EFFECT OF GENETICS ON BODY WEIGHT VARIABILITY IN CHANNEL CATFISH (*Ictalurus punctatus*) FEMALE X BLUE CATFISH (*I. furcatus*) HYBIRDS

By

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Abstract

Hybrid catfish have become common in the catfish production industry in the United States accounting for 50-70% of all catfish produced. Variable body weight of hybrid catfish can create production and marketing problems and economic loss for farmers due to undersized fingerlings and food fish and oversized food fish. Genetic effects on body weight variability in channel catfish (*Ictalurus punctatus*) female x blue catfish (*I. furcatus*) male hybrids were examined. Coefficient of variation (CV) for body weight of hybrid catfish and channel catfish fingerlings were similar and the environment had strong and equal effects on the variability of each genetic type. When evaluating hybrids produced from different strains of channel catfish and blue catfish, sire and dam affected variability both in terms of CV for body weight and population distribution. Contradictorily, crossbred channel catfish dams and crossbred blue catfish sires increased the CV for body weight, but resulted in hybrid progeny that were not extreme distances from the population mean in regards to the standard deviations. This could have major impact on the variability problem. Various families of hybrid catfish were grown in aquaria and then transferred to a split-pond. The correlation of the family CVs between hybrids grown in aquaria and a split-pond was 0.05, indicating genotype-environment or genotype-age interactions. When the families were pooled by sire genetic type or dam genetic type, the interactions decreased as the correlations were 0.51 and 0.39, respectively.

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Introduction

The first commercial catfish farming began in Kansas in the early 1930s (Dunham and Smitherman 1984). Fish farming gained momentum in the southern United States after World War II. The pioneers of fish farming began raising baitfish to meet the demands of forage for the sportfish that were being stocked into the lakes and ponds (Hargreaves and Tucker 2004). Later these farmers began to also culture sport fish for stocking to meet demands of anglers. The next logical step for the industry was the shift to farming food fish on a commercial scale. After the initial effort in Kansas, food fish culture began in Arkansas with farmers growing buffalofish, which was a regionally popular food fish. The market did not support this effort and soon farmers started polyculture of buffalofish with channel catfish (Hargreaves and Tucker 2004). By 1963, channel catfish production had expanded to become the main food fish produced with farms in operation in Arkansas, Mississippi and Alabama (Dunham and Smitherman 1984). In the early 1970's the commercial production of channel catfish expanded rapidly in Mississippi making them the leader in production after this point (Hargreaves and Tucker 2004).

When the catfish industry first started and throughout its early years, it consisted of independent farmers selling live fish for stocking or selling directly to small local markets (Hargreaves and Tucker 2004). This was done on a seasonal basis because the farmers had fish ready for harvest after the growing season in late fall. In 1967 the vertical integration of the industry finally allowed it to move past this point when eleven catfish producers in Mississippi came together to form a partnership and constructed a small processing plant (Hargreaves and Tucker 2004). Simultaneously, catfish processing also began in Alabama (Perez 2006). Farmers desired to sell their fish at the end of the summer when the fish were large enough, but to have a processing plant that is effective and sustainable a year round supply of fish is necessary. To meet the year round need of the processing plant, the farmers implemented multi-batch cropping system. The industry advanced again in 1971 when a group of catfish farmers built a feed mill in Isola, Mississippi. This allowed farmers to vastly improve fish production efficiency in ponds with feed produced specifically for catfish farmers.

The economic down turn in the early 1970's led to consolidation in the industry. Smaller less efficient producers failed and went out of business (Hargreaves and Tucker 2004). During this time the number of farms in Mississippi decreased from 563 to 199, but during this same period the average farm size grew from 18 to 35 hectares. Farmers became successful as they implemented the economics of scale in the industry by increasing their total pond area. This trend of increasing farm size while decreasing in total farms continued into the 1980's and by 1983 the average farm size was 70 ha (Hargreaves and Tucker 2004). Entering the 1980's the industry had become vertically integrated and farmers began to specialize in fingerling production or foodfish production, and some companies included processing.

Following the vertical integration of the industry the next step was the intensification. This occurred between 1980 and the early 2000's when the total pond area in the catfish industry doubled while the quantity of fish processed in the same time period increased by more than six times (Hargreaves and Tucker 2004). This was accomplished by increasing stocking densities and feeding rates. Before this intensification, the normal stocking rates were between 6,000 to 10,000 fish per hectare. The low stocking rates were used to avoid episodes of low dissolved oxygen levels in the ponds. When the farmers attempted to increase yield by increasing stocking densities and feeding rates, low dissolved oxygen levels became a chronic problem. Paddle wheel aeration was proven to be the most efficient form of aeration (Boyd and Tucker 1979) and the improvement of paddlewheel aerator design (Boyd and Ahmad 1987, Ahmad and Boyd 1988) had the greatest impact on the catfish industry prior to the end of the millennium the wide spread adoption of these paddlewheels allowed farmers to increase their yield (Boyd and Tucker 1979).

The processing plants are an integral part of the catfish industry as otherwise farmers would have to sell small amounts of live fish to local markets. The biggest challenge for the processing plants is to be constantly vigilant to prevent off-flavored catfish from entering the market (Marshall 2004). A second issue that the processors face is variability in the fish coming from a single pond. Variability is needed to keep all mechanical processing lines in operation and to meet market demands for different sized fish. However, when fish are larger or smaller than what the machine settings can handle, they must be filleted by hand, which is expensive or the fish are not marketable. Thus, farmers are paid a smaller amount for fish that fit into these categories (Marshall 2004).

The catfish industry is at an important turning point in its history. This industry has an important economic role in multiple states such as Alabama, Arkansas, Mississippi, and Texas. Although it still accounts for the majority of aquaculture production in the United States, catfish production has decreased from 347 million food size fish in 2003 to 104 million food size fish in 2013 (NASS 2003, NASS 2017). This can be attributed to multiple factors such as the recession, high fuel costs, high feed costs, and competition from imports. One of the imports that could be causing the greatest impact is the Pangasianodon catfish from Vietnam. These fish are suited for intensive aquaculture practices. They are capable of withstanding extremely low dissolved oxygen levels, which allows a much higher stocking density than the species of catfish grown in North America. Pangasianodon imports of fillets have increased from 17.4 million kilograms in 2007 to 131.2 million kilograms in 2016 (NOAA 2015). The advantages of the Pangasianodon catfish allow farmers in Asia to produce a crop much easier and cheaper than farmers in America can, which in turn allows the farmers in Asia to sell their crop cheaper. This floods the market with catfish at a price that American farmers cannot compete against. This has resulted in many of the U.S. catfish farmers being forced to leave the industry.

One key to the recovery and survival of the catfish industry is the wide spread implementation of the hybrid between channel catfish, *Ictalurus punctatus*, females and blue catfish, *I. furcatus*, males which exhibits heterosis for several traits. This channel-blue hybrid is the most powerful example of genetic improvement of farm raised fish in the world. The number of improved traits and potential economic impact far surpass any other farm raised fish. This fish has an improved feed conversion rate of 15-20% (Yant 1975), growth (especially at high densities) (Dunham et al. 2008), and survival (Dunham and Brummett 1999). The channel-blue hybrid also exhibits heterotic tolerance of low oxygen (Dunham et al. 1983), harvest by seining (Dunham and Aruge 1998), carcass yield (Yant 1975), and has more uniform growth and body shape (Dunham et al. 1982) compared to the more commonly grown channel catfish. Commercial production rates average, 13,000 kg/ha or more, double that of the average channel catfish production.

Some potential barriers for a wide spread commercially conversion over to all-hybrid catfish have emerged as more and more farmers adopt them on their farms. The current culture techniques used to produce these hybrids are not suitable for year-round harvest, and variability at harvest with a large number of oversized fish has been problematic. At this time, there are no sustainable and efficient alternate culture methods to help combat the lack of year-round harvest. The variability at harvest time could be affected by numerous factors or traits. Stocking density, feeding rates, grading, and genetics are some of these factors that might affect the growth variability in channel-blue hybrid catfish. There is much contradictory information that exists in regard to the effects of stocking density, feeding and grading on size variability.

These factors have not been extensively studied in cultured fish. Stocking density did not affect growth variability in pearlspot, *Etroplus suratensis*, (Biswas et al. 2013) or rabbitfish, *Siganus rivulatus* (Saoud et al. 2007). This is different for turbot, *Scophthalmus maximus,* (Li et al. 2013) and Channel catfish which show increased growth variability under increased stocking densities (Brooks et al. 1982). Growth variability and the frequency of oversized fish were affected by both stocking density and feeding rates in the African catfish, *Heterobranchus longifilis* (Ewa-Oboho and Enyenihi 1999). Feeding rates also affects variability and skewness in common carp, *Cyprinus carpio*, (Wohlfarth 1977) and channel catfish (Brooks et al. 1982). An increased feeding rate decreased size variability for hybrid sunfish (Wang et al. 1998).

Saoud et al. (2005) found that grading Nile tilapia, *Oreochromis niloticus*, resulted in populations with less growth variability at time of harvest, however, Yousif (2002) found that grading Nile tilapia was only a temporary fix for lowering growth variability, and that high stocking density and low feeding rates promoted growth variability. Carmichael (1994) found that grading channel catfish populations appeared to result in repartitioning of variation and the final coefficients of variation of size were not different in graded and non-graded populations. Environmental factors and genetic effects can both cause variability in different strains of ictalurid catfish, which includes channel catfish and hybrid catfish (Brooks et al. 1982, Dunham et al. 1982, Dunham et al. 2014).

The objectives of this study were to compare the growth variability of blue catfish, channel catfish and the channel catfish female X blue catfish male hybrid in ponds; determine the effect of family, genetic type of dam and genetic type of sire on the body weight variability of hybrid progeny and determine potential genotype-environment interactions on variability. The hypothesis is that using specific genetic types to make hybrids would give farmers the ability to reduce size variability and decrease the “over-size and sub-market size” fish problem.

Materials and Methods

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

Experimental Fish

The experimental fish that were used for this project were produced from brood stock that were raised at the Genetics Research Unit located on the E.W. Shell Research Center, Auburn University, Alabama. In 2015, twenty females from nine strains of channel catfish were crossed with twelve males from four strains of blue catfish to produce F1 channel catfish x blue catfish (CXB) hybrid catfish progeny (Table 1). The nine strains of female channel catfish were Tishomingo, AR, Kansas Select, Kansas Random, ARMK, Kansas × Thompson, Rio Grande, Thompson, and Mixed strains. The four strains of male blue catfish were D&B, Rio Grande, D&B × Rio Grande, and Tombigbee. Maternal half-sib, paternal half-sib, and full-sib families of F1 C×B hybrid catfish progeny were produced. However, due to poor survival of some crosses, 41 families were obtained resulting in an unbalanced design representing 26 genetic types, 11 dam types and 4 sire types. These were stocked in aquaria and later moved to a split pond.

Table 1: Strain, line or crossbreed of channel catfish (*Ictalurus punctatus*) dams and the corresponding strain or crossbreed blue catfish male (*I. furcatus*) sires mated to produce hybrid catfish for experiments in 2015.

DAM	SIRES
Unknown	TBB, RG, DB, DR
75L	DB, RG, DR
TISH	RG, DB, DR, TBB
ARMK	DB, TBB
AR	DB
KS	DR, DB
KR	RG, TBB, DR, DB
RG	TBB, DB
AC	RG, TBB
KxTH	DR
103KS	DB

Dams- ARMK- (Auburn x Rio Grande) x (Marion x Kansas), KxTH- Kansas x Thompson, AC- Adipose Clipped, RG- Rio Grande, UNK- Unknown strain, KS- Kansas Select, KR- Kansas Random, 75L- strain of fish branded 75L, TISH- Tishomingo, AR- Auburn x Rio Grande, 103KS- NWAW 103 x Kansas Select

Sires- TBB- Tombigbee, RG- Rio Grande, DB- D&B fish farm, DR- DB x RG

Kansas Random 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 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 and 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 was 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 previously initiated by the mixing of multiple strains. 103KS line was F2 from a cross of NWAW 103 (the old Uvalde strain selected for 2 generations for increased body weight using family selections) and Kansas Select.

The twelve blue catfish males (sires) were from four 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.

A subset of the resulting hybrids, three genetic types were compared in 2015. Three strains of blue catfish, Tombigbee (TBB), originating from the Tombigbee River, Alabama, D&B originating from the D&B Fish Farms in Crockett, Texas, and Rio Grande strain from the Rio Grande River in Texas were used as the sires. There were four different channel catfish genotypes evaluated in ponds in 2015, including, an unknown female x ARMK male, Tishomingo, Mix strain, and Kansas Mix, a cross between Kansas random and Kansas Select (selected for body weight for 8 generations).

In 2016, one strain of blue catfish, AR, (a F2 cross between Auburn and Rio Grande blue catfish) was used. Auburn strain of blue catfish originated from the Tensaw and Warrior Rivers in Alabama. Two sets of hybrids were produced using either Rio Grande or D&B sires. Five different channel catfish strains/lines were used Thompson, Kansas Mix, Kansas random and Marion Select. Marion was utilized and was transferred to Auburn University from the Marion State Hatchery in Marion, Alabama in the late 1960’s followed by 8 generations of selection for body weight, resulting in Marion Select.

Spawning and incubation

Beginning in May 2015 and again in May 2016, channel catfish and blue catfish brood stock were taken off feed for two days prior to being harvested from their respective ponds. These fish were loaded into a fish hauler, containing three parts per million (ppm) sodium chloride (NaCl) and the dissolved oxygen levels were maintained at or above 5mg/L, while the fish were transported to a hatchery where the fish were housed in large holding tanks. The female channel catfish were placed into mesh spawning bags and labeled with their weight and strain. These bags were then clipped to the side of the large 670 liter holding tanks where adequate water flow 11 liters per minute and dissolved oxygen levels (>5mg/L) were maintained. Each female was then artificially spawned via an intraperitoneal implant of slow release luteinizing hormone releasing hormone analog, LHRHa. The females each received a dose of LHRHa of 90 µg/kg. Ovulation normally occurred between 1,040 and 1,170 degree hours after the implantation of the LHRHa. After 36 hours, each female was checked for eggs and then rechecked every 4 hours until ovulation. If no eggs were obtained after 100 hours and the female still appeared gravid, she was re-injected with a second dose of LHRHa.

If eggs were found on the bag and ovulation was confirmed, females were anesthetized in a buffered solution of 100ppm tricaine methane sulfonate (MS-222). Once the female was properly anesthetized, she was removed from the solution, rinsed with fresh water, dried with a towel, and her eggs were hand stripped into pie pans. Crisco was applied to both the pie pan and the area around the female’s genital opening to prevent the eggs from sticking and clumping. If blood was found in the egg mass it was rinsed with 0.9% saline solution. Males were euthanized and their testes removed in preparation for sperm extraction. Testes were then rinsed in 0.9% saline solution to remove blood and weighed. The cleaned testes were then macerated and squeezed through a 100-micron mesh into a 0.9% saline solution at a ratio of 10mL of saline per one gram of testes.

After sperm solutions were prepared and eggs were collected, the eggs were fertilized by mixing sperm with the eggs for two minutes in the pie pan while adding fresh water to the pan to activate the eggs and sperm. After fertilization, the pans containing eggs were transferred to a trough with a calcium chloride ($CaCl_2$) drip to keep the water hardness above 30 ppm. The eggs were left in this trough and allowed to harden for approximately one hour. The eggs were then transferred to baskets in similar troughs that were equipped with paddlewheels to agitate the eggs, mimicking the behavior of the male during incubation. These hatching troughs had 24 egg masses each with supplemental calcium chloride drips to maintain a water hardness of 30 ppm or greater. Water flow for each tank was kept around 20L/minute, and dissolved oxygen levels maintained at or above 5mg/L. Formalin was used every 8 hours at a rate of 100ppm to prevent the growth of fungus. If eggs were found with fungus infection, the fungus was removed by hand and that trough was treated twice per day with 32ppm of copper sulfate ($CuSO_4$) in addition to single formalin treatment. All chemical treatments were ceased 12 hours prior to expected hatch.

Rearing

Once hatched, fry fell through the hatching basket and into a mesh fry catcher. These fry catchers are made from a very fine window screening material. They fit tightly to the hatching baskets to prevent them from falling off and into the bottom of the tank. After 210 fry from each family were collected, the rest of the fry were released in the hatching trough below the basket or allowed to continue hatching without the mesh fry catcher. These 210 fry were then moved to a separate aquaria with each aquaria containing only one family of fry with each family being split into three replicates in three different tanks with 70 fry per aquaria. The aquaria and troughs were checked daily to ensure adequate water flow and dissolved oxygen levels. These same aquaria and troughs were syphoned daily to remove dead fish and debris and water quality was monitored twice a week to ensure proper fish care within safe water quality ranges ammonia (NH_3) of 0 parts per million (ppm), a nitrite (NO_2) of 0 ppm, alkalinity and hardness above 40, chlorine of 0 ppm, and pH between 6.8-7.2.

Once the fry had consumed their yolk sac and began the swim up phase they were fed with Purina® AquaMax® powdered starter diet containing 50% protein until they reached a size of approximately 2.5 cm. During the entire experiment, fish were fed to satiation daily. From this 2.5 cm length until they reached 3.8 – 4 cm the fish were fed Purina® AquaMax® 100 diet containing 50% protein, from 4 – 5 cm they were fed Purina® AquaMax® 200 containing 50% protein, and after they reached 5 cm they were fed Purina® AquaMax® 300 containing 50% protein until the experiment was terminated after 323 days of growth.

The aquaria were flow-through with water supplied from a reservoir pond. The system was later converted to a recirculating system equipped with a rotating drum filter to remove solid waste, sump for holding water and solid waste settling, a biofilter for sequestering nitrogen and ammonia, and a UV filter for sterilization of water before it returned to the aquaria. Shortly after initiating the recirculating system, ammonia and nitrite problems occurred. When ammonia reached near toxic levels water exchanges were performed, and in the case for nitrite, NaCl was applied at a ratio of 9ppm NaCl to 1ppm nitrite to prevent brown blood disease.

Once the fish located in the recirculating system were large enough to be marked a sub set of each family were pit tagged and then placed into a split pond that consisted of 5 ponds. Total area of the split pond is 526 square meters with an average depth of 1.5 meters. One pond was used to house all of the fish and was connected to the other 4 ponds via 20.3 cm corrugated pipes. Each of these other 4 ponds were also connected to each other with corrugated pipes. Water is pumped in to the pond with fish at one end and at the other end the water leaves and must move through all 4 other ponds before it can reenter to the fish holding pond. During the day, the pump would run to circulate water throughout the 5 ponds, and then at night the single pond with fish in it was aerated with the pump turned off. The fish were fed ad libitum with 32% protein floating catfish pellets for the 245 days they remained in the split-pond.

Once the remaining fry consumed their yolk sac they were fed with Purina® AquaMax® powdered starter diet containing 50% protein in their respective hatching troughs until they could be stocked into 0.04 hectare (ha) earthen ponds. Each pond had a single strain of hybrid catfish stocked, blue catfish, and channel catfish stocked. For each pond in 2015 and 2016, a stocking rate of 175,000 fish/ha was the target to simulate the stocking densities found on a commercial farm (Table 2).

A large spawning barrel was placed in the pond to provide an area of protection for the newly stocked fry. The dissolved oxygen was checked daily and an aerator was ran each night to ensure proper dissolved oxygen levels were maintained. Water quality was tested once a week to check for any parameters that may cause a problem with the fish such as a spike in nitrite or ammonia. The fry continued to be fed with Purina® AquaMax® powdered starter diet containing 50% protein until they reached a size where they were able to transition to being fed Purina® AquaMax® 300. Once the fish reached a size of approximately 7-8 cm they were fed a diet of normal pelleted bulk fish feed with a 32% protein balance diet. Each day the fish were fed to satiation.

These fish were sampled twice, once in the fall/early winter and again in the spring when the experiment was terminated. Fish were sampled and harvested by seining. Once the seine had been secured 300 fish of each genetic type were then randomly selected from the seine to be individually weighed. In the case of sampling, all fish were then returned to their respective ponds after sampling.

Table 2: Stocking densities, number of fish sampled, mean body weight and coefficient of variation of body weight for channel catfish female (*Ictalurus punctatus*) x blue catfish male (*I. furcatus*) hybrids, channel catfish, and blue catfish for various genetic types stocked in separate ponds. G ponds are 0.04- hectares as well as M9. M8 and H ponds are 0.02- hectares.

2015	Genetic Type	# stocked	# sampled	Mean weight (g)	*coefficient of variation
G53	DB hybrids	4580	306	17.4	26.2%
	UxARMK 195 channel	800	36	21.5	23.8%
G51	DB hybrid	2950	137	20.6	45.8%
	Mixed channel	800	133	19.7	41.2%
R29	TBB hybrid	2200	300	11	56.3%
	Tishomingo channel	3600	290	13	48.4%
R21	RG hybrid	3900	333	12.4	51.3%
	RG channel	2100	212	11.6	47.6%
M8	DB hybrid	1200	298	26.2	29.5%
	UxARMK 195 channel	800	156	34.2	40.8%
M9	RG hybrid	4070	297	20.3	40.2%

	UxARMK 195 channel	2000	297	21.9	43.9%
*2016					
G45	RG hybrid	500	57	58.3	21.9%
	DB hybrid	450	77	49.2	28.5%
	103ks channel	500	33	80.7	28.1%
G42	AR blue	5000	105	30.8	49.6%
	KR channel	5000	107	22.8	84.5%
H15	Mix hybrid	630	128	24.7	22.2%
	MS channel	100	82	42.1	26.8%
	AR blue	100	36	28.4	30.5%
H21	RG hybrid	150	104	44.8	25.9%
	DB hybrid	200	103	38.2	20.3%
	Kmix channel	120	97	69.8	21.8%
H22	AR blue	500	103	24.9	30.6%
	Thompson channel	750	105	43.3	39.3%

*2015 Channel catfish CVs were pooled across all ponds and compared to the hybrid catfish CVs across all ponds, and no significant differences were found (P>0.05, t-test).

2016 two t-tests were conducted using the pooled CV across ponds for blue catfish compared to pooled channel catfish, and pooled hybrid catfish across ponds compared to pooled channel catfish, and no significant differences were found (P>0.05).

Blue catfish: AR- Auburn x Rio Grande

Channel catfish: UxARMK- Unknown fish crossed with (Auburn x Rio Grande) x (Marion x Kansas), RG- Rio Grande, 103KS- NWAC 103 x Kansas Select, KR- Kansas Random, MS- Marion Select, Kmix- Mix Kansas strain

Hybrid catfish sires: DB- Blue catfish from DB fish farms, RG- Rio Grande, TBB- Tombigbee

Data Analysis

Means, standard deviations, and coefficient of variation (CV) were all calculated for body weight in Microsoft Excel. Individual weights were collected from each fish from the earthen ponds and from the recirculating system aquaria. For the fish that were raised in earthen ponds the CV was calculated for each genetic type then the CVs for each genetic type were compared using a paired t-test. For fish grown in the recirculating system and transferred to the split pond, CV for body weight was calculated for individual families, genetic types, pooled by dam strain and pooled by sire strain. CVs for each classification were compared with a paired t test to Kansas Random for dams and DB for sires as a standard genetic type. The coefficient of variation (CV) was calculated for each individual set of hybrid catfish and channel catfish in each pond. These CVs were then compared to each other on a pond by pond basis. Then all the CVs were pooled together by either hybrid catfish CV or channel catfish CV and compared using a t-test. A correlation was generated between the hybrid catfish CVs and channel catfish CVs.

The weights for each individual fish from the recirculating system were pooled together to calculate the CV for the entire population and determine the population distribution by percentages of fish in each standard deviation. Then the data was sorted into different categories: families, sire strain, dam strain, CVs calculated by category and compared to a standard using a pair wise t-test. In the case of the dam genetic types, the standard was Kansas Random, and the sire genetic type was DB. Population distributions were compared within each experiment using a Fisher’s exact test (http://udel.edu/~mcdonald/statfishers.html).

Results

Earthen Ponds

The six earthen ponds in 2015 had channel catfish and hybrid catfish in each pond. The pooled CV for body weight of channel catfish and hybrid catfish in these ponds were not different ($P>0.05$, Fig. 1). A correlation of 0.90 was obtained from these two sets of numbers.

The 5 earthen ponds stocked in 2016 had one of these three sets of fish: channel catfish and blue catfish, channel catfish, blue catfish and hybrid catfish, or channel catfish, RG hybrid catfish, and DB hybrid catfish. The CV for these genetic types were not different ($P>0.05$, Fig. 2). There were no significant differences in the population distribution for 2015 or 2016 ($P>0.05$, Table 2)

Recirculating system

The hybrid population distribution based on dam genotype is found in Table 3, and again all of the population distributions had positive skewness. Three dam genetic groups had a significantly different CV from the standard family of Kansas random (Table 4). The three dam genetic types were Kansas x Thompson, ARMK, and Kansas Select. The population distribution of the hybrids pooled by sire genetic type is found in Table 5, and again all of the population distributions had positive skewness. The mean body weight and mean CV, for the individual sire types is found in Table 6. CV of the DR hybrids were higher than that of the DB standard ($P<0.005$). The mean body weight of all fish at harvest from the aquaria was 12 and the standard deviation was 4. The overall CV was 35.8.

Split Pond

The body weight distribution for the overall population of hybrids after 245 days of growth is found in Fig. 3. The CV increased from 35.8 at stocking to 48.7 at harvest (Table 7). Based on the analysis from the aquaria, this magnitude of shift in the CV is likely significant. The correlation coefficient for the CVs of the 41 families between the split-pond and recirculating system was 0.05.

However, if the data is analyzed based on the pooled sire type, the correlation is 0.51, and if by pooled dam type, the correlation is 0.39. Neither of these correlation coefficients are significant, but they approach significance (P=0.17).

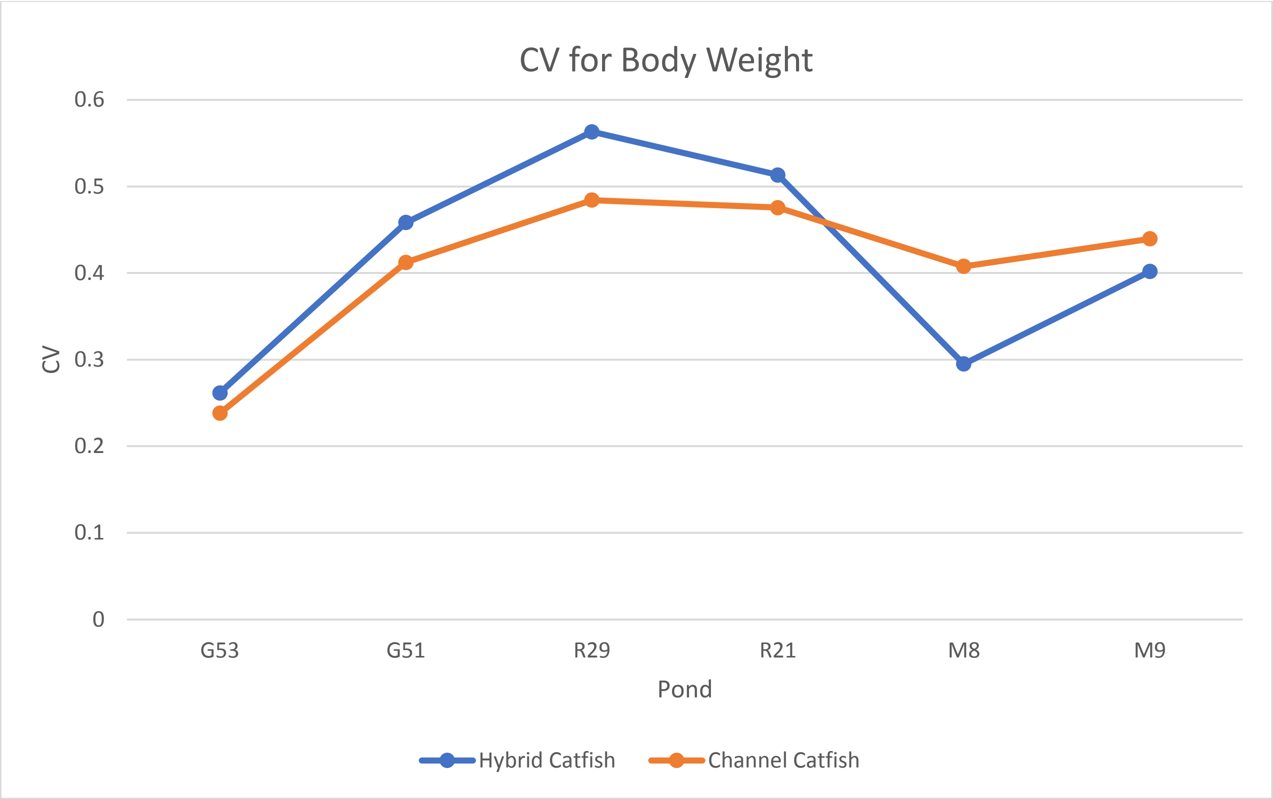


Figure 1: Coefficient of variation for body weights of channel catfish female (*Ictalurus punctatus*) x blue catfish male (*I. furcatus*) hybrids and channel catfish (*I. punctatus*) grown in six earthen ponds in 2015 at 175,000 fish/ha. The CVs were not different (t-test, $P<0.05$). The correlation between the channel catfish CV and the hybrid catfish CV was 0.90 ($P=0.79$).

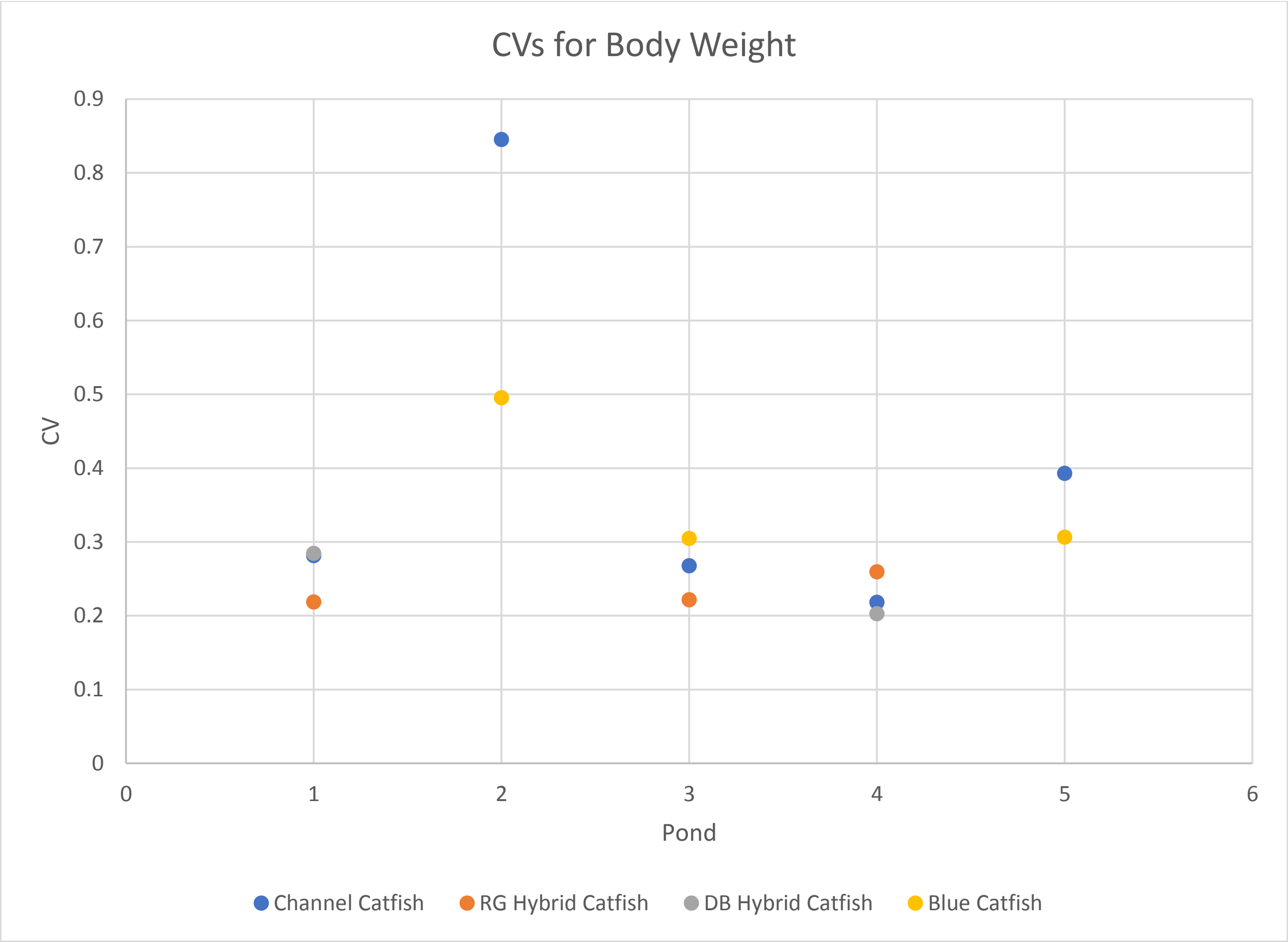
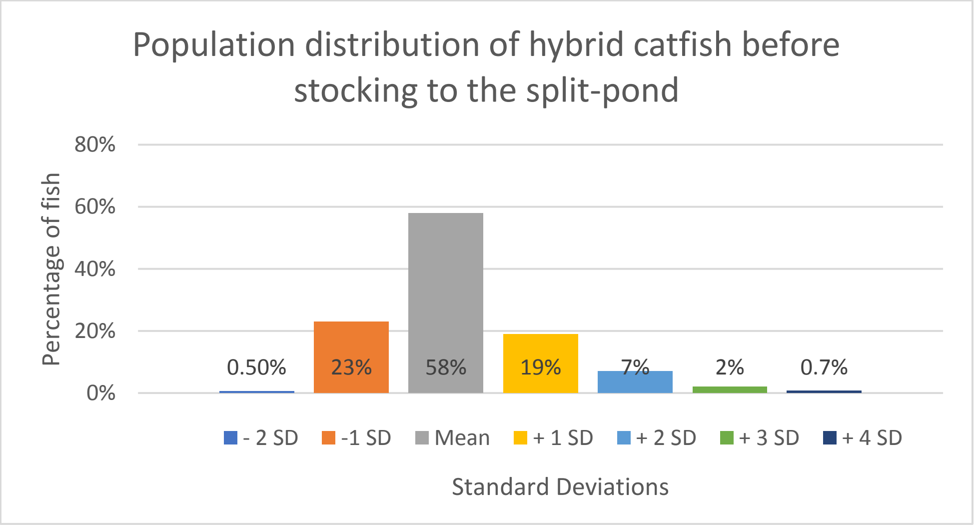


Figure 2: Coefficient of variation (CV) for body weight of channel catfish female (*Ictalurus punctatus*) x blue catfish male (*I. furcatus*) hybrids. The hybrids were of two different sire genetic types, Rio Grande (RG) and D&B fish farm sires (DB), and many dam genetic types. blue catfish (*I. furcatus*) and channel catfish (*I. punctatus*) ponds in 2016. CVs were not different (P>0.05, t-test).

Table 3: Population distribution of body weight for channel catfish female (*Ictalurus punctatus*) x blue catfish male (*I. furcatus*) hybrids, channel catfish (Ictalurus punctatus), blue catfish (I. furcatus), hybrid Catfish with Rio Grande sires, and hybrid Catfish with DB sires stocked in 2015 and 2016 in 0.04-ha ponds at 175,000 fish/ha and fed ad-libitum. This population distribution is measured in standard deviations of mean body weight. No differences were found (P>0.05) among population distributions with years (Fisher's Exact test).

SD	**2015 Channel Catfish**	**2015 Hybrid Catfish**	**2016 Channel Catfish**	**2016 Blue Catfish**	**2016 RG Hybrid Catfish**	**2016 DB Hybrid Catfish**
-4	0	0	0	0	0	0
-3	0	0	0	0	0.3	0
-2	0	0.83	0.2	0	1	2.5
-1	10	10.8	13.2	16.6	14.3	16.5
<1	73.6	74.6	68.8	62.6	67.7	64
1	16.3	14.5	18	20.6	18	19.5
2	6.5	5	3.2	5.6	5.7	5
3	2.3	2	0.8	1	1.3	0.5
4	0.5	0.33	0	0	0.3	0

A



B

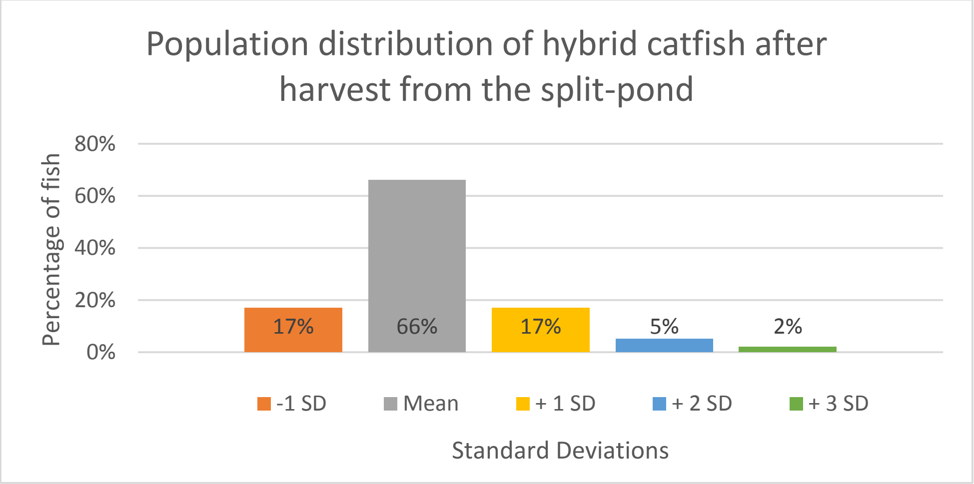


Figure 3: **A** percentage of channel catfish female (*Ictalurus punctatus*) x blue catfish male (*I. furcatus*) hybrids that falls within each standard deviation away before stocking to the split-pond. **B** the distribution of channel catfish female (*Ictalurus punctatus*) x blue catfish male (*I. furcatus*) hybrids through the standard deviations away from the mean weight after harvest from the split-pond. The mean is 194 with a standard deviation of 94.

Table 4: Population distribution of channel catfish female (*Ictalurus punctatus*) x blue catfish male (*I. furcatus*) hybrids with different genetic types of dams measured in standard deviations of mean body weight. Fish were grown in 60L aquaria in a recirculating system for 245 days at a stocking density of approximately 35 fish per aquaria.

Genetic Type						% of fish in each Standard Deviation of the mean					
	-5	-4	-3	-2	-1	<1	1	2	3	4	5
ARMK	0	0	0	0	9	69	22	8	3	1	0.1
KxTH	0	0	0	0	8	75	17	6	0	0	0
AC	0	0	0	0	8	74	18	8	2	1	0.2
RG	0	0	0	0	16	61	23	4	0.5	0.5	0
UNK	0	0	0	2	21	52	27	9	3	1	0.8
KS	0	0	0	0.2	13	65	22	8	2	0.6	0
KR	0	0	0	0.4	14	61	25	9	4	1	0.4
75L	0	0	0	0	5	85	10	2	0.6	0.2	0
TISH	0	0	0	0	16	66	18	6	2	0.3	0.2
AR	0	0	0	0	10	65	25	5	0.6	0.2	0
103KS	0	0	0	0	11	65	24	8	5	3	0

*ARMK- (Auburn x Rio Grande) x (Marion x Kansas), KxTH- Kansas x Thompson, AC- Adipose Clipped, RG- Rio Grande, UNK- Unknown strain, KS- Kansas Select, KR- Kansas Random, 75L- strain of fish branded 75L, TISH- Tishomingo, AR- Auburn x Rio Grande, 103KS- NWAW 103 x Kansas Select

Table 5: Mean body weight (BW) and coefficient of variation for body weight (CV) for channel catfish female (*Ictalurus punctatus*) x blue catfish male (*I. furcatus*) hybrids of various dam genetic types grown in 60L aquaria at a density of 35 fish per aquaria in a recirculating system.

Dam genetic type	**Number of fish**	**Mean Body wt (g)**	**Mean CV**	**Number of replicate aquaria**	**P Value**
ARMK**	583	7.69	24.5%	9	0.0265
KxTH*	63	18.19	69.9%	3	0.0128
AC	430	9.82	33.8%	7	0.3631
RG	390	8.54	24.0%	6	0.1292
UNK	1166	9.56	25.4%	20	0.1312
KS***	546	8.52	26.1%	9	0.0772
KR	1386	9.85	28.6%	24	NA
75L	1413	9.26	32.6%	22	0.2751
Tish	655	8.14	29.8%	10	0.9479
AR	624	8.80	21.9%	9	0.0039
103KS	37	11.73	32.5%	NA	NA

*CV for BW is different from that of KR (P<0.01, paired t-test)

** CV for BW is different from that of KR (P<0.05, paired t-test)

*** CV for BW is different from that of KR (P<0.10, paired t-test)

ARMK- (Auburn x Rio Grande) x (Marion x Kansas), KxTH- Kansas x Thompson, AC- Adipose Clipped, RG- Rio Grande, UNK- Unknown strain, KS- Kansas Select, KR- Kansas Random, 75L- strain of fish branded 75L, TISH- Tishomingo, AR- Auburn x Rio Grande, 103KS- NWAW 103 x Kansas Select

Table 6: Population distribution of channel catfish female (*Ictalurus punctatus*) x blue catfish male (*I. furcatus*) hybrids with different genetic types of sires measured in standard deviations of mean body weight. Fish were grown in 60L aquaria in a recirculating system for 245 days at a density of 35 fish per aquaria

Sire Genetic Type						% of fish in each Standard Deviations of the mean					
	-5	-4	-3	-2	-1	<1	1	2	3	4	5
RG	0	0	0	0.8	21	55	24	9	4	1	0.5
DB	0	0	0	0.07	16	60	24	8	2	0.7	0.3
DXR	0	0	0	0	6	82	12	3	2	0.9	0.5
TBB	0	0	0	0.2	22	55	23	7	2	1	0.4

RG- Rio Grande, DB- D&B fish farm strain, DR- F1 cross of DB and Rio Grande, TBB- strain from the Tombigbee River in Alabama.

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Table 7: Mean body weight (BW) and coefficient of variation for body weight (CV) for channel catfish female (*Ictalurus punctatus*) x blue catfish male (*I. furcatus*) hybrids of various sire genetic types grown in 60L aquaria at 35 fish per aquaria in a recirculating system

Sire genetic type	**Number of Fish**	**Mean Body Weight**	**Mean CV**	**Number of replicate aquaria**	**P Value**
RG	1710	9.38	27.7%	27	0.2877
DB	3026	8.68	25.4%	48	NA
DR*	1522	10.61	36.9%	30	0.0026
TBB	1034	8.23	27.6%	16	0.7039

* CV for BW is different from that of DB ($P<0.05$, paired t-test)

Table 8: Coefficent of variation for the initial body weights and final body weights of channel catfish female (*Ictalurus punctatus*) x blue catfish male (*I. furcatus*) hybrids with different genetic types of dams and sires. Fish were grown in a communal split-pond for 245 days. When pooled by sire type, the correlation between initial and final CV is 0.51 (P=0.17), and if pooled by dam type, the correlation is 0.39 (P=0.17).

Sire	**Initial CV**	**Final CV**
TBB	42.8	48.4
DB	32.8	44.6
DR	35.5	51.1
RG	32.6	42.2
Dams	**Initial CV**	**Final CV**
Unk	32.7	43.4
75L	33.2	47.9
ARMK	55.5	52.4
AR	33.5	42.1
KS	39.2	55.7
KR	33.3	48.6
RG	32.1	39.9
AC	30.1	52.8

Discussion

Variable body weight at time of harvest is one of the issues that farmers face when sending their fish to the processors. Farmers can obtain a higher profit when they send relatively uniform size fish to the processor, and can either be docked for or not paid for oversize and undersized fish. Variable and undersized hybrid fingerlings also present culture and marketing problems for fingerling producers. Genetics could provide a possible solution to this variability through selection for individuals or strains of sires and dams that produce more uniform hybrids. Genetics had a significant effect on growth variability, however, hybrid catfish and channel catfish fingerlings had similar CV for body weight in ponds, which changed in parallel fashion indicating that environmental factors had a stronger effect on growth variability than genetic factors. Genotype-environment or genotype-age interactions also had a very, strong impact on body weight variability. There were genetic differences for among families, dam types and sire types for body weight variability. Interestingly, use of crossbred parents increased the CV for BW, but reduced the overall spread of the population distribution.

In 2015, there were 6 ponds stocked with hybrid and channel catfish. The coefficient of variation of hybrids and channel catfish moved in parallel fashion from pond to pond suggesting that environmental effects have larger effects than genetic effects on growth variability of ictalurid catfish. The same trend was seen in 2016 when comparing blue catfish, channel catfish and hybrid catfish grown in a series of communal ponds. Environmental effects could include stocking densities, survival and its related causes various feeding variables among others (Dunham *et al*. 1990).

Both the hybrid and the channel catfish genetic types grown in ponds in each year had a distribution of fish that had more fish larger than the mean instead of those smaller than the mean. This would be beneficial for farmers producing fingerlings for sale since the price is higher for larger fingerlings per cm and price is based upon length. This could also be beneficial for food fish producers. If enough of the fish are larger than the mean, then an early partial harvest of the larger fish would be possible impacting production and cash flow in a positive way. However, a more likely impact is that these larger individuals become the oversized fish that become a processing problem, and actually decrease profitability because of the lack of market and lower price for the oversized fish (Marshall 2004). The processing plant actually needs some variability in the fish due to demand for different sizes of fish for various markets. Additionally, the plants have multiple processing lines for a different size fish. The processing plants operate more efficiently if all lines are active, thus, a degree of, but not too much variability is desirable.

Hybrids grown in aquaria also had a population distribution positively skewed with 29% of the fish more than and 23% less than one standard deviation from the mean. Significant dam genetic type effects were observed for the CV with hybrids from dams having some ancestry from Rio Grande. The highest CV was found in hybrids of the crossbred dam KxTH, which would be expected to produce gametes with much more variability. Although not significant and contradictorily, these crossbred females produced an observed smaller number of oversized and undersized fingerlings, which would be highly beneficial for all strata of the catfish industry.

A similar result was found in regard to the effect of sire genetic type. Again, the crossbred, D X R, males generated hybrid progeny with the highest CV for BW. Just as was the case with the crossbred dams, these crossbred males produced hybrid progeny with the tightest population distribution with 82% of the fish being within less than 1% of the population distribution, and only 6% between 1 and 2 SD of the mean and only 12% greater than 1 SD from the mean. This population distribution should be desirable for both fingerling and food fish producers.

It appears contradictory that these crossbred dams and sires would produce hybrid progeny that have both increased variability, but a tighter population distribution. The variability would seem to be expected as the gametes would have a high degree of variability. The heterozygosity associated with that variability could result in a population distribution with less extremes as the heterozygosity and the associated dominance and epistatic effects might produce a more standard performance, but with many more phenotypes within that standard range. Although the F1 interspecific hybrid progeny are obviously highly heterozygous, there would be differing levels of heterozygosity among different hybrid types based upon the homozygosity of the parent genetic type. Long lasting genetic maternal effects on early growth variation could explain the differences in the growth variability of hybrids from crossbred females compared to pure strain or line dams, and paternal predominance (Dunham et al. 1982) could be involved regarding the variance differences observed among the sires.

From the time that the hybrid catfish from the aquaria were stocked in the split-pond until they were harvested from the split ponds, there were observed changes in the population distribution. The CV increase from an initial 35.7% to a final CV of 48.6%, while the observed percentage of fish within one standard deviation of the mean increased. This was more greatly influenced by a shift of fish away from being undersized compared to a shift away from oversized fish although both were occurring. The correlation of the family CV between the aquaria and pond was near zero. Thus, although the overall population distribution only experienced relatively minor changes, the variation among families had very large shifts indicating genotype-environment or environment-age interactions. Research is needed to determine, which of these two explanations or a combination of the two caused this result. This will be difficult as it would require a very large number of ponds or the growth of the fish to a large size in aquaria. Perhaps, the best solution would be to use SNP or microsatellite markers so that the families could be mixed at a young age in ponds, although this approach is still relatively expensive.

These genotype-age or genotype-environment interactions were less important when the data is analyzed by pooling the hybrids by dam type or sire type. The correlations were positive and moderate, 0.39 and 0.51 for dam and sire genetic types, respectively, which was not significant, likely due to insufficient replication.

Genetic type of sire or dam has an impact on C x B hybrid catfish variability for body weight. Selection of the appropriate sire or dam genetic type could be used to produce more desirable population distributions. Crossbred sires or crossbred dams produced hybrid populations with more preferred population distributions. Additional types of crossbreds should be evaluated to confirm this trend. Also, the effect of using both a crossbred dam and sire should be evaluated to see if there is an even greater impact on body weight variability. Additionally, other traits of these hybrids from different families, strains and crossbreeds needs to be evaluated as improvements in population distribution would be negated if these less variable hybrid genetic types have decreased performance for other economically important performance traits.

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Appendix

Table 9: Coefficient of variation for each family of channel catfish female (*Ictalurus punctatus*) x blue catfish male (*I. furcatus*) hybrids in the recirculating system located in the hatchery and the split pond. These two sets of CVs were ran through a correlation test in excel and the value received was 0.05. This shows there is no correlation between the two sets of CVs.

Family	Split pond	Recirc System
CV 41	0.413297	0.295661
CV 40	0.411567	0.196641
CV 39	0.361159	0.293776
CV 37	0.312212	0.332457
CV 36	0.380080	0.311074
CV 33	0.324951	0.330714
CV 32	0.336098	0.356718
CV 31	0.430899	0.367629
CV 29	0.374714	1.149853
CV 28	0.502651	0.370642
CV 27	0.444114	0.284207
CV 26	0.338428	0.304271
CV 25	0.459506	0.345666
CV 24	0.462583	0.287721
CV 23	0.234456	0.264092
CV 22	0.629965	0.303305
CV 21	0.537735	0.435031
CV 20	0.441002	0.402754
CV 18	0.410299	0.415653
CV 17	0.427523	0.398841
CV 16	0.486328	0.319912
CV 15	0.421294	0.399950
CV 14	0.415357	0.283807
CV 13	0.495450	0.332939
CV 12	0.441432	0.305816
CV 11	0.394316	0.274606
CV 10	0.356967	0.276733
CV 9	0.526650	0.368212
CV 8	0.481692	0.414122

CV 5	0.336989	0.267883
CV 4	0.479408	0.414092
CV 3	0.519361	0.357573
CV 1	0.536771	0.452052

Table 10: Average weights for each family of channel catfish female (*Ictalurus punctatus*) x blue catfish male (*I. furcatus*) hybrids at time of stocking into the split pond and after harvest from the split-pond.

Family	Average Initial Wt (g)	Average Final Wt (g)
41	12.64	196.36
40	11.48	140
39	10.32	192.90
37	7.21	179.32
36	11.78	248.5
33	11.68	212.75
32	12.65	223.33
31	8.34	164.31
29	16.4	358
28	10.34	203.36
27	8.79	155.29
26	7.17	188.62
25	10.94	280
24	9.99	200.73
23	14	262
22	9.55	204
21	10.77	163
20	8.21	143.70
18	8.75	142.73
17	9.31	164

16	12.54	286.88
15	9.37	207.59
14	7.57	133.62
13	8.94	196.10
12	8.10	147.36
11	12.47	240.26
10	11.41	178.53
9	14.20	255.71
8	11.45	214.19
5	10.16	224.44
4	8.64	199.43
3	10.54	197.43
1	9.94	165.10