the challenge. DO was measured using a YSI (Yellow Springs, Ohio) proDSS dissolved oxygen probe. Dissolved oxygen was measured in a different location for each measurement to ensure that the oxygen was dropping at the same rate throughout the tank. When the DO level ceased declining naturally, water level was reduced from 19 cm to 13 cm. and from 345 L to 230 L, respectively. Once the DO level ceased declining a second time, at 3.19 mg/l, DO level was reduced chemically by addition of a solution of sodium sulfite with cobalt chloride as a catalyst (Vanderhorst and Lewis 1969). Sodium sulfite was added at a rate of 7.9 ppm per 1 ppm DO and cobalt chloride was added at a rate of 8 ppm (Cavano 2007). Since the addition of this much sodium sulfite would immediately drop the DO level to essentially 0, the amount of chemical added was enough to lower the DO by ~1 ppm every 15 min. At 2 h and 5 min after initiation of the experiment, sodium sulfite was first added. Over the next 1 h and 35 min, 0.89 g sodium sulfite and 0.115 g cobalt chloride was added in 12 additions. Additions were spaced evenly as possible to allow the DO to fall at a steady rate. Sodium sulfite and cobalt chloride were diluted in a 13 L solution and spread evenly throughout the tank to help ensure an even decrease of DO throughout the tank. Chemicals were weighed on an Ohaus (Parsippany, New Jersey, USA) scale.

Fish were removed from their baskets when they became moribund, as defined by complete loss of equilibrium. Time of death, weight in grams, basket number, and replicate group were recorded for each fish removed from the baskets. Fish were weighed using Ohaus scales and ranged from 0.7g to 2.9g in body weight. The Challenge was terminated when 50% mortality was reached, and the DO had dropped to 0.66. Upon completion of the challenge, aeration and water flow was restored to the tank. The survivors from each basket were counted,

weighed collectively, and the replicate groups and family numbers recorded. Survivors were placed in separate aquaria by family.

Statistical Analysis

Statistical analysis was conducted with SAS version 9.4, Cary, NC, USA. The Proc Mixed function was used to attain the mean squares values for the dam, sire, and dam × sire interactions. GCA for the dams and sires are equal to dam and sire variances while SCA is equal to the variance of dam-sire crosses (Cotterill et al. 1986). ANOVA was used to compare the sources of variation for survival rate, TTD (including survivors), and TTD (no survivors). Differences in variances of individual dams were calculated using unpaired t-tests. Differences in variances between individual sires were calculated using unpaired t-tests. Differences in variances between individual crosses were calculated using unpaired t-tests. ANOVA was used to calculate the effect of mean family weight on survival rate, TTD (including survivors), and TTD (no survivors). The Proc Reg function was used to calculate the R^2 and fit statistics for the linear regression of mean family weight on survival rate. ANOVA was used to calculate the effect of dam strain, sire strain, and dam-sire cross strains on survival rate, TTD (with survivors), and TTD (no survivors). The Proc GLM function was used to determine the presence of observed differences between dam strains, between sire strains, and between strains in individual dam-sire crosses. Tukey-Kramer adjustment for multiple comparisons was used to identify where observed differences lied amongst individual dam strains, individual sire strains, and strains in dam-sire crosses (using least squares means) for survival rate, TTD (survivors included), and TTD (no survivors). All significance in this study was tested at $\alpha = 0.05$.

The variation between mean weights of families was enough to warrant adjusting survival rate based on mean weights of each replicate. Survival rate was adjusted for using the formula $Y_A = Y - b_{Xy}(\overline{X}_i - \overline{X}_P)$, where Y_A is adjusted survival of a replicate, Y is the actual survival of the replicate, b_{xy} is the regression coefficient, $\overline{X}_i$ is the mean replicate weight, and $\overline{X}_P$ is the mean weight of all the replicates.

Results

Combining Ability

The genetic variation for survival rate of fingerlings during the low dissolved oxygen challenge was mostly explained by the dam GCA of 10% (Figure 1) Sire GCA was 1% for survival rate, while the SCA for specific dam × sire crosses was 0% (Table 1, Figure 1). This indicates that additive genetic variation had the greatest influence on variation, with the females having almost total genetic influence over survival rate. Dominant gene interactions of specific combining ability had no influence.

The genetic variation for time to death of fingerlings during the low dissolved oxygen challenge, with survivor data included, was mostly explained by the dam GCA of 25% (Table 1, Figure 2). Sire GCA was 3% for TTD with survivors, while the SCA for specific dam × sire cross was 0%. Additive genetic variation from the dam had almost total influence over the TTD when survivor data was included, with sires contributing very little. Dominant gene interactions from specific combining ability had no influence.

The genetic variation for time to death of fingerlings during the low dissolved oxygen challenge, when only accounting for data from moribund fish, was mostly explained by the specific combining ability of specific dam × sire cross (17%, Table 1, Figure 3). There was also an observed influence from dam GCA (8%, Table 1, Figure 3, Appendix Table 7). Sire GCA percentage was 0 (Table 1, Figure 3). This suggests that dominant gene interaction had the most influence over the time to death, when only accounting for data from dead fish. Additive genetic variation from females also had an observed influence, but to a lesser degree.

Table 1: Estimates of general combining ability variance for dam (σ^2GCA_d) and sire (σ^2GCA_s) and specific combining ability variance (σ^2SCA), error variance (σ^2_E) and corresponding standard errors for average survival rate (SR) per family and average time to death (TTD) per family for tolerance to low dissolved oxygen of channel catfish (*Ictalurus punctatus*) female × blue catfish (*I. furcatus*) male F1 hybrid progeny during the low dissolved oxygen challenge.

Low DO resistance indices	Genetic parameter estimates			
	σ^2GCA_d (± SE)	σ^2GCA_s (± SE)	σ^2SCA (± SE)	σ^2_E
Survival rate	0.01 (0.01)	0.0006 (0.004)	0.00 (-)	0.09
TTD including survivors	0.09 (0.06)	0.01 (0.01)	0.00 (-)	0.26
TTD all dead	0.02 (0.02)	0.000093 (0.01)	0.04 (0.02)	0.18

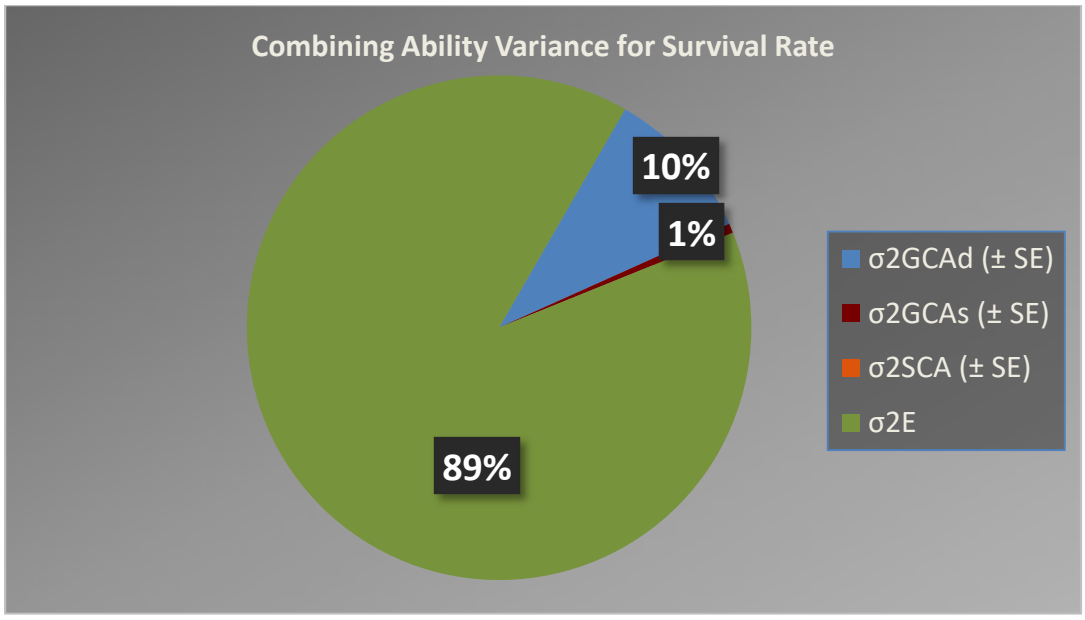

Figure 1: Comparison the general combining ability (GCA) estimate percentages for female channel catfish (*Ictalurus punctatus*) dams and male blue catfish (*I .furcatus*) sires, channel × blue specific combining ability (SCA), and the error variance in survival rate during the low dissolved oxygen challenge. Dam GCA is σ2GCAd, sire GCA is σ 2GCAds, SCA is σ2SCA, and σ2E is non-genetic variation.

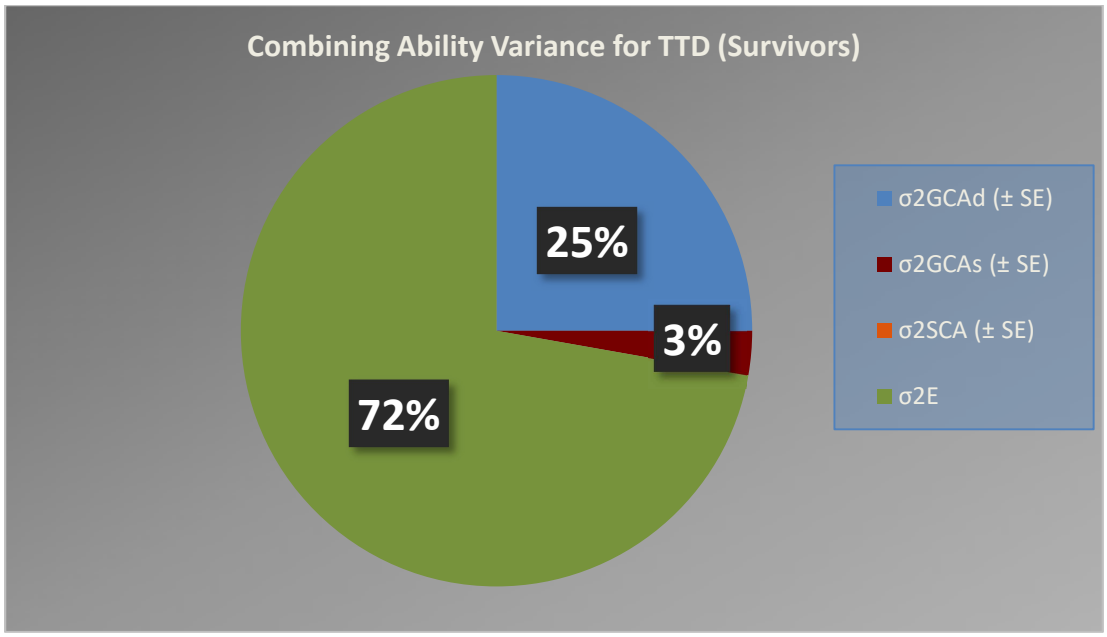

Figure 2: Comparison of general combining ability (GCA) estimate percentages for female channel catfish (*Ictalurus punctatus*) dams and male blue catfish (*I. furcatus*) sires, channel × blue specific combining ability (SCA), and the error variance in time to death (TTD) when survivor data is included in the analysis of the low dissolved oxygen challenge. Dam GCA is σ2GCAd, sire GCA is σ 2GCAds, SCA is σ2SCA, and σ2E is non-genetic variation.

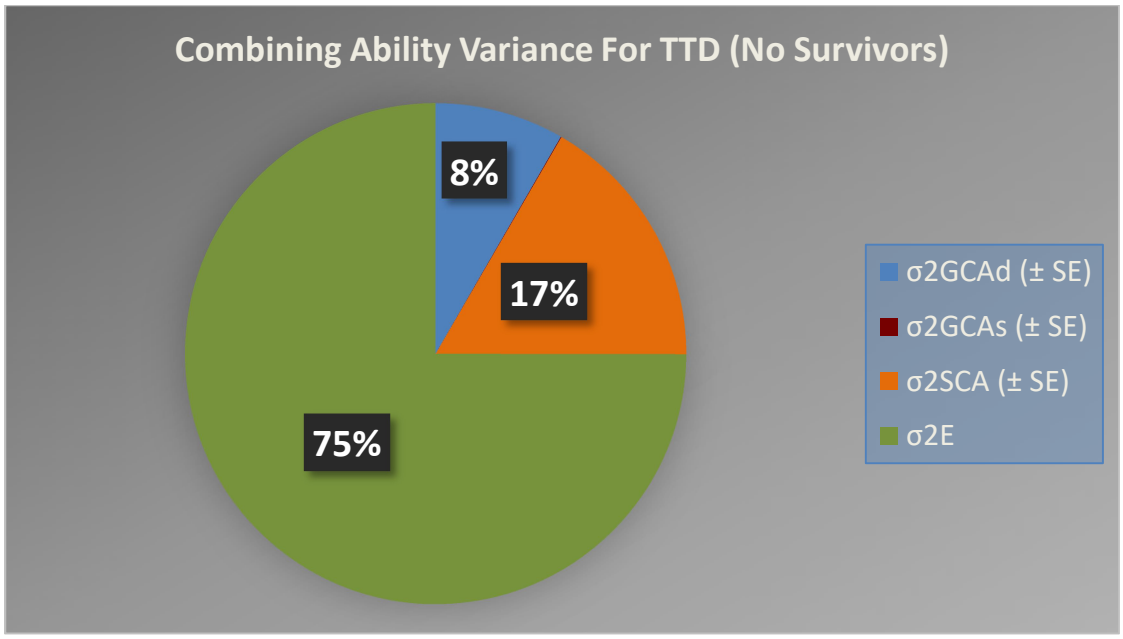

Figure 3: Comparison of the general combining ability (GCA) estimate percentages for female channel catfish (*Ictalurus punctatus*) dams and male blue catfish (*I. furcatus*) sires, channel × blue specific combining ability (SCA), and the error variance in time to death (TTD) when only accounting for the fish that died during the low dissolved oxygen challenge. Dam GCA is σ2GCAd, sire GCA is σ 2GCAds, SCA is σ2SCA, and σ2E is non-genetic variation.

Combining ability analysis was conducted on the survival rates adjusted for body weight. Covariance estimates gave a dam GCA estimate of 0.009548, but a sire SCA estimate of 0, and an SCA estimate of 0 (Appendix table 16). Overall, dam additive genetic effects accounted for 9.53% of the genetic variation in survival rates adjusted for body weight (Figure 4). This indicates that when adjusting for body weight differences, additive genetic effects from the dam provides all of the genetic variation when compared to additive genetic effects of the sire and dominance effects of the specific dam-sire cross.

GCA estimates for individual dams showed an observed difference between Pkmix332 and the other females used in the study. Pkmix332 had an observed lower GCA estimates for survival and time to death (with and without survivor data) meaning her progeny performed an observed worse than the progeny of other females in the study (Figures 5, 6, 7, 8, and 9). SCA estimates for specific dam-sire crosses showed four crosses with an observed improved performance in comparison to other crosses and four crosses with an observed decreased performance in comparison to the other crosses (Figure 8).

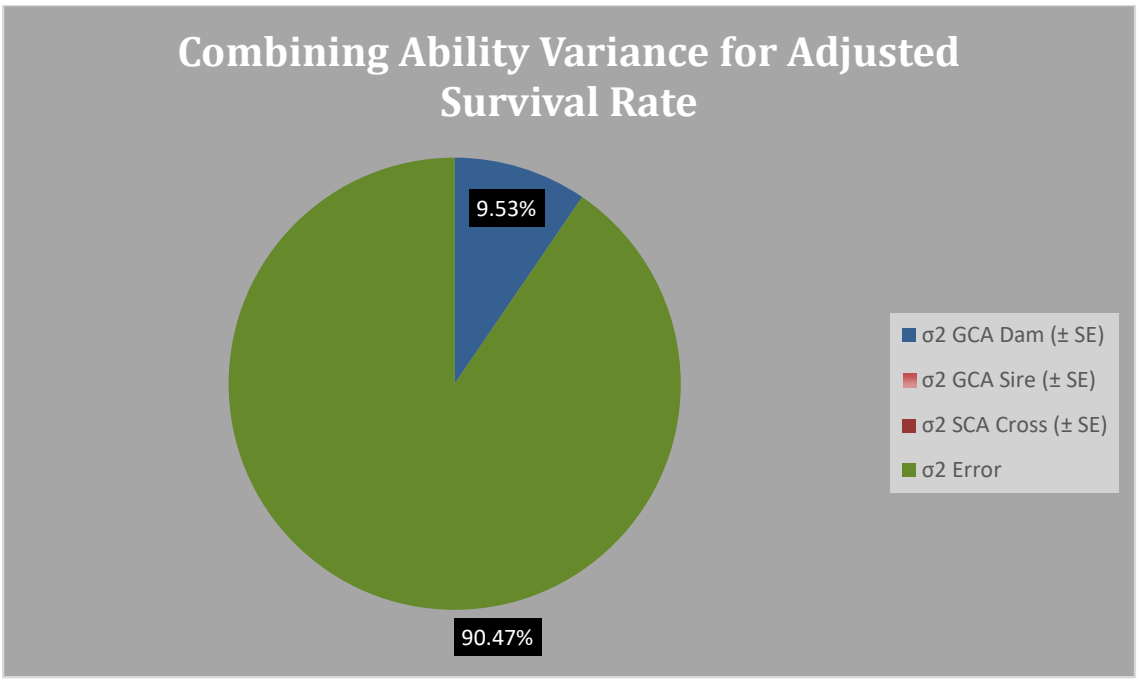

Figure 4: Comparison of the general combining ability (GCA) estimate percentages for female channel catfish (*Ictalurus punctatus*) dams and male blue catfish (*I. furcatus*) sires, channel × blue specific combining ability (SCA), and the error variance in adjusted survival rate, for tolerance to low oxygen. Dam GCA is σ^2 GCA Dam , sire GCA is σ^2 GCA Sire, SCA is σ^2 SCA Cross, and σ^2 Error is non-genetic variation.

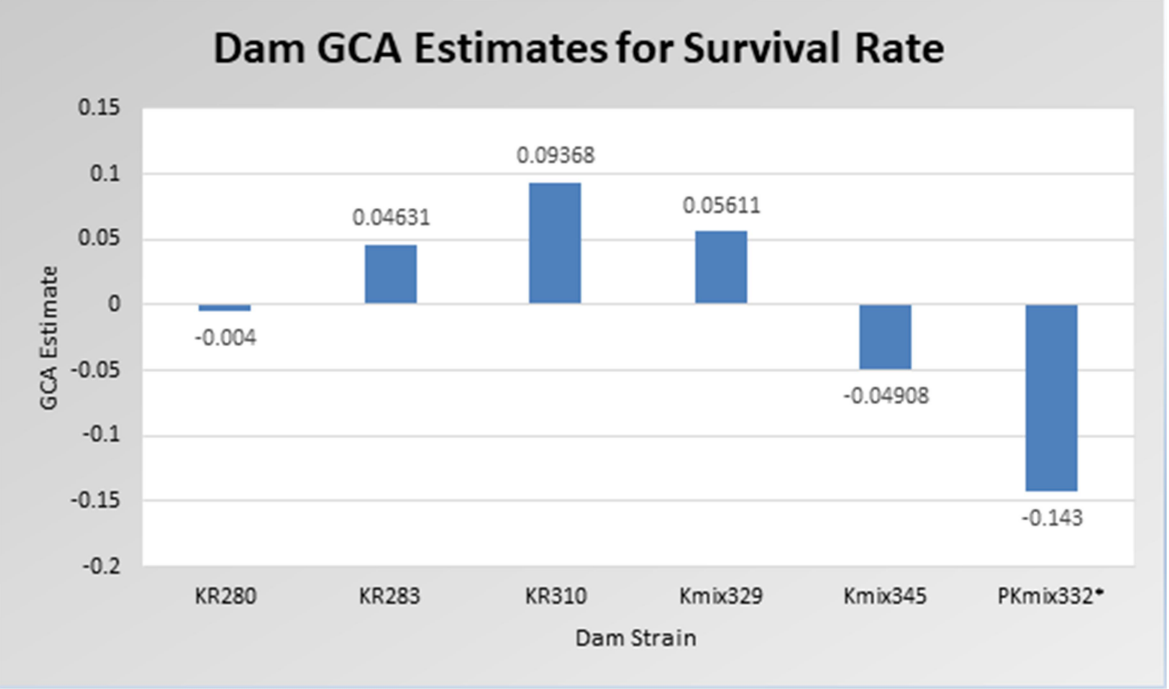

Figure 5: Comparison of the individual general combining ability (GCA) estimates for female channel catfish (*Ictalurus punctatus*) dams for survival rate during the low DO challenge. Negative values indicate dam produced progeny with lower survival rate during challenge. Positive values indicate dam produced progeny with higher survival rate during challenge. KR indicates Kansas Random strain, Kmix indicates Kansas Mix strain, PKmix indicates Probable Kansas Mix strain. GCA estimates with asterisk (*) are different among others ($p<0.05$, *t*-test).

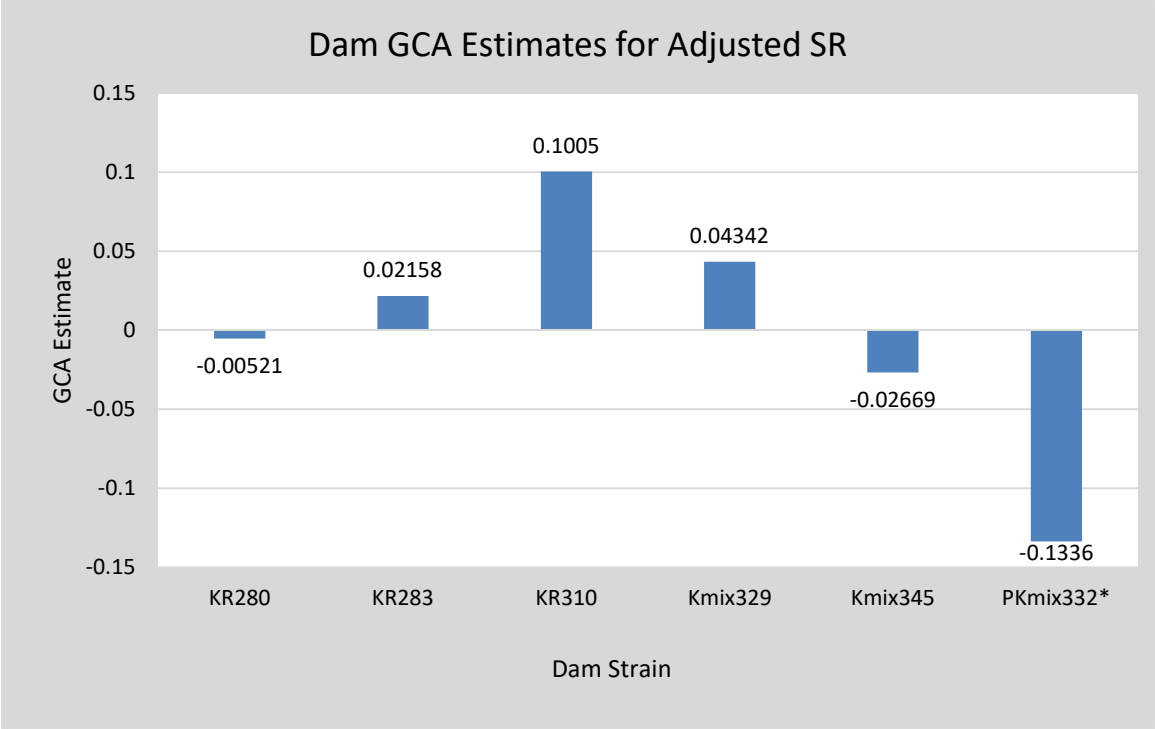

Figure 6: Comparison of the individual general combining ability (GCA) estimates for female channel catfish (*Ictalurus punctatus*) dams for adjusted survival rate during the low DO challenge. Negative values indicate dam produced progeny with lower survival rate during challenge. Positive values indicate dam produced progeny with higher survival rate during challenge. KR indicates Kansas Random strain, Kmix indicates Kansas Mix strain, PKmix indicates Probable Kansas Mix strain. GCA estimates with asterisk (*) are different among others ($p<0.05$, *t*-test).

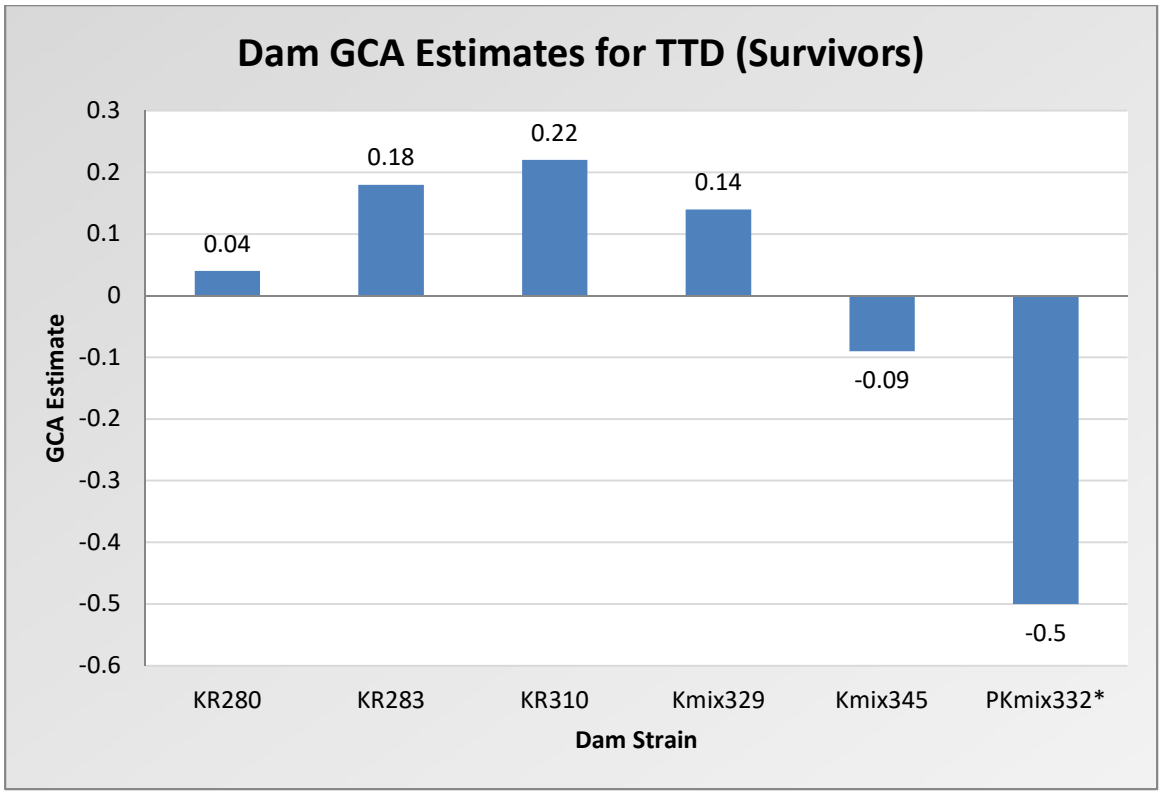

Figure 7: Comparison of the individual general combining ability (GCA) estimates for female channel catfish (*Ictalurus punctatus*) dams for time to death (TTD) during the low DO challenge when survivor data is included in the analysis. Negative values indicate dam produced progeny with lower survival rate during challenge. Positive values indicate dam produced progeny with higher survival rate during challenge. KR indicates Kansas Random strain, Kmix indicates Kansas Mix strain, PKmix indicates Probable Kansas Mix strain. GCA estimates with asterisk (*) are different from others ($p<0.05$, *t*-test).

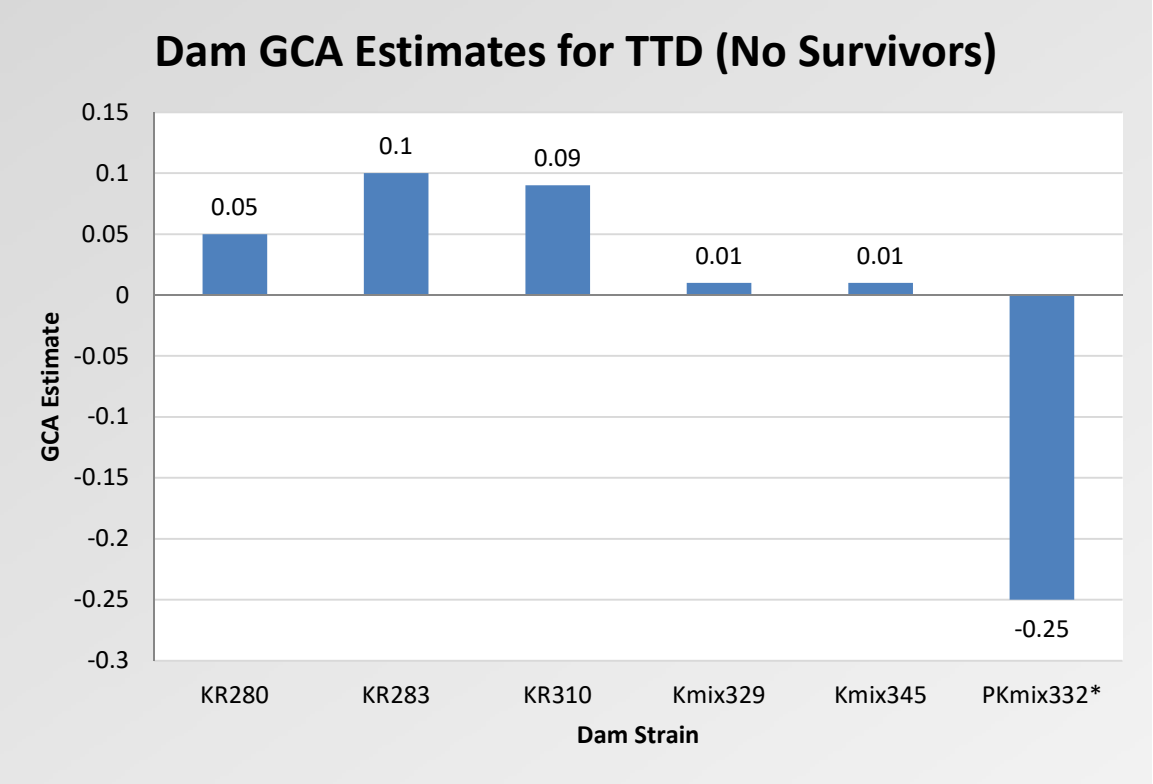

Figure 8: Comparison of the individual general combining ability (GCA) estimates for female channel catfish (*Ictalurus punctatus*) dams for time to death (TTD) when only data from fish that died is included in the analysis. Negative values indicate dam produced progeny with lower survival rate during challenge. Positive values indicate dam produced progeny with higher survival rate during challenge. KR indicates Kansas Random strain, Kmix indicates Kansas Mix strain, PKmix indicates Probable Kansas Mix strain. GCA estimates with asterisk (*) are d different from others ($p<0.05$, *t*-test).

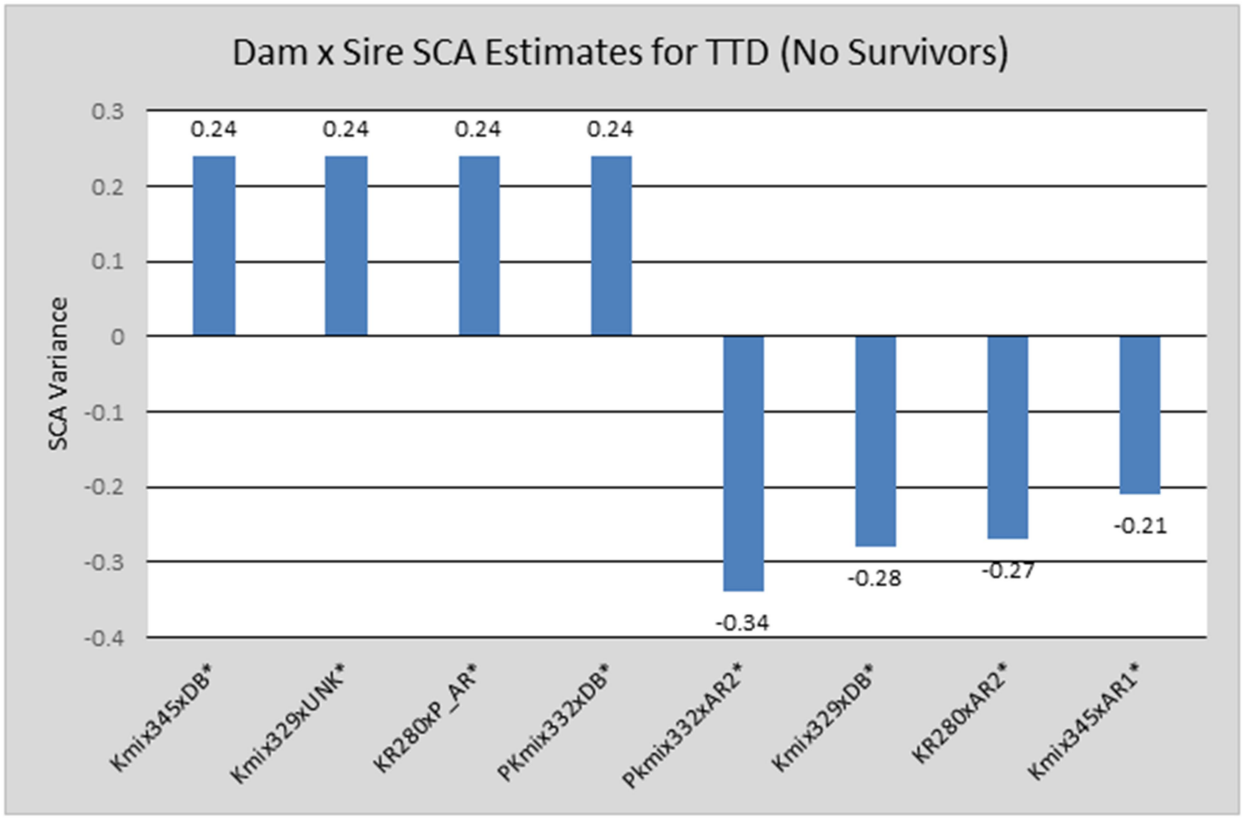

Figure 9: Comparison of specific combining ability (SCA) estimates for specific dam-sire crosses of female channel catfish (*Ictalurus puntatus*) dams × male blue catfish (*I. furactus*) sires during the low DO challenge that had observed differences with the SCA of other crosses. Among dams, KR indicates Kansas Random strain, Kmix indicates Kansas Mix strain, PKmix indicates Probable Kansas Mix strain. Among sires, AR indicates Auburn-Rio strain, DB indicates a strain of males from the DB Farm (Texas), and UNK indicates a sire of unknown strain. GCA estimates with asterisk (*) are different from others ($p<0.05$, *t*-test).

Effect of Body weight on Survival and Time to Death

Mean square analysis of variance indicated no effect (P >0.05, Appendix table 16) of body weight on time to death (TTD) with survivor data included. Mean square analysis of variance indicated no effect (P >0.05, Appendix table 16) for the effect of body weight on time to death without survivor data included. Body weight had an effect on survival rate (P=0.024, Table 2). Heavier fingerlings had a lower survival rate than smaller fingerlings (regression coefficient of -0.3755) The R^2 value for the relationship between body weight and survival rate was 0.1410 (Figure 9, Appendix table 16), and only a small portion of the measured survival rate was explained by body weight.

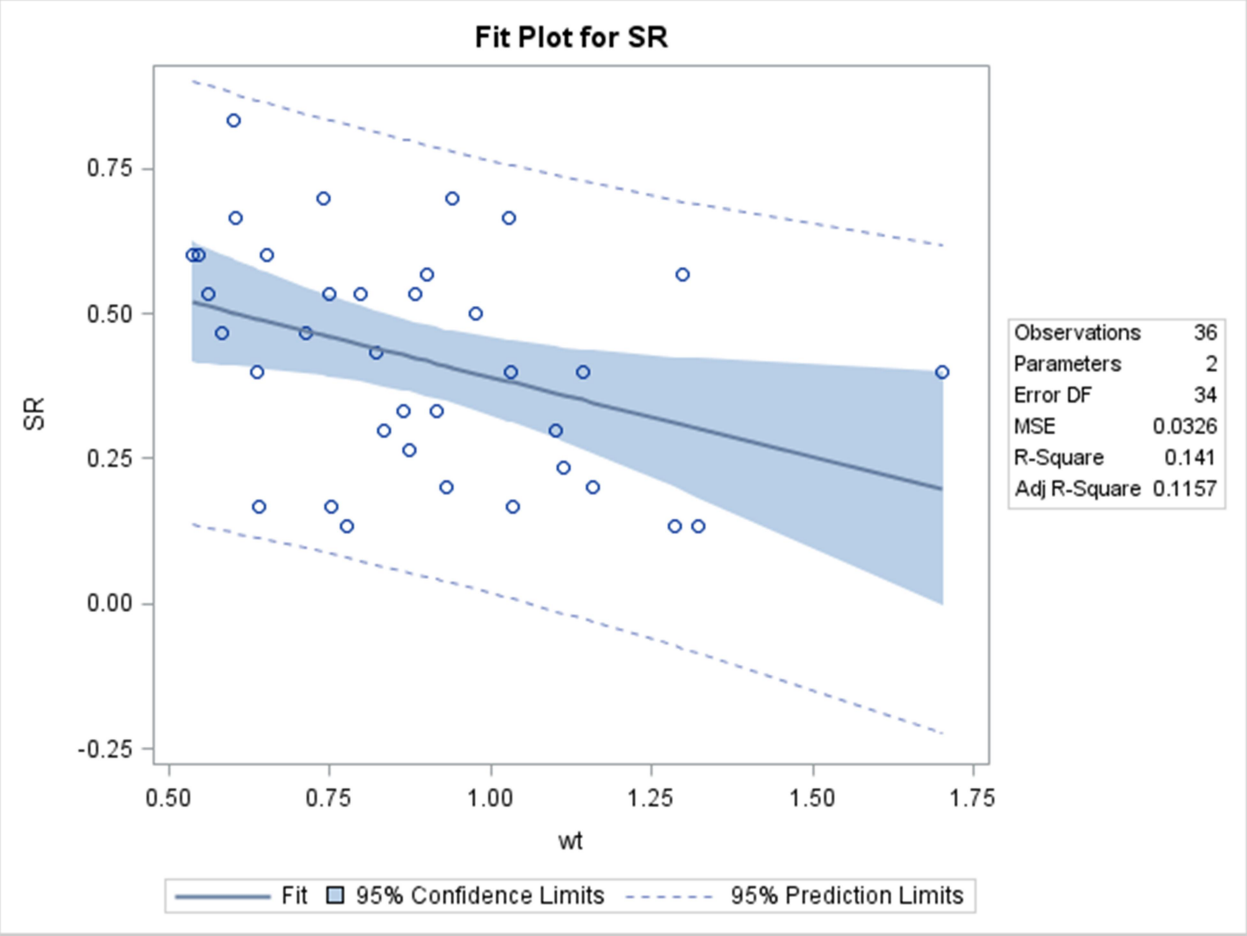

Figure 10: Linear regression of family weight and survival rate of channel catfish (*Ictalurus punctatus*) female × blue catfish (*I. furcatus*) male F1 hybrid progeny during low DO challenge.

Effect of Strain

The strain of dam, strain of sire, or individual dam-sire crosses did not affect the survival or time to death in the study (Appendix tables 11, 12, 13; Table 3). The strain of dam did not have an observed affect survival rate, adjusted survival rate, or time to death (with and without survivor data). It is notable that the least squares means of the Kansas Random strain were all greater than the Kansas Mix strain (Table 3). The sire strain did not have an observed affect the survival rate, adjusted survival rate, or time to death (with and without survivor data). It is notable that the least squares means of the Auburn-Rio strain were all smaller than for other sire strains (Table 4). The individual dam-sire strain crosses did not have an observed affect the survival rate, adjusted survival rate, or time to death (with and without survivor data). However, the Kmix-AR cross produced the smallest observed least squares means for adjusted survival rate and time to death (with and without survivor data), as well as the next to lowest least squares means value for survival rate (Table 5). There was no effect of body weight as the observed means for body weight were similar, 0.85 for Kansas Random hybrids and 0.89 for Kmix hybrids (Table 6). Strain analysis was done without dam "P_kmix332" and sire "P_AR" as the strains of these fish could not be confirmed.

Table 3: GLM procedure least squares means analysis with Tukey-Kramer adjustment for multiple comparisons for the difference between individual channel strains in the factorial mating design component of channel catfish (*Ictalurus punctatus*) female × blue catfish (*I. furcatus*) male F1 hybrid progeny. Test for the effect of individual channel strains on survival rate, adjusted survival rate, TTD (survivors included), and TTD (no survivors) during the low DO challenge.

Dam Strain	**SR LSMEAN**	**Adjusted SR LSMEAN**	**TTD LSMEAN**	**TTDns LSMEAN**
KR	0.5040	0.4990	5.3576	4.7263
Kmix	0.4450	0.4443	5.2612	4.5815

Table 4: GLM procedure least squares means analysis with Tukey-Kramer adjustment for multiple comparisons for the difference between individual sire strains in the factorial mating design component of channel catfish (*Ictalurus punctatus*) female × blue catfish (*I. furcatus*) male F1 hybrid progeny. Test for the effect of individual channel strains on survival rate, adjusted survival rate, TTD (survivors included), and TTD (no survivors) during the low DO challenge.

Sire Strain	**SR LSMEAN**	**Adjusted SR LSMEAN**	**TTD LSMEAN**	**TTDns LSMEAN**
AR	0.3444	0.3767	5.0145	4.4780
DB	0.5222	0.4932	5.3734	4.7441
DR	0.4389	0.4796	5.2662	4.6421
Unk	0.4238	0.4020	5.2041	4.5077

Table 5: GLM procedure least squares means analysis with Tukey-Kramer adjustment for multiple comparisons for the difference between individual sire strains in the factorial mating design component of channel catfish (*Ictalurus punctatus*) female × blue catfish (*I. furcatus*) male F1 hybrid progeny test for the effect of individual channel-blue crosses on survival rate, adjusted survival rate, TTD (survivors included), and TTD (no survivors) during the low DO challenge.

Dam Strain	Sire Strain	SR LSMEAN	Adjusted SR LSMEAN	TTD LSMEAN	TTDns LSMEAN
KR	**AR**	0.4056	0.3947	5.2827	4.6195
KR	**DB**	0.6000	0.5901	5.4337	4.8748
KR	**DR**	0.6111	0.6495	5.4590	4.7108
KR	**Unk**	0.4143	0.4442	5.2780	4.5216
Kmix	**AR**	0.3417	0.3877	4.9255	4.4805
Kmix	**DB**	0.4333	0.3903	5.3292	4.6344
Kmix	**DR**	0.3167	0.4243	5.3248	4.7248
Kmix	**Unk**	0.5667	0.4794	5.4741	4.7373

Table 6: Mean weight (g) of dead fish and mean weight of alive fish from families of channel catfish (*Ictalurus punctatus*) female × blue catfish (*I. furcatus*) male F1 hybrid progeny during the low DO challenge.

Family	dead mean weight	alive mean weight
Kmix329xAR1	1.0550	1.1833
Kmix329xAR2	0.8537	0.7923
Kmix329xDB	0.9573	0.6150
Kmix329xDR	1.0480	0.9733
Kmix329xP_AR	1.0000	0.5520
Kmix329xUnk	0.8254	0.6875
Kmix345xAR1	0.9738	0.7667
Kmix345xAR2	0.8889	0.9600
Kmix345xDB	0.8050	0.7875
Kmix345xDR	1.3667	1.2250
Kmix345xP_AR	1.0520	0.9000
Kmix345xUnk	0.5400	0.5333
KR280xAR1	1.0600	0.9800
KR280xAR2	0.8295	0.8444
KR280xDB	0.6733	0.6111
KR280xDR	1.0360	1.3706
KR280xP_AR	0.9392	0.7625
KR280xUnk	0.5762	0.5222
KR283xAR1	0.6327	0.5286
KR283xAR2	0.6363	0.6636
KR283xDB	0.9214	0.8500
KR283xDR	0.9817	0.8412
KR283xP_AR	0.6750	0.4750
KR283xUnk	-	1.7000
KR310xAR1	0.7867	1.0917
KR310xAR2	0.9380	0.9571
KR310xDB	1.0922	1.0000
KR310xDR	0.8060	0.7095
KR310xP_AR	0.7027	0.5550
KR310xUnk	1.2375	0.9714
PKmix332xAR1	1.3609	1.0750
PKmix332xAR2	1.1356	1.2500
PKmix332xDB	0.7850	0.6214

PKmix332xDR	0.6579	0.5600
PKmix332xP_AR	0.7185	1.1500
PKmix332xUnk	0.7608	0.7000

Discussion

To obtain the most efficient growth and best disease resistance proper dissolved oxygen levels must be maintained from fertilization through harvest. Modern intensive catfish production utilizes mechanical aeration in culture units with electric powered paddlewheels and blowers to ensure dissolved oxygen levels do not reach levels harmful to the fish. Mechanical failures and power outages have the potential to disrupt the added aeration and cause huge losses to cultured fish. Selecting genetic types of fish that have better survival and or longer time to death under acute low dissolved oxygen may give fish culturists a longer window to correct issues and restore adequate oxygen levels. This, in turn, will lead to less money lost by the farmer to crop loss and more fish available for harvest.

The dam GCA accounted for 9.5% of the variance for survival when the hybrid progeny were exposed to 0.6 ppm, whereas the sire GCA and SCA were zero. Thus, selecting for channel catfish females with increased tolerance of low dissolved oxygen should improve, and would be predicted to be the best mechanism to enhance, the genetic ability of the hybrid progeny to tolerate low dissolved oxygen. Similar combining ability results were seen in survival of channel-blue hybrid fingerlings averaging 58g when challenged with hypoxic conditions (Drescher, 2017). Dam GCA was 25%. Additive genetic variation from channel catfish appears to have increased as hybrid fingerlings grew. An alternative explanation could be that more strains of channel catfish females were utilized in the Drescher (2017) study leading to increased additive genetic variation.

Interpretation of the time to death was more complicated. If the surviving fish are included in the analysis, dam GCA accounts for 25% of the variation, sire GCA only 3% and

SCA, 0%. Again, the best genetic enhancement approach will be to select of channel catfish lines resistant to low oxygen. However, if the survivors are not considered in the analysis, the genetics of hybrid tolerance of low oxygen changes. Dam GCA reduces to 8%, sire GCA reduces to 0% and SCA becomes the most important genetic factor, increasing to 17% of the total variance. How the trait is defined and analyzed has large impact.

Variation for increased time to death (when only including dead fish in analysis) in channel-blue hybrid catfish fingerlings under hypoxic conditions is comprised of both additive genetic effects from the dam and dominance effects from the specific dam-sire cross, with dominance effects having twice the influence. The objective of this trait is that the fish to live long enough that the culturist can discover and correct the problem before the fish die. Therefore both methods of examining this trait are important. The best approach might be to conduct a two-tiered program, selecting for lines or pairs of channel catfish and blue catfish that combine well to utilize the positive dominance effects, while also selecting for channel catfish with low dissolved oxygen tolerance. This would cause all genetic gain to be made from both the positive dominance effects and the positive additive effects.

Age, size, and genetic background affect the combining ability for time to death. Dam GCA and SCA had the most influence in the current study. Sire GCA, at 15% of total variation, was the most important genetic factor for the larger hybrids (Drescher 2017) followed by dam GCA. The differences in the results of these studies suggest that when hybrid catfish are younger time to death performance under acute low oxygen is most controlled by additive genetic effects from the dam and dominance effects from the specific cross but as the fish ages additive genetic effects from the sire become more important.

Strain did not affect low oxygen tolerance in hybrid catfish. However, hybrid offspring from Kansas Random dams had better mean performance for survival rate, time to death (with survivors), and time to death (no survivors) than offspring from Kansas Mix dams. There were no strain effects for the blue catfish sires but hybrid offspring from DB sires had better mean performance for survival rate, time to death (with survivors), and time to death (no survivors) than offspring from other sires. Strain also did not have effect on low oxygen tolerance but the KRxDR offspring had better mean performance for survival rate, and time to death (with survivors) than other crosses.

Drescher (2017) also found the use of high performing dam strains will result in hybrids with increased survival under acute low oxygen, in this case as large fingerlings. The Kansas Random channel strain had better survival in older hybrids (along with Kansas Select and Rio Grande strains which were not used in this study) than other dam strains (Drescher 2017). Similarly, Kansas Random channel catfish strain also showed improved performance in the current study. Thus, when desiring to produce a hybrid fish with genetically increased survival traits under acute low oxygen, hybrid progeny from Kansas Random dams will have better survival as both fingerlings and fingerlings compared to other genetic types of hybrids.

Time to death (no survivors) was influenced by a combination of dam GCA and SCA of specific crosses, with SCA having more influence than dam GCA. The results suggest the presence of strain effects for this trait. Hybrid offspring from the Kansas Random strain had longer mean time to death than offspring from the Kansas Mix channel catfish line. However, dams used in crosses with the longest time to death (Kmix345, Pkmix332, KR280, Kmix329) were also found in crosses with the shortest time to death. Although overall strain performance

for time to death for offspring of Kansas Random dams was better than Kansas Mix dams, there was considerable variation among individual females.

In this study, strains of Sire were not different from each other with regard to time to death, but when crossed with different dams, performance differences for specific crosses were seen. For older hybrid offspring, those from DR strain of blue catfish had the longest mean time to death for 58g fingerlings (Drescher 2017). While sire strains were not observed different from each other for mean time to death in the current study, all pairings involving DR males had a positive SCA, while other males had both positive and negative SCAs. DR sires produced hybrid offspring with good low oxygen survival traits as both fingerlings and fingerlings.

Body weight had a observed, but small negative impact, r = -0.38 on survival when the hybrid progeny were exposed to low dissolved oxygen. However, this caused no change for the GCA and SCA estimates, thus correcting for body weight did not alter the conclusions regarding the genetics of oxygen tolerance of hybrid catfish. Drescher (2017) also found a negative correlation between body weight and survival under acute low oxygen conditions for hybrid progeny when they were ~ 58g. The r value was much smaller, -0.10, despite the family means being more variable than in the current study. However, the relationship between low oxygen tolerance and survival is not consistent across *Ictalurids.* Wang et al. (2017), found that channel catfish averaging 180g had a positive correlation between body weight and survival under low oxygen conditions.

Effects of temperature as it relates to genotype-environment interactions related to low oxygen tolerance traits was not examined in the current study. Strains of channel catfish and blue catfish of southern origin may be more tolerant to low oxygen conditions than those from

northern origins. Strains of southern channel catfish, blue catfish, and their hybrid crosses had greater thermal tolerance than did strains of northern channel catfish, blue catfish, and their hybrid crosses (Stewart and Allen, 2014). Warm water holds less dissolved oxygen than cooler water and thus other southern strains and genetic types may be naturally selected for increased survival and time to death under acute low oxygen in comparison to hybrids produced from northern strains of channel catfish and blue catfish. Results of Dunham et al. (2014) support this hypothesisas hybrid catfish from Rio Grande blue catfish sires, the southernmost extent of the geographic range of blue catfish had better tolerance to lower dissolved oxygen than hybrid offspring of other sires at varying temperatures.

As there were differences in our results and those of Drescher (2017), future studies should also be conducted on market size hybrids, using the same strains as examined in these first two studies, to provide a complete analysis of combining ability for performance in acute low oxygen over commercially relevant life stages hybrid catfish. Role of temperature and genotype-environment interactions related to temperature should be evaluated. In addition, studies conducted in commercial size ponds should be conducted to examine genotype-environment interactions for low oxygen tolerance for field verification. Lastly, recurrent and reciprocal recurrent selection programs should be conducted for these low oxygen survival traits to confirm the predictions from the combining ability analysis.

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Appendix

Table 7: Type 3 Mixed Procedure analysis of variance of the factorial mating design component of channel catfish (*Ictalurus punctatus*) female × blue catfish (*I. furcatus*) male F1 hybrid progeny test and error means squares for the effect of body weight on survival rate during the low DO challenge. P=0.05.

Analysis of Variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	1	0.18205	0.18205	5.58	0.0240
Error	34	1.10940	0.03263		
Corrected Total	35	1.29145			

Table 8: Mean squares (MS) for the Type 3 Mixed Procedure analysis of variance of the factorial mating design component of channel catfish (*Ictalurus punctatus*) female × blue catfish (*Ictalurus furcatus*) male F1 hybrid progeny test and error means squares average for survival rate (SR) per family and average time to death (TTD) per family for tolerance to low dissolved oxygen of channel catfish female × blue catfish male F1 progeny.

Low DO Tolerace indi**ces**		Source of variation			
		Dam	Sire	Dam × Sire	Error
Survival rate (%)	d.f.	5	5	25	70
	MS	0.28 *	0.10 ns	0.08 ns	0.10
TTD including survivors	d.f.	5	5	25	70
	MS	1.83 *	0.38 ns	0.23 ns	0.27
TTD no survivors	d.f.	5	5	24	428
	MS	2.89 *	0.53 *	0.65 *	0.18

After ANOVA *F*-test: * = p<0.05n and ns = p>0.05

d.f. = degrees of freedom

Table 9: Covariance parameter estimates for general and specific combining ability (GCA/SCA) estimates for average adjusted survival rate (SR) per family for tolerance to low dissolved oxygen of channel catfish (*Ictalurus punctatus*) female × blue catfish (*Ictalurus furcatus*) male F1 hybrid progeny. Combining ability estimated from covariance estimate.

Covariance Parameter Estimates					
Cov Parm	**Ratio**	**Estimate**	**Standard Error**	**Z Value**	**Pr > Z**
dam	0.1054	0.009548	0.009255	1.03	0.1511
sire	0	0	.	.	.
cross	0	0	.	.	.
Residual	1.0000	0.09060	0.01281	7.07	<.0001

Table 10: Type 3 Mixed Procedure analysis of variance of the factorial mating design component of channel catfish (*Ictalurus punctatus*) female × blue catfish (*Ictalurus furcatus*) male F1 hybrid progeny test and error means squares for the effect of body weight on TTD (with survivor data). P=0.05.

Analysis of Variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	1	0.01263	0.01263	0.07	0.7990
Error	34	6.51963	0.19175		
Corrected Total	35	6.53225			

Table 11: Type 3 Mixed Procedure analysis of variance of the factorial mating design component of channel catfish (*Ictalurus punctatus*) female × blue catfish (*Ictalurus furcatus*) male F1 hybrid progeny test and error means squares for the effect of body weight on time to death (without survivor data). P= 0.05.

Analysis of Variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	1	0.02263	0.02263	0.29	0.5964
Error	34	2.69173	0.07917		
Corrected Total	35	2.71436			

Table 12: Fit statistics for model 1 linear regression of family weight and survival rate channel catfish (*Ictalurus punctatus*) female × blue catfish (*Ictalurus furcatus*) male F1 hybrid progeny during the low DO challenge.

Root MSE	0.18064	R-Square	0.1410
Dependent Mean	0.42130	Adj R-Sq	0.1157
Coeff Var	42.87634		

Table 13: Type 3 Mixed Procedure analysis of variance of the factorial mating design component of channel catfish (*Ictalurus punctatus*) female × blue catfish (*Ictalurus furcatus*) male F1 hybrid progeny test and error means squares for the effect of dam strain on survival rate, adjusted survival rate and time to death (without survivor data) during the low DO challenge. P= 0.05.

Dam Strain Source	DF	Type III SS	Mean Square	F Value	Pr > F
Survival Rate	1	0.0678226	0.0678226	0.63	0.431
Adjusted SR	1	0.0583326	0.0583326	0.57	0.452
TTD	1	0.1811294	0.1811294	0.7	0.406
TTDns	1	0.3278362	0.3278362	2.87	0.095

Table 14: Type 3 Mixed Procedure analysis of variance of the factorial mating design component of channel catfish (*Ictalurus punctatus*) female × blue catfish (*Ictalurus furcatus*) male F1 hybrid progeny test and error means squares for differences in performance of sire strains on survival rate, adjusted survival rate and time to death (without survivor data) during the low DO challenge. P= 0.05.

Sire Strain Source	DF	Type III SS	Mean Square	F Value	Pr > F
Survival Rate	3	0.39763	0.13254	1.41	0.2456
Adjusted SR	3	0.22941	0.07647	0.81	0.4899
TTD	3	1.79844	0.59948	1.99	0.1227
TTDns	3	0.82196	0.27399	2.42	0.0735

Table 15: Type 3 Mixed Procedure analysis of variance of the factorial mating design component of channel catfish (*Ictalurus punctatus*) female × blue catfish (*Ictalurus furcatus*) male F1 hybrid progeny test and error means squares for the effect of individual dam-sire strain crosses on survival rate, adjusted survival rate and time to death (without survivor data) during the low DO challenge. P= 0.05.

Dam-Sire Cross Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Survival Rate	3	0.36625	0.12208	1.19	0.3207
Adjusted SR	3	0.20862	0.06954	0.68	0.5651
TTD	3	0.70343	0.23448	0.92	0.4358
TTDns	3	0.34325	0.11442	1.09	0.3611

Table 16: the population means and phenotypic standard deviations (SD) for average survival rate (SR) per family and average time to death (TTD) per family for tolerance to low dissolved oxygen of channel catfish (*Ictalurus punctatus*) female × blue catfish (*Ictalurus furcatus*) male F1 hybrid progeny.

Low DO resistance indices	Population mean	Phenotypic SD
Survival rate (%)	57.83	32.33
Time to death (hr) including survivors	5.17	0.58
Time to death (hr) all dead	4.45	0.49

Table 17: General and specific combining ability (GCA/SCA) estimates for average survival rate (SR) per family and average time to death (TTD) per family for tolerance to low dissolved oxygen of channel catfish (*Ictalurus punctatus*) female × blue catfish (*Ictalurus furcatus*) male F1 hybrid progeny.

	Parents	GCA/SCA estimates		
		Survival rate	TTD including survivors	TTD no survivors
Dam	KR280	-0.004	0.04	0.05
	KR283	0.05	0.18	0.10
	KR310	0.09	0.22	0.09
	Kmix329	0.06	0.14	0.01
	Kmix345	-0.05	-0.09	0.01
	PKmix332	-0.14*	-0.50*	-0.25*
Sire	AR1	-0.01	-0.05	-0.001
	AR2	-0.003	-0.04	-0.001
	DB	0.01	0.06	0.001
	DR	0.001	0.03	0.001
	P_AR	0.003	-0.01	-0.0002
	Unk	0.001	0.01	0.00007
Family (cross)	KR280xAR1	-	-	0.01
	KR280xAR2	-	-	-0.27*
	KR280xDB	-	-	0.09
	KR280xDR	-	-	0.07
	KR280xP_AR	-	-	0.24*
	KR280xUnk	-	-	-0.06
	KR283xAR1	-	-	0.09

KR283xAR2	-	-	-0.01
KR283xDB	-	-	0.04
KR283xDR	-	-	0.04
KR283xP_AR	-	-	-0.003
KR310xAR1	-	-	-0.03
KR310xAR2	-	-	0.18
KR310xDB	-	-	0.09
KR310xDR	-	-	0.06
KR310xP_AR	-	-	0.02
KR310xUnk	-	-	-0.15
Kmix329xAR1	-	-	-0.09
Kmix329xAR2	-	-	0.14
Kmix329xDB	-	-	-0.28*
Kmix329xDR	-	-	0.14
Kmix329xP_AR	-	-	-0.13
Kmix329xUnk	-	-	0.24*
Kmix345xAR1	-	-	-0.21*
Kmix345xAR2	-	-	-0.10
Kmix345xDB	-	-	0.24
Kmix345xDR	-	-	0.14
Kmix345xP_AR	-	-	-0.16
Kmix345xUnk	-	-	0.10
PKmix332xAR1	-	-	-0.19
PKmix332xAR2	-	-	-0.34*

PKmix332xDB	-	-	0.24*
PKmix332xDR	-	-	0.04
PKmix332xP_AR	-	-	-0.07
PKmix332xUnk	-	-	-0.09

GCA/SCA estimates with asterisk (*) are different from others (p<0.05, *t*-test).

Table 18: Type 3 Mixed Procedure analysis of variance of the factorial mating design component of channel catfish (*Ictalurus punctatus*) female × blue catfish (*Ictalurus furcatus*) male F1 hybrid progeny test and error means squares for the effect of dam strain on survival rate, adjusted survival rate and time to death (without survivor data) during the low DO challenge. P= 0.05.

Dam Strain Source	DF	Type III SS	Mean Square	F Value	Pr > F
Survival Rate	1	0.0678226	0.0678226	0.63	0.431
Adjusted SR	1	0.0583326	0.0583326	0.57	0.452
TTD	1	0.1811294	0.1811294	0.7	0.406
TTDns	1	0.3278362	0.3278362	2.87	0.095

Table 19: Type 3 Mixed Procedure analysis of variance of the factorial mating design component of channel catfish (*Ictalurus punctatus*) female × blue catfish (*Ictalurus furcatus*) male F1 hybrid progeny test and error means squares for differences in performance of sire strains on survival rate, adjusted survival rate and time to death (without survivor data) during the low DO challenge. P= 0.05.

Sire Strain Source	DF	Type III SS	Mean Square	F Value	Pr > F
Survival Rate	3	0.39763	0.13254	1.41	0.2456
Adjusted SR	3	0.22941	0.07647	0.81	0.4899
TTD	3	1.79844	0.59948	1.99	0.1227
TTDns	3	0.82196	0.27399	2.42	0.0735

Table 20: Type 3 Mixed Procedure analysis of variance of the factorial mating design component of channel catfish (*Ictalurus punctatus*) female × blue catfish (*Ictalurus furcatus*) male F1 hybrid progeny test and error means squares for the effect of individual dam-sire strain crosses on survival rate, adjusted survival rate and time to death (without survivor data) during the low DO challenge. P= 0.05.

Dam-Sire Cross Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Survival Rate	3	0.36625	0.12208	1.19	0.3207
Adjusted SR	3	0.20862	0.06954	0.68	0.5651
TTD	3	0.70343	0.23448	0.92	0.4358
TTDns	3	0.34325	0.11442	1.09	0.3611

Figures

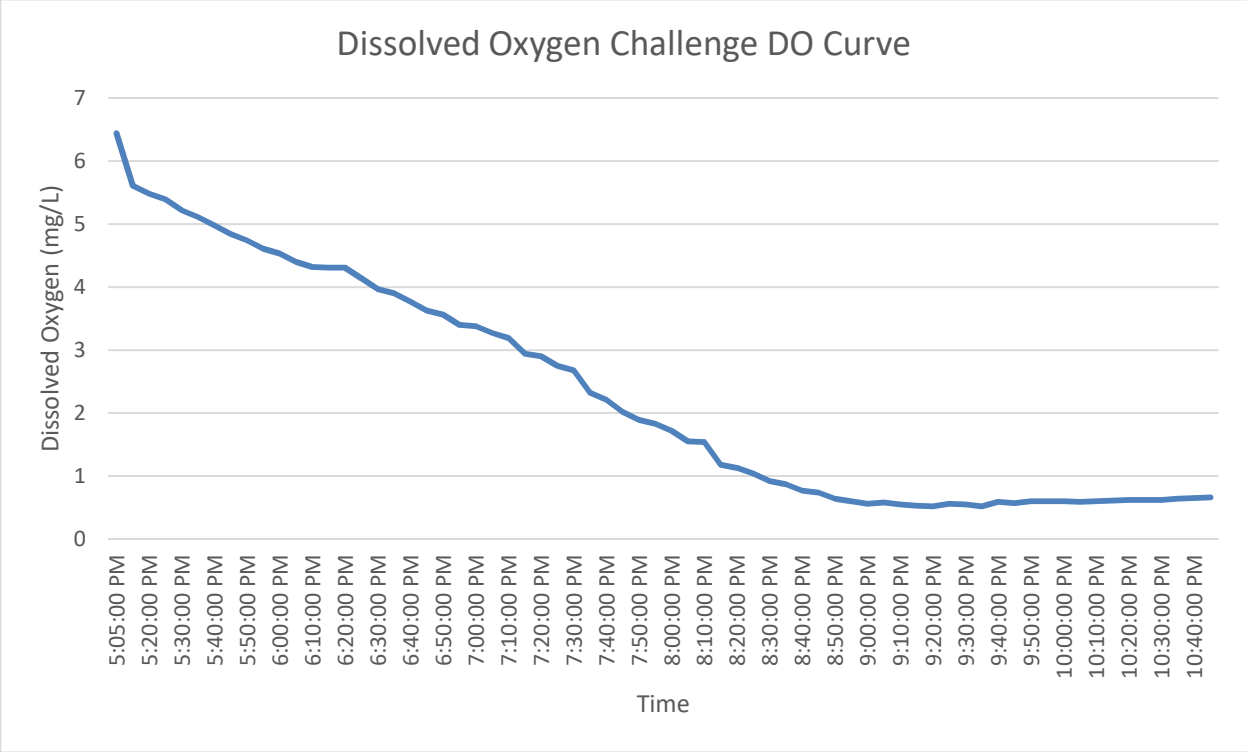
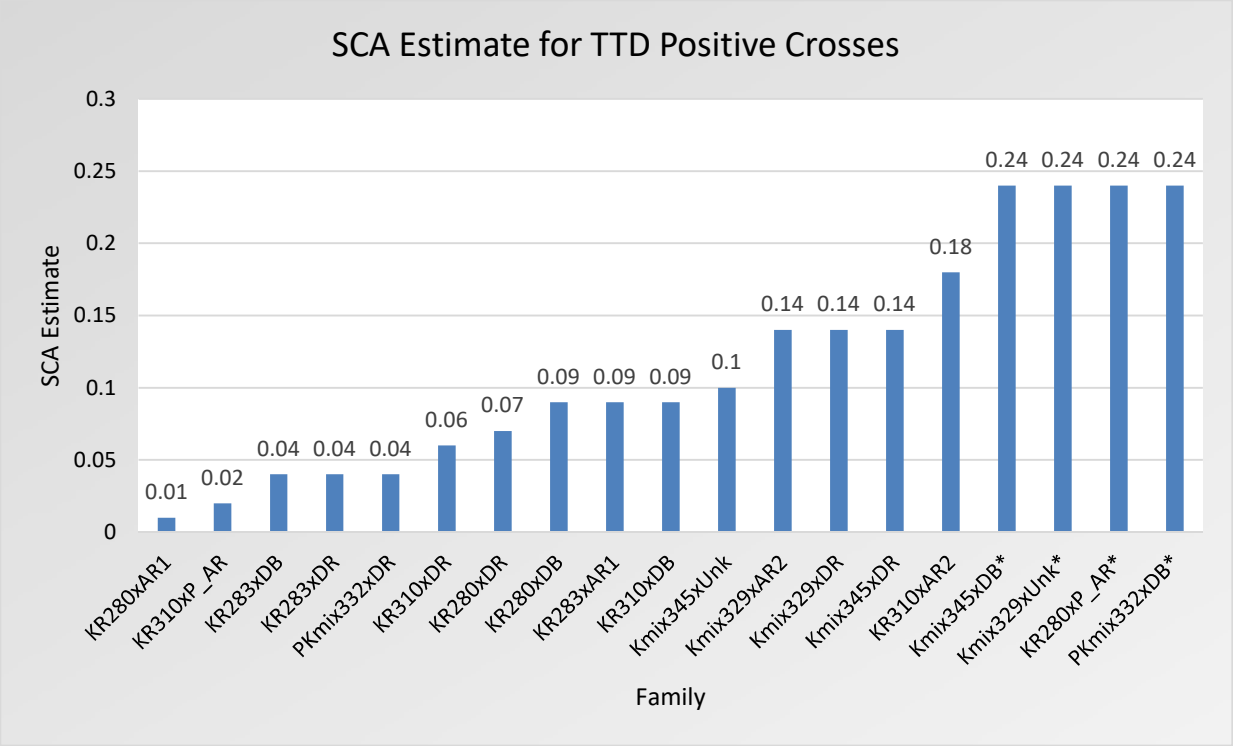
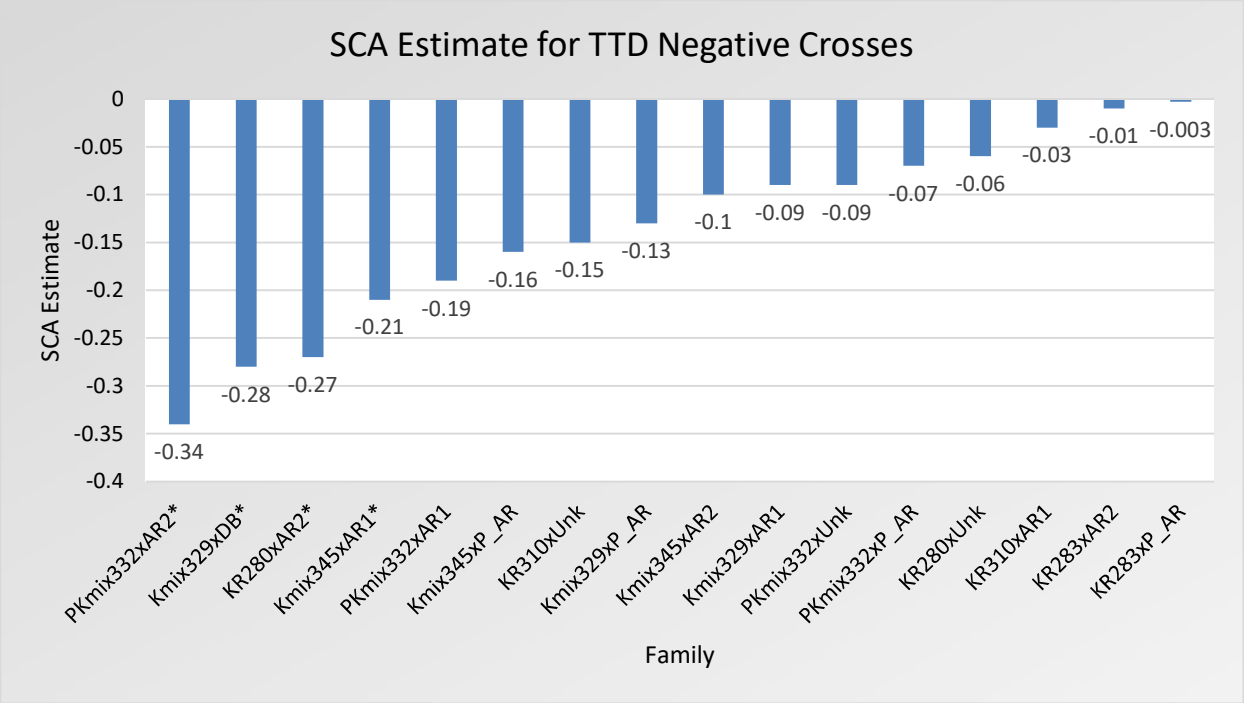


Figure 11: Measured levels of dissolved oxygen (mg/L) in ten minute increments during the low dissolved oxygen challenge of channel catfish (*Ictalurus punctatus*) female × blue catfish (*Ictalurus furcatus*) male F1 hybrid progeny.



SCA estimates with asterisk (*) are observed different among others ($p<0.05$, *t*-test).

Figure 12: Comparison of specific combining ability (SCA) estimates for all crosses of female channel catfish (*Ictalurus puntatus*) dams × male blue catfish (*Ictalurus furctus*) sires with negative estimated specific combining ability during the low DO challenge. Among dams, KR indicates Kansas Random strain, Kmix indicates Kansas Mix strain, PKmix indicates Probable Kansas Mix strain. Among sires, AR indicates Auburn-Rio Grande strain, DB indicates a strain of males from the DB Farm (Texas), DR indicates a strain of fish with DB females × Rio Grande males, P_AR indicates a probable Auburn-Rio strain, and UNK indicates a sire of unknown strain.



SCA estimates with asterisk (*) are observed different among others ($p<0.05$, *t*-test).

Figure 13: Comparison of specific combining ability (SCA) estimates for female channel catfish (*Ictalurus puntatus*) dams × male blue catfish (*Ictalurus furctus*) sires that were deemed observed observed in comparison with other crosses during the low DO challenge. Among dams, KR indicates Kansas Random strain, Kmix indicates Kansas Mix strain, PKmix indicates Probable Kansas Mix strain. Among sires, AR indicates Auburn-Rio strain, DB indicates a strain of males from the DB Farm (Texas), and UNK indicates a sire of unknown strain.