Growth and Morphology of a Synthetic Channel Catfish (*Ictalurus punctatus*)-Blue Catfish *(Ictalurus furcatus)* Backcross Breed and the Hybrid Between the Backcross Female and Blue Catfish Male

by

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Synthetic Backcross Catfish, Growth Rate, Body Shape, Hybrid

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Abstract

The F1 hybrid between a channel catfish female (*Ictalurus punctatus*) × a blue catfish (*Ictalurus furcatus*) outperforms both parental species in commercial environments for numerous traits. However, reproductive isolation mechanisms between the species make mass production of the F1 hybrid labor intensive and the hybrid is not 100% disease resistant. A synthetic breed between channel catfish and blue catfish was produced by 3 generations of backcrossing with channel catfish followed by one generation of closed breeding. This synthetic channel-blue breed was hybridized with blue catfish males to determine if there could be benefits from both multiple generations of backcrossing followed by heterosis from hybridization. The growth of this hybrid was approximately 30% faster than both the parental synthetic backcross breed and that of channel catfish. The growth rate of synthetic backcross catfish was similar to channel catfish which is not surprising as selection for growth rate was not part of the backcrossing program. The hybrid between the backcross female and blue catfish male had the highest relative body area than backcross catfish and channel catfish, which predicts that it would also have the higher dressout and fillet percentage. The correlation among morphometric traits was variable among genotypes. Skewness for body weight tended to be low to moderate for all genetic types, which is important considering the oversized fish problem in the commercial industry. Skewness for relative body area was highly negative for the backcross × hybrid, negative for the backcross and positive, but near zero for the channel catfish.

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List of Abbreviations

AU	Auburn University
AU-IACUC	Auburn University Institutional Animal Care and Use Committee
B	Blue catfish
BC	Backcross catfish
C	Channel catfish
CB	Channel-Blue hybrid catfish
ESC	Enteric Septicemia of Catfish
ERM	University of Hawaii
FAO	Food and Agriculture Organization
LHRHa	Luteinizing Hormone Releasing Hormone analog
RTFS	Rainbow Trout Fry Syndrome

Introduction

With the increase of population around the world, people need reliable, sustainable, and environmental-friendly protein sources. Human societies are facing the enormous challenge of providing food and livelihoods to meet more than 19 billion people's requirements, while addressing the disproportionate impacts of climate change and environmental degradation on the resource base (FAO 2018). Aquaculture must continue to grow to meet the fish protein requirement of the future. Fish production around world peaked at about 171 million tonnes in 2016, which aquaculture contributed 47 % of the total. The global total capture fisheries production was 90.9 million tonnes in 2016 which is a decrease in comparison to the last two years (FAO 2018), and the global aquaculture production in 2016 was 110.2 million tones (FAO 2108). Production per unit area needs to be improved for efficient aquaculture and wise stewardship of resources. As production intensifies, the fish need to have the ability to adapt to increasingly stressfull environments (Dunham 2011). Genetic enhancement could be a key to solve problems associated with the increase of stocking density and to meet future demands (Dunham 2011). There are different approaches for genetic enhancement, including strain selection, interspecific hybridization, polyploidy, transgenic modification, marker-assisted selection and others.

Catfish, a member of the order *Siluriformes* or *Nematognathi*, is one of the most diverse and biogeographically ubiquitous groups of teleosts. Around the world, catfish have more than 3,000 species and 37 recognized families. Catfish represent almost 11% of all fish and 5.5% of all vertebrates (Armbruster 2011). They are one of the foremost aquaculture species in the world due to their diversity, handling tolerance, high disease resistance, high fecundity

, and good performance in feed conversion (Jin et al. 2016). The most common cultured catfish

species worldwide are African catfish (*Clarias garienpinus*), walking catfish (*C. batrachus*), hybrid walking catfish (female *C. microcephalus*×male African catfish), tra (*Pangasionodon hyphphthalmus*), blue catfish (*Ictalurus furcatus*), channel catfish (*Ictalurus punctatus*), and the F_1 hybrid of female channel catfish × male blue catfish (Khan et al. 2009, Jin et al. 2016).

Channel catfish is extensively cultured in the United States. In 2015, catfish production accounted for around 68% of all freshwater production in the United States (NMFS, 2015). However, in recent years, the U.S. catfish industry has shown a significant decrease in production. From 2003 to 2014, catfish production in the U.S. fell from 300,278 metric tons to 136,531 (NMFS 2015). This 54% decrease was caused by several different reasons. The increased import of frozen catfish from Asian countries was the main reason for the rapid decrease in U.S. production (Hanson and Sites 2015). The import of frozen catfish has increased rapidly from 13,607 metric tonnes in 2005 to 108,408 metric tonnes in 2014. The rate of increase was almost 977%. Furthermore, around 97,618 metric tonnes of catfish were Vietnamese.

In 2016, the import of Vietnamese *Pangasianodon* reached a peak of more than 131,000 metric tons (NOAA 2018). After 2016, the import of Vietnamese *Pangasianodon* decreased to 96,460 metric tonnes, and 2018 imports totaled 97,920 metric tonnes while the production of catfish in the US is only 68,038 metric tonnes (NOAA 2018).

As a commercial species, *Pangasianodon* has many good traits. *Pangasianodon* has fast growth rate, and their ability to breathe air allows them to be produced at incredibly high density. *Pangasianodon* production can reach 300 tonnes per hectare (FAO 2019). This high production of *Pangasianodon* has led to Vietnam capturing most of the US catfish fillet market (NOAA 2018). Thus, it is necessary to improve the competitiveness of U.S. catfish industry.

Two Main North American Catfish Species

Channel catfish was the primary species of the catfish industry. Channel catfish tolerate low temperatures, low dissolved oxygen, and high stocking densities (Torrans et al. 2012). Also, channel catfish have a high fillet yield (Torrans et al. 2012). Moreover, these good traits made channel catfish excellent candidate for aquaculture. Compared with other species, channel catfish growth to food size faster and has higher disease resistance (Torrans et al. 2012).

Early in the 1900s, catfish culture and farming began. The evolution of an organism better suited for aquaculture environment begins even without directed selection (Dunham et al. 2000). In channel catfish, an increase growth rate of 3-6% per generation was observed (Dunham et al. 2000). Genetic enhancement programs including evaluation of strain effects, intraspecific cross-breeding, interspecific hybridization, and mass selection were processed on catfish for additional gains in performance. These experiments have successfully improved the traits such as growth rate, feed conversion, survival rate, tolerance to low dissolved oxygen, seinability, higher carcass yields, disease resistance, and the performance of reproductive (Smitherman et al. 1983, Dunham 1983, Dunham 1987, Rezk et al. 2003, Dunham 2008, Dunham 2010).

Blue catfish (*Ictalurus furcatus*) is hard to breed. Culturists recognized that the blue catfish have a poor tolerance of low oxygen, poor disease resistance, and slow sexual maturation (Dunham and Smitherman 1984). Blue catfish have a high disease resistance to enteric septicemia of catfish (ESC), channel catfish virus (CCV), and proliferative gill disease (PGD) (Torrans et al. 2012). Compared with channel catfish, blue catfish are more resistant to nitrite, which could cause methemoglobinemia, or brown blood disease (Schoore et al. 1995). Blue catfish are easy to seine (Dunham and Argue 1998), and this is probably because they prefer to stay in midwater (Graham 1999), and virtually 100% can be harvested in a single seine haul. Blue catfish normally shows a uniform growth (Brooks et al. 1982, Dunham et al. 1982, Jiang et al. 2008), and

higher carcass yield (headed, gutted, skinned) when compared with channel catfish (Morrison 1992, Li et al. 2008). Their high response to the feed makes them a desirable species for use in pay-lakes (Collins 1988, Tidwell and Mims 1990). Moreover, their interspecific hybrid with channel catfish females has heterotic growth (Giudice 1966).

Selection

Using selection to improve performance has been successfully applied to various aquaculture species. Selection was used to improve of disease resistance to endemic furunculosis in salmonids by more than 50%. Another successful selection was improving brook trout (*Salvelinus fontinalis*) resistance to endemic furunculosis by three generations of selection, improving survival rate from 2% in the original population to 69% in the selected population (Embody and Hayford, 1925). In rainbow trout (*Oncorhynchus mykiss*), additive genetic variation existed for resistance to redmouth disease (ERM), rainbow trout fry syndrome (RTFS) and viral hemorrhagic septicemia 5 (VHS) (Henryon et al. 2005). All of these three diseases could be improved by selection (Wetten et al. 2007, Kjoglum et al. 2008). IPN (Okamoto et al. 1993) and bacterial cold-water disease (BCWD) (Leeds et al. 2010) resistant strains of rainbow trout have also been developed.

The genetic variance of catfish showed the possibility of improving the disease resistance to Enteric septicemia (ESC) through selection (Wolters and Johnson 1995). However, there was no response to selection for enteric septicemia of catfish (ESC) caused by *Edwardsiella ictaluri* after one generation of selection in Kansas strain (Dunham et al. 1994). Additionally, mass selection did not improve resistance to *Aeromonas hydrophila* in walking catfish (*Clarias macrocephalus*) (Na-Nakorn et al. 1995).

Intraspecific Crossbreeding

Strains of channel catfish, Auburn, Marion, Kansas and the Rio Grande, were evaluated

crossed (Dunham and Smitherman 1983). Crossbred progeny had a better performance in overall viability, weight gain, fecundity and lower mortality. Crossbreeding is an efficient method to improve disease resistance. The frequency improvement of disease resistance when F_1 crossbreeds are produced in various aquaculture species was 50-70% for the crosses examined (Dunham 2011).

The crossbred between AU-M female channel catfish and AU-K males had fast growth rate and exhibited heterosis for disease resistance and tolerance of low oxygen (Dunham and Smitherman 1985, Padi 2004). The effect of crossing three channel catfish strains, Red River, Norris, and Marion × Kansas (MXK), to improve the resistance to *Edwardsiella ictaluri* was also evaluated with a diallel crossing experiment design. The result of the *E. ictaluri* infection challenge showed that the progeny of Norris × MK had the highest survival rate (mean: 90.0 ± 1.5) when comparing to the control, Norris × Norris and MK×MK (Wolters and Johnson 1995). In Thailand, Prarom et al. (1990) improved the resistance to *Aeromonas hydrophila* of Gunther's walking catfish by using crossbreeding.

Sometimes, crossbreeding will only improve one trait, but can sometimes improve multiple traits. In channel catfish, the progeny of Marion female × Kansas male show improvement for multiple traits, including growth rate, sexual maturity, angling vulnerability, low oxygen tolerance and disease resistance (Dunham 2011). Crossbred AU-M×AU-K brood stock exhibit heterosis for early sexual maturity as these fish had high spawning rate at 3-year old compared to their parents (Dunham and Smitherman 1985).

Interspecific hybridization

The interspecific hybrid (female × male) of brook trout × rainbow trout shows an improve-

ment in disease resistance to viral hemorrhagic septicemia virus (VHSV) and infectious hemopoietic necrosis virus (IHNV) when compared to the parental species (Dorson et al. 1991). The interspecific hybrid of brown trout female and Atlantic salmon (*Salmo salar*) male exhibited intermediate resistance to skin fluke, *Gyrodactylus salaries* (Bakke et al. 1999) and over 69% improvement for resistance to amoebic gill disease (AGD) when compared to its parents (Maynard et al. 2016).

The reciprocal hybrids of *Xiphophorus maculatus* and *Xiphophorus variatus* showed higher resistance to *Ichthyophthirius multifiliis* when compared with their parents (Clayton and Price 1994). Koi (*Cyprinus carpio*)×goldfish (*Carassius auratus*) hybrids exhibited a higher survival rate (65%) when challenged with koi herpesvirus isolate E (KHV-E) compared to koi×crucian carp hybrids (91% mortality) and koi without any history of disease (100% mortality) (Bergmann et al. 2010). Hybrids of Nile tilapia (*Oreochromis niloticus*) female×blue tilapia (*Oreochromis aureus*) male exhibit higher resistance to *Aeromonas sobria* (Cai et al. 2004).

Interspecific hybridization does not always improve the traits of the hybrid progeny. The hybrid progeny of Atlantic salmon×Arctic char (*Salvelinus alpinus*) did not exhibit improved disease resistance to sea louse (*Lepeophtheirus salmonis*) when compared to the parents (Fleming et al. 2014). Arctic Char were susceptible to infectious pancreatic necrosis virus (IPNV), but resistant to viral haemorrhagic septicemia virus (VHSV). Their hybrid was susceptible to IPNV and partially resistant to VHSV (Dorson et al. 1991).

North American catfish interspecific catfish hybrids and their 7 parent species have been evaluated (Dunham and Smitherman 1984), and only the hybrid between channel catfish female and blue catfish males (CB hybrid) exhibited significant improvement in economically important traits when compared with both parent species. Despite its good aquaculture performance, the

CB hybrid was not commercialized for three decades due to reproductive barriers (Su et al. 2013).

With the development of effective hormone induction protocols, the CB hybrid is now an attractive choice for pond production. CB hybrid has superior growth rate, resistance to many diseases, higher survival and production in high-density ponds, tolerance of low dissolved oxygen, carcass yield and harvestability (Dunham and Masser 2012). Wolters et al. (1996) found that the channel-blue hybrid catfish has an intermediate resistance to *Edwardsiella ictaluri* compared with the channel catfish and blue catfish, and blue catfish has almost total resistance to *E. ictaluri.* In an *E. ictaluri* injection challenge, the survival and antibody levels were also intermediate between those of the channel and blue catfish. The CB catfish exhibit improvement of resistance to columnaris (Dunham and Masser 2012). When challenged with *Flavobacterium columnare* BGFS27 (genomovar II) strain, CB hybrid catfish exhibited lower mortality rate (31%) comparing with both parental species with blue catfish being the most sensitive species, 87% mortality rate, and the mortality rate of channel catfish was 74% (Arias et al. 2012). when challenged with *Flavobacterium columnare* ARS-1 (genomovar I) strain, a weaker strain, a genotype-environment interaction occurred with the channel catfish having the highest mortality rate (32%). Blue catfish and hybrid catfish were less susceptible to ARS-1, showing minimal mortalities, 4 and 9%, respectively (Arias et al. 2012).

Transgenesis

Transgenic technology is an efficient tool that could be used to improve the production of catfish in the future. Growth hormone gene has been transferred to various species such as Atlantic salmon, coho salmon (*Oncorhynchus kisutch*) (Devlin et al. 1995) and mud loach (*Misgurnus*

mizolepis) (Nam et al. 2001). The transgenic Atlantic salmon grew 2 to 6-fold faster when compared with non-transgenic controls (Du et al. 1992). Inserting the growth hormone (GH) gene resulted in 41% additional growth rate for channel catfish, which had already been mass-selected for five generations to improve growth rate (Dunham and Liu 2003). GH gene transfer in channel catfish also increases percent protein, lowers fat percentage and improves flavor and texture. Transgenesis can also be used to improve disease resistance of channel catfish. Cecropin gene from the moth (*Hyalophora cecropia*) improved disease resistance when transferred to channel catfish (Wang et al. 2019). When challenged with *E.ictaluri,* transgenic channel catfish carrying the cecropin B construct exhibited higher survival (40.7%) than the non-transgenic control channel catfish (14.8%). Only control channel catfish died while all cecropin transgenic fish survived during a natural epizootic of *F. columnare* in an earthen pond. Transgenic technology can also be used to improve cold tolerance (Wang et al. 2019), develop novel ornamental fish (Gong et al. 2003), monitor environmental pollution (Amanuma et al. 2000, Cachot et al. 2007), modify body composition (Yoshizaki et al. 2007, Cheng et al. 2014), and transgenically sterilize fish (Su 2012, Li et al. 2017, Li et al. 2018).

DNA markers and marker-assisted selection

All organisms are subject to mutations as a result of normal cellular operation or interaction with the environment. Mutation leads to a veritable and discernible genetic variation within and among individual species, and higher-order taxonomic groups. The heritable and discernible variation can be useful in aquaculture genetic research (Liu and Cordes 2004). DNA marker technology changed the way aquaculture genetics research was conducted. Theoretically, it is possible to observe and exploit genetic variation in the entire genome with DNA markers and genetic markers, such as allozymes, mitochondrial DNA, RFLP, RAPD, AFLP, microsatellite, SNP and

EST markers. The application of these DNA markers allowed various research, including investigations of genetic variability and inbreeding, parentage assignments, species and strain identification, the construction of high-resolution genetic linkage maps and fine chromosomal level genomes for aquaculture species (Liu and Cordes 2004, Liu 2017). The earliest markers used in aquaculture genetics were allozymes, allelic variants within proteins (enzymes) (Johnson et al. 1987, Liu et al. 1992). However, the low level of genetic resolution required sacrifice of the organisms, and issues related to silent or synonymous substitutions limited the usage of allozymes (Liu and Cordes 2004). RFLPs could detect genetic variation based on the DNA fragment lengths difference generated by restriction endonuclease (Botstein et al. 1980). In the 1980s, this marker was widely used in aquaculture and conservation research (Funkenstein et al. 1990, Karl and Avise 1992, Russell et al. 2000). However, RFLP markers have low polymorphism and require sequence information for target loci (Liu and Cordes 2004). These two disadvantages limit their usage in aquaculture species.

RAPD is a genetic marker that uses 8-10 arbitrary primers to randomly amplify anonymous segments of nuclear DNA (Welsh and McClelland 1990, Williams et al. 1990). Although RAPD markers are cost-effective and there are no requirements for the known target sequence. But RAPDs are inherited as dominant Mendelian markers, which makes it difficult to distinguish between homozygotes and heterozygotes (Liu and Cordes 2004). Low reproducibility further limits the use of this marker (Vos et al. 1995). Compared with RFLP and RAPD, AFLP provides better informative contents and higher resolution (Liu and Cordes 2004). AFLP markers have a large number of polymorphisms, high reproducibility and moderate costs. However, the need for special equipment for electrophoretic analysis and the poor genetic information on per marker basis hampered the widely spread use of this marker (Bensch and Akesson 2005) as well as the fact

that this is also a dominant marker.

Single nucleotide polymorphisms (SNPs) are generated by a point mutation that gives rise to alternative alleles at a given nucleotide position within a locus (Liu and Cordes 2004). Such sequence differences generated by base substitutions were discovered in 1997, but it was not until the late 1990s that SNP genotyping in large numbers of samples became possible. SNPs markers have abundance in the genome (coding and non-coding), low cost of genotyping, low genotyping error rate, ease of multiplexing, great reproducibility, amenability to high throughput processes and high level of resolution (Liu and Cordes 2004). These advantages allowed SNPs markers to quickly gained the center stage of aquaculture and conservation genetic research (Group 2001, Morin et al. 2004), except it is still quite costly if large numbers of markers are used. In recent years, SNPs have been broadly applied to aquaculture genetics studies for various purposes, such as species and hybrid identification, genetic diversity and resource analysis of aquaculture stocks, parental assignments and reproductive contribution, DNA markers, quantitative trait loci (QTL) and marker-assisted selection (MAS), EST markers in BAC contig mapping and integration of maps (Liu and Cordes 2004).

Marker-assisted selection (MAS) is now used for the genetic enhancement of aquaculture species (Zenger et al. 2019). MAS is the selection process which breeders choose high performing brood stock based on molecular markers (Liu and Cordes 2004) that are associated with the phenotype rather than selecting the phenotype directly. The assumption is that markers associate at high frequency with the gene or QTL (such as growth rate and disease resistance) due to genetic linkage. Selection based on these trait-associated markers can facilitate efficient and precise genetic enhancement programs. Many performance and production traits are complex and quantitative. Therefore, the core step of MAS is to correlate genetic and phenotypic variation through

procedures like QTL mapping and genome-wide association studies (GWAS) (Abdelrahman et al. 2017).

SNP arrays have been developed for many aquaculture species, such as Atlantic salmon (Lien et al. 2011, Houston et al. 2014, Xu et al. 2014, Yanez et al. 2016), Catfish (*I punctatus* and *I. furcatus*) (Liu et al. 2011, Zeng et al. 2017), Common carp *(Cyprinus carpio*) (Xu et al. 2014), European oysters (*Crassostrea Gigas* and *Ostrea edulis*) (Gutierrez et al. 2017), Pacific oyster (*Crassostrea Gigas*) (Hedgecock et al. 2015, Gutierrez et al. 2017, Qi et al. 2017), Pacific-white shrimp (*Litopenaeus vannamei*) (Jones et al. 2017), Rainbow trout (*Oncorhynchus mykiss*) (Palti et al. 2015), and Silver-lipped pearl oyster (*Pinctada maxima*) (Jones et al. 2013). The development and application of SNPs arrays greatly accelerate the progress of MAS. These high-density SNP genotyping arrays provide robust data for downstream QTL or GWAS analysis, some of the best examples of MAS include growth improvement in oyster, disease resistance in catfish (Geng et al. 2015, Zhou et al. 2017), and sex determination in salmon species (Pedersen et al. 2013, Ayllon et al. 2015, Barson et al. 2015).

Synthetic channel-blue backcross catfish and their hybrid with blue catfish

The synthetic channel-blue catfish breed used in the current study was produced by a series of backcrossing of channel-blue hybrid catfish with channel catfish (Fig. 1). The first generation backcross, channel catfish female × F1 male, grew at the same rate as the F1 hybrid, slower than the F2 and faster than channel catfish and blue catfish as fingerlings in ponds, but at a very low density, 22,250 fry/ha (Argue et al. 2014). However, during the second year for food fish production and at a high-density, 16,300 fingerlings/ha, the F1 backcross catfish had the slowest growth rate among these genetic types as there were genotype-age or genotype-environment interactions

(Argue et al. 2014). F1 backcross catfish also had the lowest dress out and fillet percentage among these genetic types but was most similar to channel catfish (Argue et al. 2003).

Interspecific backcrosses are a useful and efficient tool for genetic linkage mapping to investigate genome structure, function, and evolution. Channel-blue backcross progenies have been used as major resources for linkage and QTL analysis necessary for marker-assisted selection (Liu 1998 ab).

Liu et al. (2015) constructed a high-density genetic linkage map using the hybrid catfish system including F1interspecific hybrid catfish and F1 backcross catfish. This was followed by a series of GWAS experiments. The backcross catfish were used for contribute to the gene association identification including bone development, disease resistant, growth rate, heat stress and low oxygen tolerance (Jin et al. 2016, Li et al. 2017, Geng et al. 2017, Li et al. 2018, Tan et al. 2018, Wang et al. 2019).

The overall objective was to explore the potential of synthetic backcross catfish and the hybrid between backcross catfish females and blue catfish males for potential commercial application. A specific objectives of the current study was to determine the relative performance of channel catfish, synthetic channel blue backcross catfish and the hybrid between channel-blue backcross female and blue catfish male grown in earth ponds for body weight, relative body area (body area / (body area + head area)), and morphology, including total length, body length, body depth, head length, head depth, head width, caudal depth. Another objective was to determine if the channel-blue backcross catfish had sufficient genetic similarity to channel catfish that would result in heterosis when hybridized with blue catfish males.

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 brood stock used in this study were spawned and cultured at the Fish Genetics Unit, Auburn University Alabama. Initial development of these fish is described in Liu et al. (1998ab).

Channel catfish females were hybridized with blue catfish to produce F1 interspecific hybrids (F1). Then, F1 CB hybrid catfish males were backcrossed with channel catfish females to produce F1 backcross catfish. Next F1 backcross catfish males were backcrossed with channel catfish to produce F2 backcross catfish, and these males were backcrossed with channel catfish females, resulting in F3 backcross catfish. F3 backcross catfish were mated with each other to produce the synthetic channel-blue backcross catfish. The same F3 backcross catfish females were also hybridized with blue catfish males producing half-sib hybrids to the synthetic channel-blue backcross catfish.

Artificial spawning and fertilization

To induce ovulation, female catfish were injected intraperitoneally with luteinizing hormone releasing hormone analog (LHRHa). The priming injection was 20 μg/kg. After 12 hours, the second injection was administered as a dose of 100 μg/kg (Dunham and Masser 2012). Females were placed in wet spawning bags, and the spawning bag was labeled with a number and fish weight. The spawning bags were then immersed in flow-through tanks, 242.5 cm × 61 cm × 60 cm, 887.5L, with the fish set at a depth approximately half-way from bottom of the tank. The dissolved oxygen levels were maintained by diffusion of air into the tank via airstones and the

dissolved oxygen was maintained above 6 mg/L. Water temperature was 27 °C (Dunham and Masser 2012). The first check of spawning bags for eggs was 24 hours after the administration of the resolving dose and then every 4 hours.

When eggs were discovered on the spawning bags, the female was anesthetized with 100ppm tricaine methane sulfonate (MS 222) solution buffered with sodium bicarbonate. The female was not be removed from the MS 222 until the female was immobilized, but the gills were still slowly moving. When removed from the solution, the fish was rinsed with water to remove the remaining anesthetic. A dry and clean towel will be used to cover the female catfish. The eggs will be striped into a 30.5 cm metal pie pan coated with Crisco vegetable shortening. Any bloody eggs were rinsed with 0.9% saline solution made by adding 34 g of Morton's pickling salt to 3.75L of distilled water. Clumps and blood were removed before fertilization (Dunham and Masser 2012).

Before the female was stripped, the male catfish were sacrificed, and their testes were removed. Males used in this study were euthanized by blunt force trauma to the head followed by pithing. Their testes were extracted with scalpel and forceps. The incision was opened from the anus anteriorly three-fourths of the way to the head. The testes were gently cut away from the mesentery. The testes were rinsed with 0.9% saline solution to remove blood and dried gently with a towel. 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 (Dunham and Masser 2012).

The solution of sperm was added to metal pie pans and mixed with eggs at a rate of 10 mL per 100 grams of eggs. When the sperm solution was added and mixed thoroughly among eggs, a small amount of hatchery water was added to the pan to activate the sperm and eggs. After 5

minutes, the pans were transferred and submerged in a hatching trough with a calcium chloride drip (at a concentration of 50ppm). The eggs were kept in the tank for one hour and during this stage the eggs water harden, which is the effect of calcium chloride. The eggs were transferred to individual hatching baskets suspended in hatching troughs with flow water through. Water flow were maintained at 15 L per min (Dunham and Masser 2012). A motorized paddlewheel provided water agitation. To maintain hardness at a minimum of 50 ppm (Dunham and Masser 2012), a calcium chloride drip was placed at the inlet end of the hatching trough.

Eggs were checked every day for the growth of fungus. To control fungus, chemical treatment was implemented three times per day at 8-hour intervals. For the first of treatment, eggs were treated with 100 ppm formalin for an hour. The next treatment was copper sulfate (32 ppm) for 15 min, followed 8 hours later with formalin (100 ppm) for 15min. These 2 treatments were then alternated until eggs were near hatching. During the treatment, the water flow was turned off. When necessary, the fungus was manually removed.

Culture and Rearing

After fertilization, the embryos hatched in 5-6 days at a water temperature is between 23-28 C. The synthetic channel-blue backcross catfish and the hybrids were grown in tanks, 303.5 cm × 61 cm × 29.2 cm, 540L, for two months and fry were feed with 50% protein powdered feed. Then the fish were transferred to another larger tank ,631.1cm × 92.7 cm × 61cm, 3580L, water was supplied by an earth pond, for further.

Fingerlings were stocked into two 0.04 ha ponds (two genotypes per pond) at the end of 2018. The first pond (G16) was stocked with backcross channel and channel-blue synthetic backcross catfish females × blue catfish males at 9,550 fish/ha. Second pond (G17) was stocked with channel catfish and channel-blue synthetic backcross catfish female × blue catfish male hybrids

at 11,100 fish/ha. Ponds were fed with 32% protein floating catfish feed every day in summer and three times per week in winter. An aerator was utilized as needed to maintain dissolve oxygen above 3.0 mg/L, and water quality was checked three times per week. After 6 months, the fish were harvested and data collected, including body weight (BWT) and body shape.

Morphometrics and body area measurement

Individual picture of each fish were taken for future measurement. The following measurements were taken total length (TL), body length (BL), body depth (BD), head length (HL), body width (BW) and caudal depth (CD). Measurements were: total length from tip of snout to the posterior end of the tail fin; standard length form tip of snout to the posterior end of the caudal peduncle; body depth the maximum distance (find where the deepest vertical measurement can be made) between the dorsal and ventral portions of the fish; head length tip of snout to posterior edge of the operculum; body width posterior edge of the operculum and caudal depth at the minimum depth of the caudal peduncle (Fig. 2). Head area is the area of catfish between tip of snout to posterior edge of the operculum. Body area is the area between posterior edge of the operculum to the posterior end of the caudal peduncle. Body area and head area were measured by the function of ruler in Photoshop version 21.1.1 (Wang et al. 2015). Ruler function could measure the area you choose then transfer into a real area with the scale you set. Relative body area is calculated by dividing total body area (body area + head area). However, relative body shape changes as fish grow (Dunham et al. 1984; Dunham et al., 1986, Hutson et al. 2014) and absolute morphometric measurements are strongly influenced by absolute body weight and total length. Therefore, body shape measurements were standardized by dividing by total length.

Data analysis

Statistical analysis was conducted with SAS version 9.4. T-test and non-parametric test (Wilcoxon's rank-sum test) were used to analyze the body weight and relative body area. Correlation analysis was performed between two morphology measurements. ANOVA was used to compare the variation of morphology measurements in the four genotypes (G16 backcross catfish, G16 backcross × blue catfish, G17 backcross × blue catfish, and G17 channel catfish). The significant level of the all the tests was $P < 0.05$.

Results

Body weight

Backcross × blue catfish were 37.2% larger (139.8g) for body weight than the backcross catfish (107.0g) ($P < 0.001$) (Table 1). The skewness of backcross × blue catfish (Sk=1.013) and backcross catfish (Sk=0.534) were moderate and positive. The body weights of both genotypes were not normally distributed ($P < 0.05$) (Table 1, Fig.3).

Backcross × blue catfish was 31.3% larger (177.0g) for body weight than channel catfish (128.7) ($P < 0.001$) (Table 1). The body weights of both genotypes were not normally distributed ($P < 0.05$) (Table 1). The skewness of backcross × blue catfish (Sk=1.219) and channel catfish (Sk= 0.577) were moderate and positive (Table 1, Fig4).

Relative body area

Backcross × blue catfish (0.872) had a 1.6% larger for relative body area than the backcross catfish (0.857) ($P < 0.0001$) (Table 2). The relative body area of both genotypes was not normally distributed ($P < 0.0001$). The skewness of backcross × blue catfish (Sk=-5.195) and backcross catfish (Sk=-0.833) were significantly different from zero ($P < 0.0001$) (Table 2, Fig. 5)

Backcross × blue catfish (0.855) had a 1.0% larger for relative body area than channel catfish

(0.846) (Table 2). Relative body area of backcross × blue catfish (Sk=-2.473) was not normally distributed ($P < 0.0001$) (Table 2), and the population distribution for channel catfish was normal and positive (Sk=0.575) (Table 2, Fig.6).

Morphometrics

The mean HL/TL for G17 BC × B, G16 BC × B, BC, and C were 0.187, 0.175, 0.181, and 0.200, respectively, with C having the longest head and G16 BC × B having the shortest head ($P < 0.05$) (Table 3). The mean HW/TL for G17 BC × B, G16 BC × B, BC, and C were 0.103, 0.106, 0.104 and 0.096, respectively, with C having the narrowest head ($P <0.05$) (Table 3). The mean HD/TL for G17 BC × B, G16 BC × B, BC, and C were 0.125, 0.115, 0.118, and 0.123, respectively, with C and G17 BC × B having the deepest heads ($P <0.05$).The mean BW/TL for G17 BC × B, G16 BC × B, BC, and C were 0.119, 0.123, 0.130, and 0.122, respectively with BC having the widest body ($P <0.05$).The mean BD/TL for G17 BC × B, G16 BC × B, BC, and C were 0.169, 0.160, 0.150, and 0.158, respectively, with G17 BC × B having the deepest body ($P <0.05$) (Table 3).The mean CD/TL for G17 BC × B, G16 BC × B, BC, and C were 0.073, 0.074, 0.075 and 0.071, respectively, with C having the lowest caudal depth ($P <0.05$).The mean eye diameter for G17 BC × B, G16 BC × B, BC, and C were 0.94, 0.83, 0.81 and 0.90 ,respectively, with BC and 16 BC × B, having the smallest eye diameter ($P <0.05$) (Table 3).

Correlations among traits

BWT and HL were only correlated and weakly in backcross × blue catfish grown in pond G17 ($r=0.24$, $P < 0.05$) (Table 4). The correlation of BWT and HW was moderate to weak in backcross × blue catfish ($r=0.48$, $P < 0.0001$) and backcross catfish ($r=0.26$, $P < 0.05$) grown in pond G16. The correlation between BWT and BD was weak in backcross × blue catfish grown in pond G16

(r=0.15, P < 0.05) and channel catfish grown in pond G17 (r=0.36, P < 0.05). No significant correlation was found between BW and the following traits, HD, BW and CD, for any genotype. In general, BWT had little impact on the morphometric ratios at this life stage. The few correlations involving BWT and the morphometric ratios were not consistent among genetic types and were not consistent between the two groups of BC × B. In general, and as expected, the correlations between TL and the morphometric ratios were similar to those found between BWT and the morphometric ratios, but even weaker.

The correlation of relative BA and HL shows a weak correlation (r=-0.23, P < 0.05) in backcross catfish, and as expected as HL decreased relative BA increased (Table 4). The correlation of BA and HD was consistently negative and moderate across all genotypes and were as follows: backcross × blue catfish grown in pond G17 (r=-0.29, P < 0.05), backcross × blue catfish grown in pond G16 (r=-0.30, P < 0.05), backcross catfish G16 (r=-0.47, P < 0.0001) and channel catfish (r=-0.62, P < 0.0001).As head depth decreased, relative body area increased. Body and caudal traits had little impact as only BW was negatively correlated (r= -0.29, P < 0.05) with BA (Table 4).

Among the head traits, the correlation between HL and HW was weak and positive for all fish of mixed ancestry, G17 backcross × blue catfish (r=0.32, P < 0.05), G16 backcross × blue catfish (r=0.35, P < 0.0001), backcross catfish G16(r=0.26, P < 0.05), but not different from zero for channel catfish. The correlation of HL and HD was weak and positive for G17 backcross × blue catfish (r=0.25, P < 0.05), backcross catfish (r=0.21, P < 0.05) and channel catfish (r=0.40, P < 0.05), but was not different from zero for G16 backcross × blue catfish. The correlation of HW and HD shows a weak positive correlation in G17 backcross × blue catfish (r=0.26, P < 0.05), G16 backcross × blue catfish channel catfish (r=0.21, P < 0.05), channel catfish (r=0.41, P < 0.05) and

was not different from zero for backcross catfish (Table 4). In general, the head traits were moderately correlated with each other, but this appeared less significant and less common in backcross catfish.

The correlation between HL and BW was weak and moderate for G17 backcross × blue catfish ($r=0.23$, $P < 0.05$), and G16 backcross × blue catfish ($r=0.42$, $P < 0.0001$) and not different from zero for the other 2 genetic types. The correlation of HW and BD was weak and only significant for G17 backcross × blue catfish ($r =0.22$, $P < 0.05$). The correlation of HW and BW was moderately high and positive for G17 backcross × blue catfish grown ($r=0.68$, $P < 0.0001$), G16 backcross × blue catfish grown ($r=0.65$, $P < 0.0001$) and channel catfish ($r=0.51$, $P < 0.0001$). The correlation of HL and BD was low and variable for G17 backcross × blue catfish grown ($r=0.26$, $P < 0.05$), backcross catfish ($r=-0.28$, $P < 0.05$) and channel catfish ($r=0.39$, $P < 0.05$) (Table 4).

HD had a moderate correlation with BD in G17 backcross × blue catfish ($r=0.42$, $P < 0.001$), G16 backcross × blue catfish($r=0.30$, $P < 0.0001$), backcross catfish grown ($r=0.46$, $P < 0.0001$) and channel catfish($r=0.44$, $P < 0.05$). The two backcross × blue hybrid catfish both had positive low correlations, r=0.30-0.38, between HD and BW (Table 4).

The only significant correlation between HW and CD was $r=0.22$ ($P < 0.05$) for G17 BC × B, and the only one between HD and CD was $r=0.31$ ($P < 0.05$) for G16 BC × B. All HL-CD correlations were not different than zero (Table 4).

The correlation of BD and BW was weak and positive for G17 backcross × blue catfish ($r=0.22$, $P < 0.05$). All genetic types had a weak to moderate positive correlation for BD and CD ,and were G17 backcross × blue catfish ($r=0.28$, $P < 0.05$), G16 backcross × blue catfish ($r=0.45$, $P < 0.0001$), backcross catfish ($r=0.30$, $P < 0.05$) and channel catfish ($r=0.42$, $P < 0.05$).There were no significant correlations between BW and CD (Table 4).

As expected, BWT had a strong correlation with TL in G17 backcross × blue catfish ($r=0.90$, $P < 0.0001$), G16 backcross × blue catfish ($r=0.90$, $P < 0.0001$), backcross catfish grown ($r=0.93$, $P < 0.0001$) and channel catfish ($r=0.96$, $P < 0.0001$). Neither body weight nor total length was correlated with relative body area ($P > 0.05$) (Table 4).

Discussion

Backcross × blue catfish had a 37.5% higher body weight than backcross catfish, and a 31.3 % higher body weight than channel catfish. The growth rate of the backcross channel catfish appears to be similar to channel catfish. Apparently, the three generations of backcrossing to channel catfish results in a fish that is enough channel-like that its hybrid with blue catfish males exhibits heterotic growth. When F1 hybrid catfish (channel catfish ♀ × blue catfish ♂) were hybridized or backcrossed to blue catfish males, they grew more slowly than all parental types (Argue et al. 2014) apparently exhibiting negative epistasis. On average those fish would have been 2/3 blue catfish, thus it is not surprising there was no heterosis or growth improvement. The amount of heterosis for body weight observed for the backcross catfish × blue catfish was similar to that exhibited by channel catfish × blue catfish (Giudice 1966, Dunham et al. 1990, Argue et al. 2014).

Skewness coefficients for the weight of channel catfish can be affected by feeding competition which could cause by feeding rate, food size and other conditions (Moav and Wohlfarth 1973). In our study, the body weight distribution showed a moderate positive skewness for backcross × blue catfish and weak positive skewness for channel catfish and backcross catfish as in each population there are several individuals growing faster than the positive tail of the normal distribution. There were several backcross × blue catfish that were much larger than their cohorts. This skewness could aggravate competition for food as individuals with a slightly larger body size can obtain

more food and magnify this advantage (Dunham, 2011; Wohlfarth,1977). In an environment in which catfish rely more on an artificial food supplement, the feeding type (sinking or floating) and feed particle size affect population distributions for the body weight of channel catfish (McGinty, 1980). The feed which allow fish to have more time to consume will contribute to the size uniformity and the less skewness coefficients for the body weight. The magnification effect caused by feed has both genetic and environmental components. When food is plentiful, the skewness of body weight of understocked fingerling channel catfish gradually decreases as they grow to market size. The individual body weight distribution is close to symmetrical (Green et al. 2004)

Skewness is much more severe in common carp (Nakamura and Kasahara 1951, 1955,1956,1957; Moav and Wohlfarth 1973) and is associated with feeding. The skewness of body weight caused by competition for food was also found in pygmy sunfish (*Elassoma*) (Kimmel et al. 1986). In Nile tilapia, Azaza et al. (2010) found that skewness increased steeply in the groups that were offered large food particle size, indicating that few fish gained a major growth advantage over others if they good access the large particle size.

The relative body area of backcross × blue catfish was 1.6% larger than backcross catfish, and 1.0 % larger than channel catfish. The relative body area should be a good predictor of dress out percent and fillet percent as a larger relative body area equates to more edible flesh. The only weakness as a means of prediction is that it does not allow estimation of visceral waste. Thus, the backcross × blue catfish should have a higher carcass yield than channel catfish and backcross catfish. The channel × blue F1 hybrid catfish also has higher dress out % and fillet % compared to its parent species (Argue et al. 2003). The F1 backcross, channel catfish female crossed with F1 hybrid catfish male had very low dress out percent and fillet percentage compared to channel catfish, blue catfish and F1 hybrid catfish and all other backcross combinations (Argue et al. 2003).

This result for the F1 generation of channel catfish female × F1 hybrid male may be a result of negative epistasis. After three generations of backcrossing to channel catfish females and one generation of closed breeding, the relative body area leads to the prediction that the backcross catfish would still have a lower carcass yield than channel catfish.

The correlation between two traits varied among genotypes, however, some tendencies from the results were obvious. The weak negative correlation between relative body area and head depth existed in all genotypes. In all genotypes, body width, head depth and caudal depth were not significant correlated with relative body area. These results were similar to the correlation between these morphological traits and dress out percentage of channel catfish found by Dunham et al. (1983, 1985). However, for the correlation between relative body area and head length only a weak negative correlation ($P < 0.05$) was detected in backcross catfish. In another similar study, the correlation between head length (head length/total length) and dressing percentage among blue, channel, white and hybrid catfish were moderately negative (Dunham et al. 1983).

Individual selection for some traits is difficult such as dressing percentage since the fish must be sacrificed to measure the trait. However, if the trait is highly correlated with a second trait, indirect selection for these lethal traits might be practical provided that there is a correlated response (Dunham et al. 1985). The potential for genetic improvement of slaughter yield in common carp and sea bass (*Dicentrarchus labrax*) through indirect selection by using morphological predictors was proved possible (Vanpeputte et al. 2007; Prchal et al. 2018).

The backcross catfish and backcross × blue catfish have potential application in the commercial catfish industry. Channel catfish and blue catfish have their own culture advantages and disadvantages. Currently, the best genotype for catfish farming is the channel catfish female × blue catfish male hybrid, which has good performance for growth rate, food conversion rate, tolerance

of low dissolved oxygen, disease resistance, and carcass yield. Although this hybrid is perhaps the best example of overall genetic improvement of multiple traits in aquaculture genetics history, it can be improved further and it does not have total disease resistance (Wolters et al. 1996, Elaswad et al. 2019). An option to improve upon the F1 hybrid is developing a synthetic breed by interspecific backcrossing, the channel-blue backcross catfish. However, such a synthetic breed may not exhibit heterosis for the multitude of traits like what is observed in the F1 channel-blue hybrid catfish. Once a "channel-like" breed is established, hybridizing with blue catfish males might produce heterosis for multiple traits similar to what was seen for F1 channel-blue hybrid catfish.

Similarly, the backcross hybrid striped bass (*Morone saxatilis*) (sunshine bass female × striped bass male) perform as well as F1 hybrid striped bass (sunshine bass: white bass female × striped bass male) for many economically important traits such as growth rate (Lindell et al. 2004, Jenkins et al. 2007). And in tilapia, a F1 cold tolerant backcross tilapia was developed by hybridization (female *T. aurea* × male red tilapia, heterozygous for redness) followed by backcrossing (female *T. aurea* × male red tilapia, red phenotype). The F1 backcross tilapia shows similar tolerance traits with *Tilapia aurea* which is a cold tolerant species (Behrends et al. 1984).

Given that channel catfish have better resistance to columnaris and poor resistance to ESC, blue catfish are highly resistant to ESC, and there were promising results for growth rate and relative body area of backcross and backcross × blue catfish from current research, future studies should investigate the disease resistance traits in backcross catfish and backcross × blue. In addition, the SNP markers associated with disease resistance in catfish been investigated (Tan et al. 2018, Wang et al. 2019), thus, marker assistant selection to improve the disease resistance of backcross catfish and backcross × blue catfish should be examined.

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Table 1 Mean body weight of backcross catfish (BC) (derived from channel catfish (C), *Ictalurus punctatus*, and blue catfish (B), *I. furcatus*) and backcross catfish ♀ × blue catfish ♂ (BC × B) grown in Pond G16 (0.04-ha pond at 9550 fish / ha) for 9 months and BC × B and channel catfish (C) grown in pond G17 (0.04-ha pond at 11,100 fish/ha) for 9 months[1,2]

	Body Weight (g)			
	G17 BC × B	G16 BC × B	G16BC	G17C
Number	145	195	136	66
Mean ±SD	177.0±61.4	139.8±66.9	107.0a±52.4	128.7±46.7
CV	34.7	47.9	49.0	36.3
Maximum	449.0	439.0	257.0	255.0
Minimum	75.5	34.0	22.0	55.0
Skewness	1.21	1.01	0.53	0.58

[1] BC × B is the largest genetic type in each pond (non-parametric test (Wilcoxon's rank-sum test), $P < 0.05$)

[2]SD is standard deviation, CV is coefficient variation

Table 2 Mean relative body area1 of backcross catfish (BC) (derived from channel catfish (C), *Ictalurus punctatus*, and blue catfish (B), *I. furcatus*) and backcross catfish ♀ × blue catfish ♂ (BC × B) grown in Pond G16 (0.04-ha pond at 9550 fish / ha) for 9 months[2,3]

	Relative Body Area			
	G17 BC × B	G16 BC × B	G16BC	G17C
Number	107	198	133	43
Mean ±SD	0.854±0.022	0.871±0.021	0.857±0.026	0.846±0.027
CV	2.34	2.92	2.42	3.55
Maximum	0.898	0.921	0.917	0.926
Minimum	0.712	0.777	0.644	0.795
Skewness	-2.47	-5.19	-0.833	0.57

[1]Relative body area is the body area divided by the total area (body area + head area)

[2] BC × B is the largest genetic type in each pond (non-parametric test (Wilcoxon's rank-sum test), $P < 0.05$)

[3]SD is standard deviation, CV is coefficient variation

Table 3 Morphological measurement among relative body area (BA), total length (TL) body depth (BD), caudal depth (CD), body width (BW), head width (HW), head depth (HD) and head length (HL) of backcross catfish (BC) (derived from channel catfish (C), *Ictalurus punctatus*, and blue catfish (B), *I. furcatus*) and backcross catfish ♀ × blue catfish ♂ (BC × B) grown in Pond G16 (0.04-ha ponds at a density of 9550 fish / ha) for 9 months and BC × B and channel catfish (C) grown in pond G16 (0.04-ha ponds at a density of 11,100 fish/ha) for 9 months[1][2]

	G17 BC × B			G16 BC × B			G16 BC			G17 C		
Trait	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
Total Length	29.2[a]	3.1	10.7	27.1[b]	4.1	48.2	24.8[c]	4.0	46.7	26.0[ab]	3.5	13.3
Eye Diameter	0.94[a]	0.09	10.0	0.83[c]	0.09	3.0	0.81[c]	0.09	2.3	0.90[b]	0.10	3.2
Relative Body Area	0.855[bc]	0.022	2.6	0.872[a]	0.025	3.0	0.857[b]	0.021	2.4	0.846[c]	0.026	3.2
Standard Length	0.821[a]	0.043	5.5	0.805[b]	0.036	4.4	0.800[b]	0.034	4.4	0.824[a]	0.035	3.1
Body depth	0.169[a]	0.011	7.2	0.160[b]	0.010	7.0	0.150[c]	0.010	6.6	0.158[b]	0.010	5.7
Head Length	0.187[b]	0.011	7.1	0.175[d]	0.012	6.6	0.181[c]	0.014	6.7	0.200[a]	0.016	7.3
Caudal Depth	0.073[a]	0.07	8.7	0.074[a]	0.005	5.5	0.075[a]	0.008	9.2	0.071[b]	0.006	6.4
Body width	0.119[c]	0.019	16.2	0.123[b]	0.009	8.0	0.130[a]	0.022	11.1	0.123[bc]	0.014	9.8
Head width	0.103[a]	0.014	13.7	0.106[a]	0.011	11.0	0.104[a]	0.011	11.8	0.096[b]	0.014	12.0
Head depth	0.125[b]	0.010	6.9	0.115[a]	0.010	7.7	0.118[a]	0.010	8.1	0.123[b]	0.012	10.0

[1] body depth (BD), caudal depth (CD), body width (BD), head width (HW), head depth (HD) and head length (HL) were standard by dividing by total length. Relative body area is the body area divided by the total body area (body area + head area).
[2]Means followed by the same letter in the same row are not different (F- test, $P > 0.05$)
[3]SD is standard deviation, CV is coefficient variation

Table 4 Correlations among body weight (BWT), relative body area (BA), total length (TL) body depth (BD), caudal depth (CD), body width (BW), head width (HW), head depth (HD) and head length (HL) of backcross catfish (BC) (derived from channel catfish (C), *Ictalurus punctatus*, and blue catfish (B), *I. furcatus*) and backcross catfish ♀ × blue catfish ♂ (BC × B) grown in Pond G16 (0.04-ha pond at 9550 fish / ha) for 9 months and BC × B and channel catfish (C) grown in pond G16 (0.04-ha pond at 11,100 fish/ha) for 9 months[12].

	Correlation			
	Genetic Type			
Traits	G17 BC × B	G16 BC × B	G16 BC	G17 C
BWT-HL/TL	0.24*	0.07	0.01	0.05
BWT-HW/TL	0.05	0.48**	0.26*	0.06
BWT-HD/TL	0.07	0.07	-0.06	0.18
BWT-BD/TL	0.11	0.15*	0.16	0.36*
BWT-BW/TL	0.05	0.14	0.06	-0.16
BWT-CD/TL	0.01	0.11	0.03	0.13
TL-HL/TL	0.24*	-0.05	0.02	-0.03
TL-HW/TL	0.06	-0.05	0.25*	0.03
TL-HD/TL	-0.19	-0.13	-0.19*	0.10
TL-BD/TL	-0.17	0.09	-0.01	0.21
TL-BW/TL	-0.19	-0.05	0.02	-0.14
TL-CD/TL	-0.07	0.03	-0.05	0.03
BA-HL/TL	-0.20	-0.05	-0.23*	-0.03
BA-HW/TL	-0.18	-0.03	-0.11	0.03
BA-HD/TL	-0.29*	-0.30*	-0.47**	-0.62**
BA-BD/TL	0.05	0.04	0.02	-0.29
BA-BW/TL	-0.29*	-0.14	-0.05	0.01
BA-CD/TL	0.00	0.14	0.08	0.14
HL/TL -HW/TL	0.32*	0.35**	0.26*	-0.10
HL/TL -HD/TL	0.25*	0.21*	-0.13	0.40*
HL/TL -BD/TL	0.26*	0.02	-0.28*	0.39*
HL/TL -BW/TL	0.23*	0.42	0.05	-0.09
HL/TL -CD/TL	0.01	-0.07	-0.08	0.04
HD/TL-BD/TL	0.42**	0.30**	0.46**	0.44*
HD/TL-BW/TL	0.38**	0.30**	0.05	0.02
HD/TL-CD/TL	0.14	0.31**	0.09	0.05
HW/TL-HD/TL	0.26*	0.21*	0.11	0.41*
HW/TL- BD/TL	0.22*	0.06	0.06	0.09
HW/TL -BW/TL	0.68**	0.65**	0.11	0.51*
HW/TL-CD/TL	0.22*	0.10	0.08	-0.08
BD/TL-BW/TL	0.22*	0.02	0.10	0.09

BD/TL-CD/TL	0.28*	0.45**	0.30*	0.42*
BW/TL-CD/TL	0.13	0.13	-0.01	-0.15
BWT-TL	0.90**	0.90**	0.93**	0.96**
BWT-BA	0.10	0.10	0.06	-0.21
TL-BA	0.12	0.12	0.07	-0.18

[1] body depth (BD), caudal depth (CD), body width (BW), head width (HW), head depth (HD) and head length (HL) were standard by dividing by total length (TL). Relative body area is the body area divided by the total body area (body area + head area).
[2]Correlation is significant * ($P < 0.05$) and ** ($P < 0.0001$)

Fig. 1 Pedigree for development of synthetic channel catfish *(Ictalurus punctatus*)-blue catfish (*Ictalurus furcatus*) backcross breed and the hybrid between the backcross female and blue catfish male

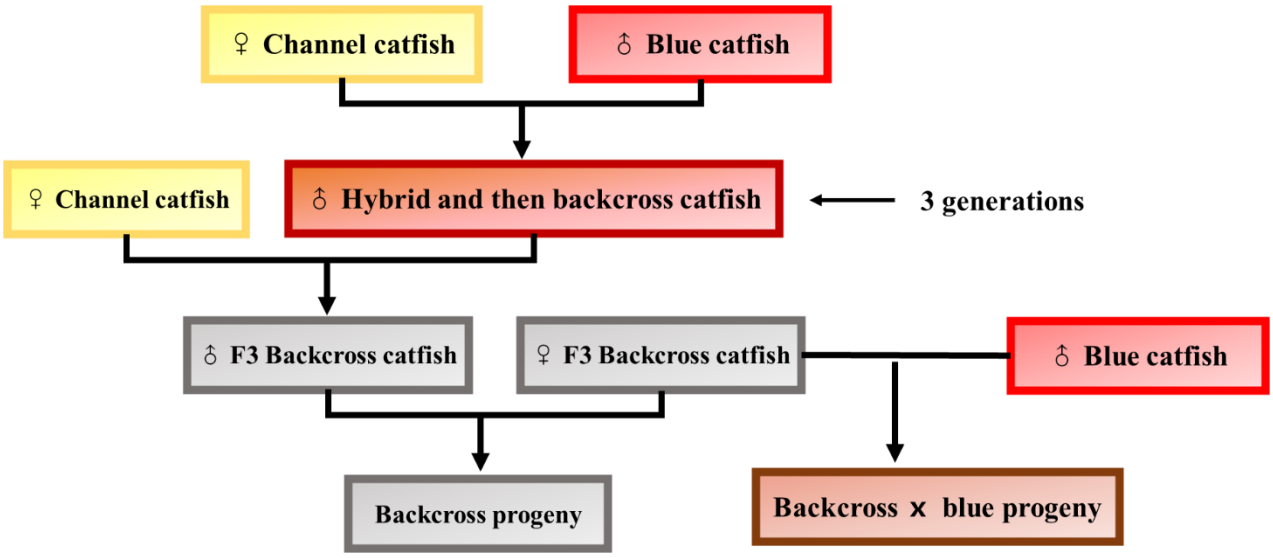
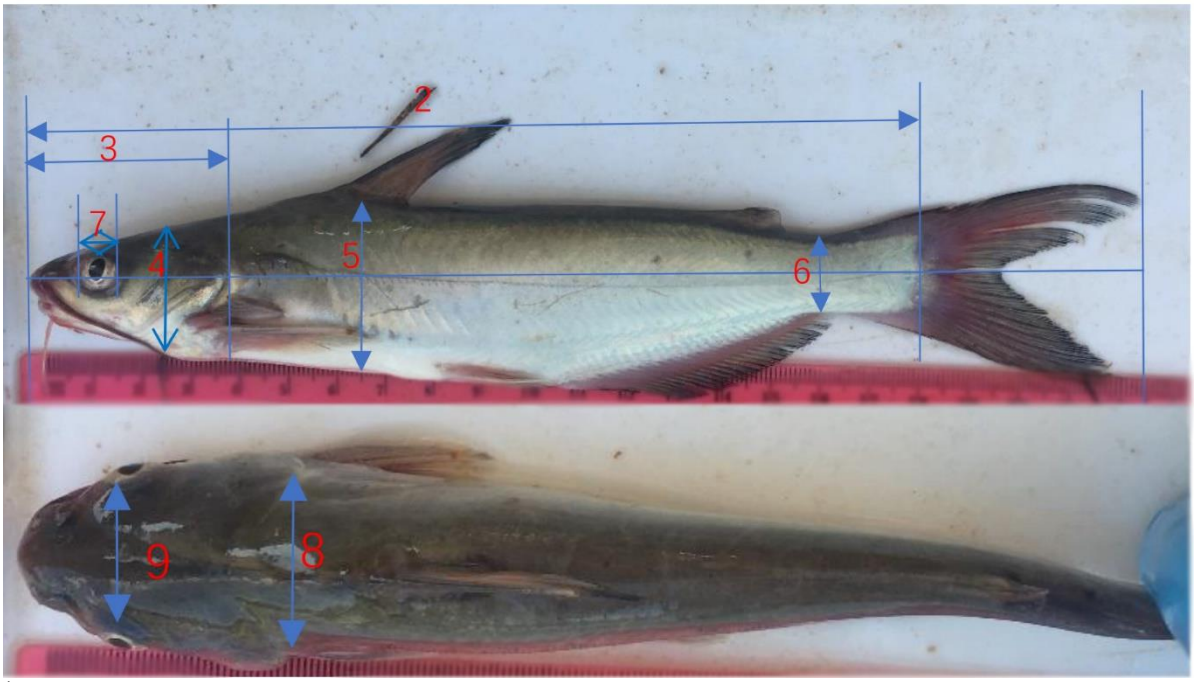


Fig. 2 Morphological measurement for total length, standard length, body depth, caudal depth, body width, head width, head depth and head length of backcross catfish (BC) (derived from channel catfish (C) , *Ictalurus punctatus*, and blue catfish (B), *I. furcatus*) and backcross catfish ♀ × blue catfish ♂ (BC × B) grown in pond G16 (0.04-ha ponds at a density of 9550 fish / ha) for 9 months and BC × B and channel catfish (C) grown in pond G16 (0.04-ha ponds at a density of 11,100 fish/ha) for 9 months[1]



[1]1 Total length: from tip of snout to the posterior end of the tail fin, 2 standard length: body length form tip of snout to the posterior end of the caudal peduncle, 3 head length: tip of snout to posterior edge of the operculum, 4 head depth: greatest vertical depth of the head, 5 body depth: maximum distance (find where the deepest vertical measurement can be made) between the dorsal and ventral portions of the fish, 6 caudal depth: minimum depth of the caudal peduncle, 7 eye diameters: largest horizontal length, 8 body width: measured as the greatest width of the body, 9 head width: distance between eyes

Fig. 3 Body weight distribution of backcross catfish (BC) (derived from channel catfish (C), *Ictalurus punctatus*, and blue catfish (B), *I. furcatus*) and backcross catfish ♀ × blue catfish ♂ (BC × B) grown in 0.04-ha pond at 9550 fish / ha for 9 months

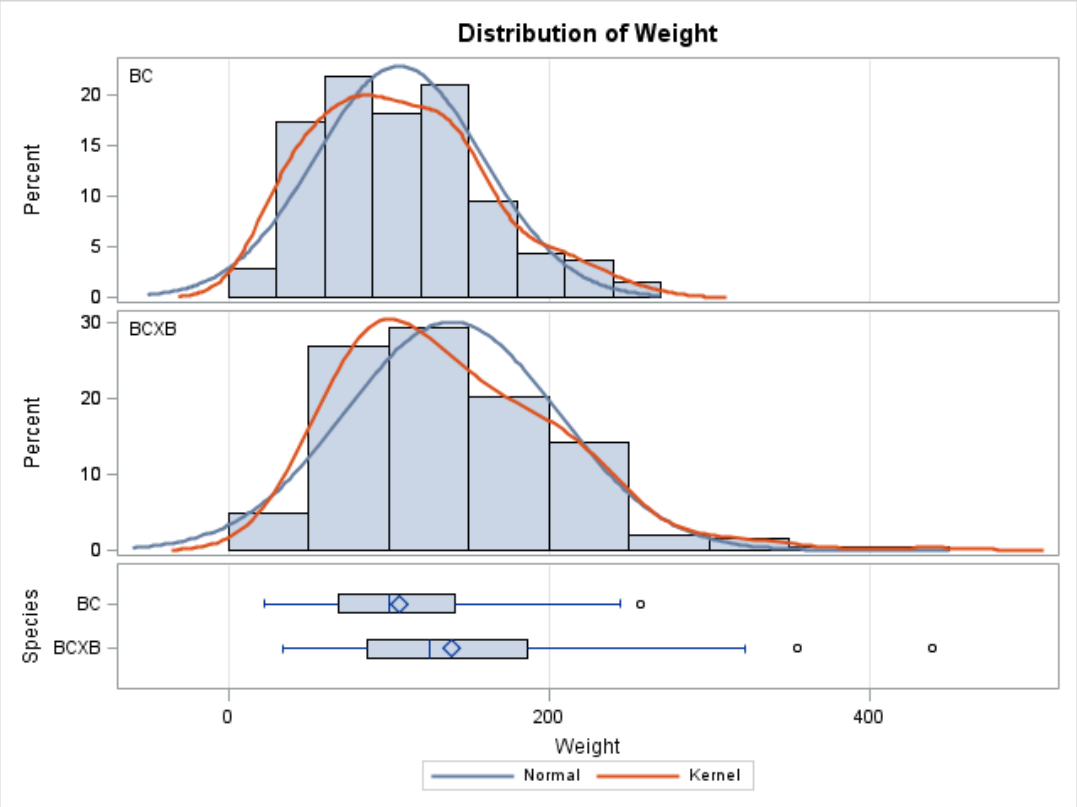


Fig. 4 Body weight distribution of backcross (derived from channel catfish (C), *Ictalurus punctatus*, and blue catfish (B), *I. furcatus*) catfish (BC) ♀ × blue catfish ♂ (BC × B) and channel catfish (C) grow in 0.04-ha ponds at a density of 11,100 fish / ha for 9 months

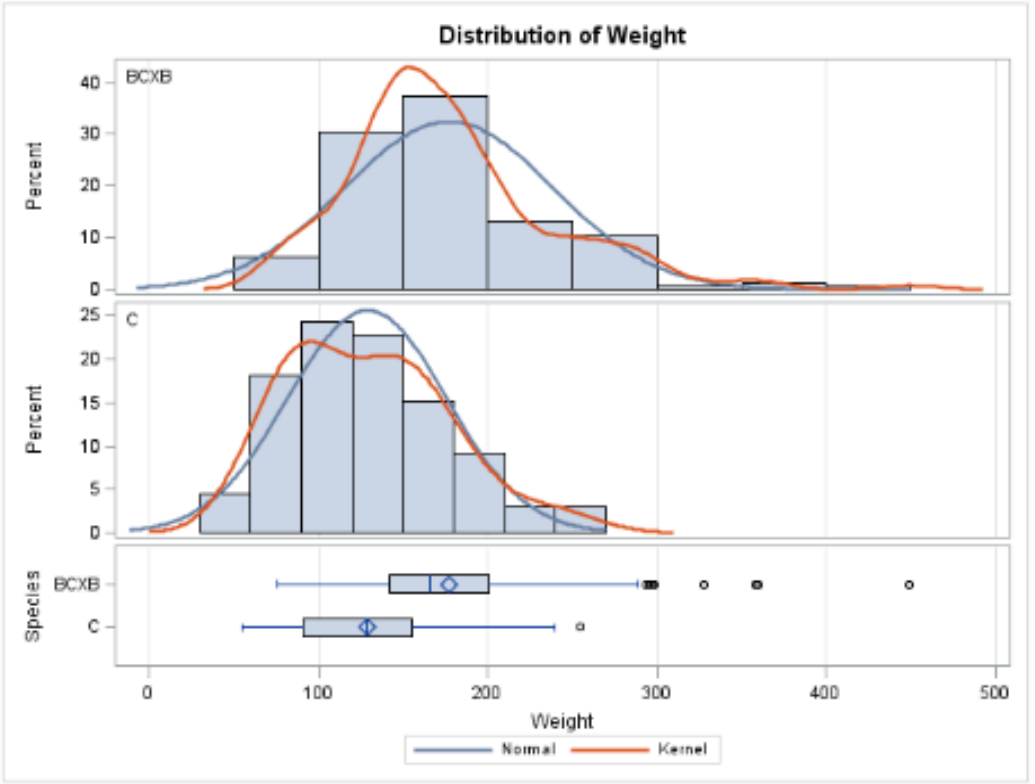
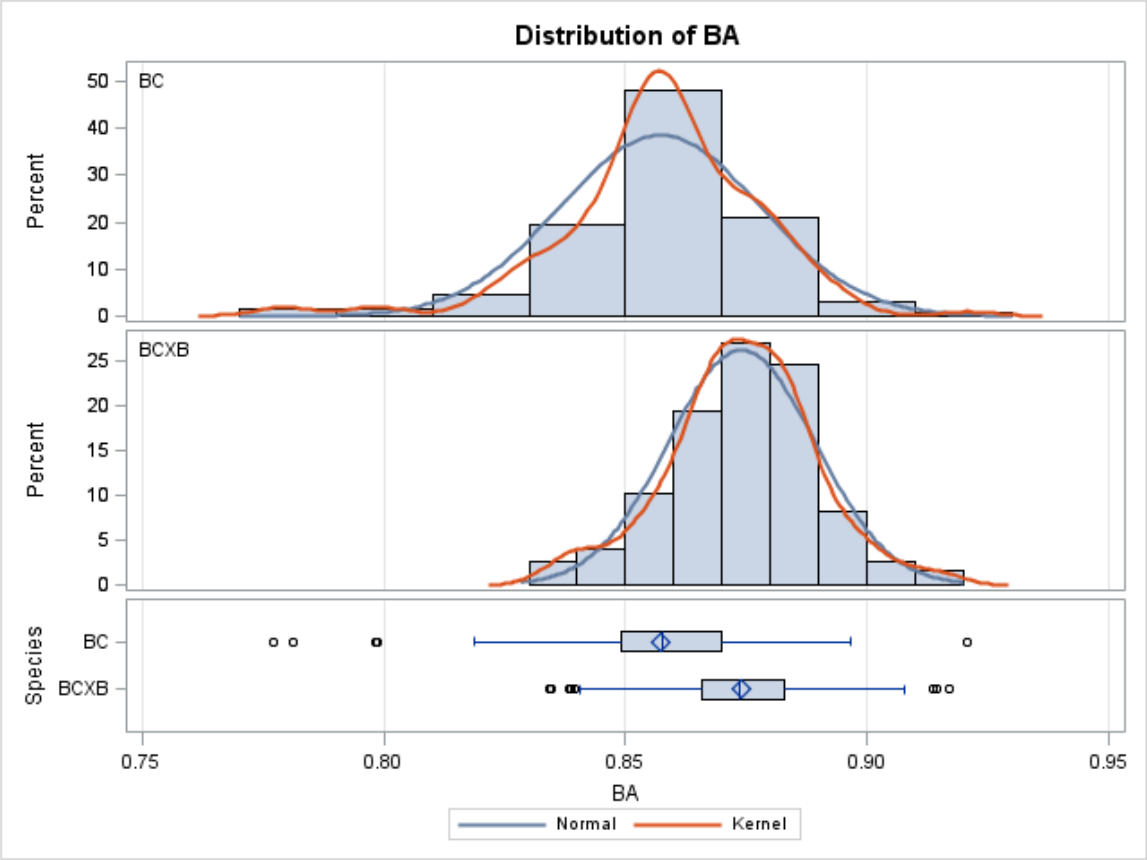
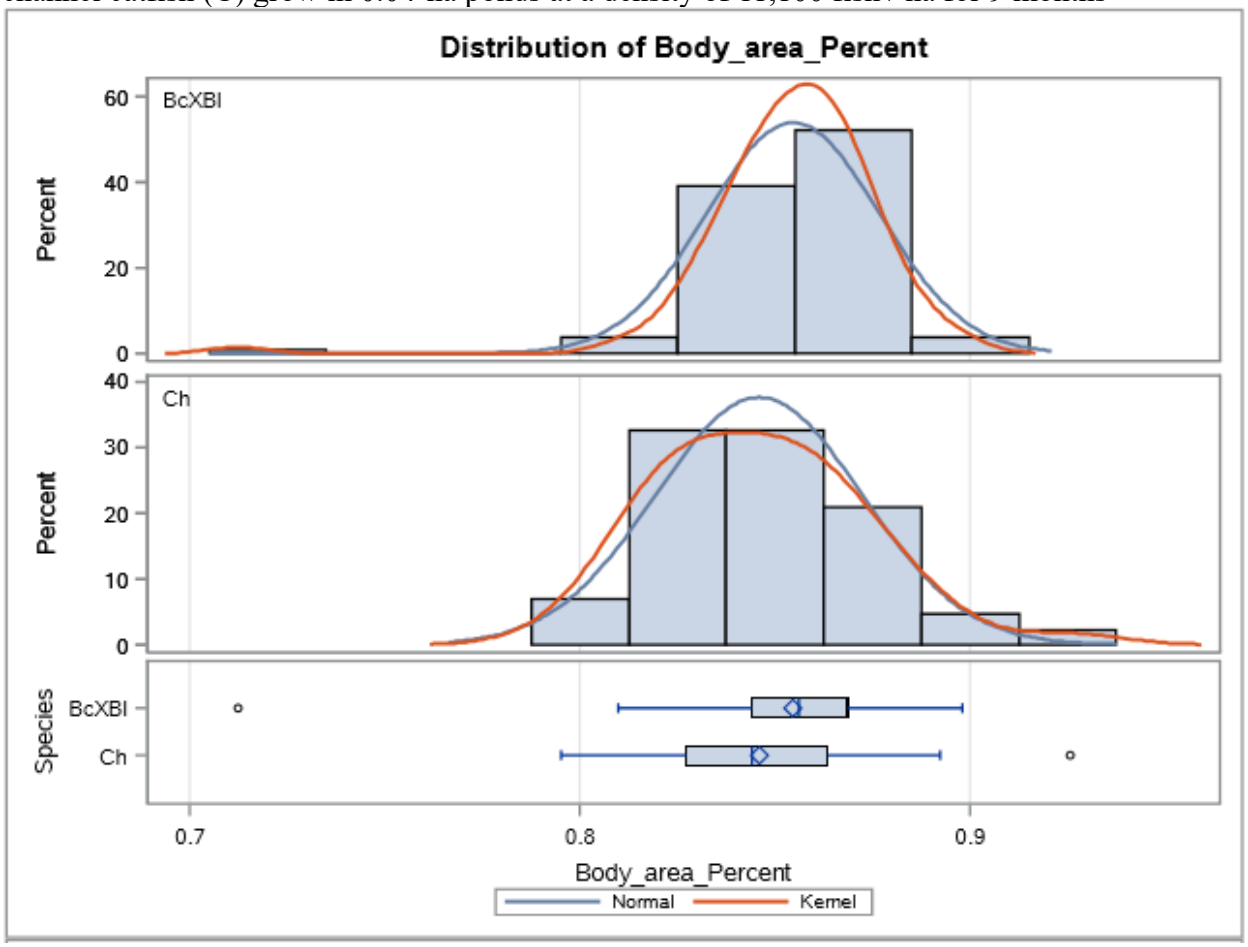


Fig. 5 Relative body area[1] distribution of backcross catfish (BC) (derived from channel catfish (C), *Ictalurus punctatus*, and blue catfish (B), *I. furcatus*) and backcross catfish ♀ × blue catfish ♂ (BC × B) grown in 0.04-ha pond at 9550 fish / ha for 9 months



[1]Relative body area is the body area divided by the total area (body area + head area)

Fig. 6 Relative body area[1] distribution of backcross (derived from channel catfish (C), *Ictalurus punctatus*, and blue catfish (B), *I. furcatus*) catfish (BC) ♀ × blue catfish ♂ (BC × B) and channel catfish (C) grow in 0.04-ha ponds at a density of 11,100 fish / ha for 9 months



[1]Relative body area is the body area divided by the total area (body area + head area)

Appendix

Table1 T-test analysis procedure of weight of backcross catfish (BC) (derived from channel catfish (C), *Ictalurus punctatus*, and blue catfish (B), *I. furcatus*) and backcross catfish ♀ × blue catfish ♂ (BC × B) grown in 0.04-ha pond at 9550 fish / ha for 9 months

Method	**Variances**	**DF**	**t-Value**	**Pr > |t|**
Pooled	Equal	340	-4.84	<.0001
Satterthwaite	Unequal	331.82	-5.06	<.0001

Equality of Variances				
Method	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Folded F	203	137	1.6	0.0033

Table 2 No-parametric procedure of weight of backcross catfish (BC) (derived from channel catfish (C), *Ictalurus punctatus*, and blue catfish (B), *I. furcatus*) and backcross catfish ♀ × blue catfish ♂ (BC × B) grown in 0.04-ha pond at 9550 fish / ha for 9 months

Wilcoxon Two-Sample Test	
Statistic	15479.5
Normal Approximation	
Z	-7.7323
One-Sided Pr < Z	<.0001
Two-Sided Pr > |Z|	<.0001
t Approximation	
One-Sided Pr < Z	<.0001
Two-Sided Pr > |Z|	<.0001

Table 3 T-test analysis procedure of relative body area of backcross (derived from channel catfish (C), *Ictalurus punctatus*, and blue catfish (B), *I. furcatus*) catfish (BC) ♀ × blue catfish ♂ (BC × B) grow in 0.04-ha ponds at a density of 11,100 fish / ha for 9 months[1]

The TTEST Procedure

Method	**Variances**	**DF**	**t Value**	**Pr > |t|**
Pooled	Equal	148	2.05	0.0419
Satterthwaite	Unequal	66.922	1.9	0.0613

Equality of Variances

Method	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Folded F	42	106	1.42	0.1504

[1]Relative body area is the body area divided by the total area (body area + head area)

Table 4 No-parametric procedure of relative body area[1] of backcross (derived from channel catfish (C), *Ictalurus punctatus*, and blue catfish (B), *I. furcatus*) catfish (BC) ♀ × blue catfish ♂ (BC × B) grow in 0.04-ha ponds at a density of 11,100 fish / ha for 9 months[1]

Wilcoxon Two-Sample Test	
Statistic	15479.5
Normal Approximation	
Z	-7.7323
One-Sided Pr < Z	<.0001
Two-Sided Pr > |Z|	<.0001
t Approximation	
One-Sided Pr < Z	<.0001
Two-Sided Pr > |Z|	<.0001

[1]Relative body area is the body area divided by the total area (body area + head area)

Table 5 Normality test of body weight of backcross catfish (BC) ♀ (derived from channel catfish (C), *Ictalurus punctatus*, and blue catfish (B), *I. furcatus*) × blue catfish ♂ (BC × B) grow in 0.04-ha pond at 9550 fish /ha for 9 months[1]

Test	Statistic		p Value		Skewness
Shapiro-Wilk	W	0.923237	Pr < W	0.0015	1.013
Kolmogorov-Smirnov	D	0.121012	Pr > D	0.1469	
Cramer-von Mises	W-Sq	0.545776	Pr > W-Sq	0.0536	
Anderson-Darling	A-Sq	2.945009	Pr > A-Sq	0.0124	

[1]As the sample number is less than 2000, the result of Shapiro-Wilk is more accrue

Table 6 Normality test of backcross catfish (BC) (derived from channel catfish (C), *Ictalurus punctatus*, and blue catfish (B), *I. furcatus*) (BC) grow in 0.04-ha pond at 9550 fish /ha for 9 months[1]

Test	Statistic		p Value		Skewness
Shapiro-Wilk	W	0.939079	Pr < W	<0.0001	0.534
Kolmogorov-Smirnov	D	0.10143	Pr > D	<0.0100	
Cramer-von Mises	W-Sq	0.420184	Pr > W-Sq	<0.0050	
Anderson-Darling	A-Sq	0.420184	Pr > A-Sq	<0.0050	

[1]As the sample number is less than 2000, the result of Shapiro-Wilk is more accrue

Table 7 Normality test of body weight of backcross (derived from channel catfish (C), *Ictalurus punctatus*, and blue catfish (B), *I. furcatus*) catfish (BC) ♀ × blue catfish ♂ (BC × B) grow in 0.04-ha ponds at a density of 11,100 fish / ha for 9 months[1]

Test		Statistic	p Value		Skewness
Shapiro-Wilk	W	0.923237	Pr < W	<0.0001	1.219
Kolmogorov-Smirnov	D	0.121012	Pr > D	<0.0001	
Cramer-von Mises	W-Sq	0.545776	Pr > W-Sq	<0.0050	
Anderson-Darling	A-Sq	2.945009	Pr > A-Sq	<0.0050	

[1]As the sample number is less than 2000, the result of Shapiro-Wilk is more accrue

Table 8 Normality test of body weight of channel catfish (C), *Ictalurus punctatus*, grow in 0.04-ha ponds at a density of 11,100 fish / ha for 9 months[1]

Test	Statistic		p Value		Skewness
Shapiro-Wilk	W	0.958198	Pr < W	0.0259	0.577
Kolmogorov-Smirnov	D	0.125459	Pr > D	0.0107	
Cramer-von Mises	W-Sq	0.112196	Pr > W-Sq	0.0794	
Anderson-Darling	A-Sq	0.747202	Pr > A-Sq	0.0492	

[1]As the sample number is less than 2000, the result of Shapiro-Wilk is more accrue

Table 9 Normality test of relative body area[1] of backcross catfish (BC) (derived from channel catfish (C), *Ictalurus punctatus*, and blue catfish(B), *I. furcatus*) ♀ × blue catfish ♂ (BC × B) grow in 0.04-ha ponds at a density of 9550 fish /ha for 9 months[2]

Test		Statistic	p Value		Skewness
Shapiro-Wilk	W	0.621214	Pr < W	<0.0001	-5.195
Kolmogorov-Smirnov	D	0.164834	Pr > D	<0.0001	
Cramer-von Mises	W-Sq	1.870503	Pr > W-Sq	<0.0050	
Anderson-Darling	A-Sq	11.4063	Pr > A-Sq	<0.0050	

[1]Relative body area is the body area divided by the total area (body area + head area)
[2]As the sample number is less than 2000, the result of Shapiro-Wilk is more accrue

Table 10 Normality test of relative body area[1] of backcross catfish (derived from channel catfish (C), *Ictalurus punctatus*, and blue catfish (B), *I. furcatus*) (BC) grow in 0.04-ha ponds at a density of 9550 fish /ha for 9 months[12]

Test	Statistic		p Value		Skewness
Shapiro-Wilk	W	0.942282	Pr < W	<0.0001	-0.833
Kolmogorov-Smirnov	D	0.099808	Pr > D	<0.0001	
Cramer-von Mises	W-Sq	0.311542	Pr > W-Sq	<0.0050	
Anderson-Darling	A-Sq	1.821014	Pr > A-Sq	<0.0050	

[1]Relative body area is the body area divided by the total area (body area + head area)
[2]As the sample number is less than 2000, the result of Shapiro-Wilk is more accrue

Table 11 Normality test of relative body area[1] of backcross catfish (BC) ♀ (derived from channel catfish (C), *Ictalurus punctatus* ,and blue catfish (B), *I. furcatus*) × blue catfish ♂ (BC × B) grown in 0.04-ha ponds at a density of 11,100 fish / ha for 9 months[12]

Test		Statistic	p Value		Skewness
Shapiro-Wilk	W	0.8388	Pr < W	<0.0001	-2.472
Kolmogorov-Smirnov	D	0.086548	Pr > D	0.0473	
Cramer-von Mises	W-Sq	0.230085	Pr > W-Sq	<0.0050	
Anderson-Darling	A-Sq	1.686844	Pr > A-Sq	<0.0050	

[1]Relative body area is the body area divided by the total area (body area + head area)
[2]As the sample number is less than 2000, the result of Shapiro-Wilk is more accrue

Table 12 Normality test of relative body area[1] of channel catfish (C), *Ictalurus punctatus*, grown in 0.04-ha pond at 11,100 fish / ha for 9 months[12]

Test		Statistic		p Value	Skewness
Shapiro-Wilk	W	0.975179	Pr < W	0.4690	0.584
Kolmogorov-Smirnov	D	0.076474	Pr > D	>0.1500	
Cramer-von Mises	W-Sq	0.035539	Pr > W-Sq	>0.2500	
Anderson-Darling	A-Sq	0.262962	Pr > A-Sq	>0.2500	

[1]Relative body area is the body area divided by the total area (body area + head area)
[2]As the sample number is less than 2000, the result of Shapiro-Wilk is more accrue

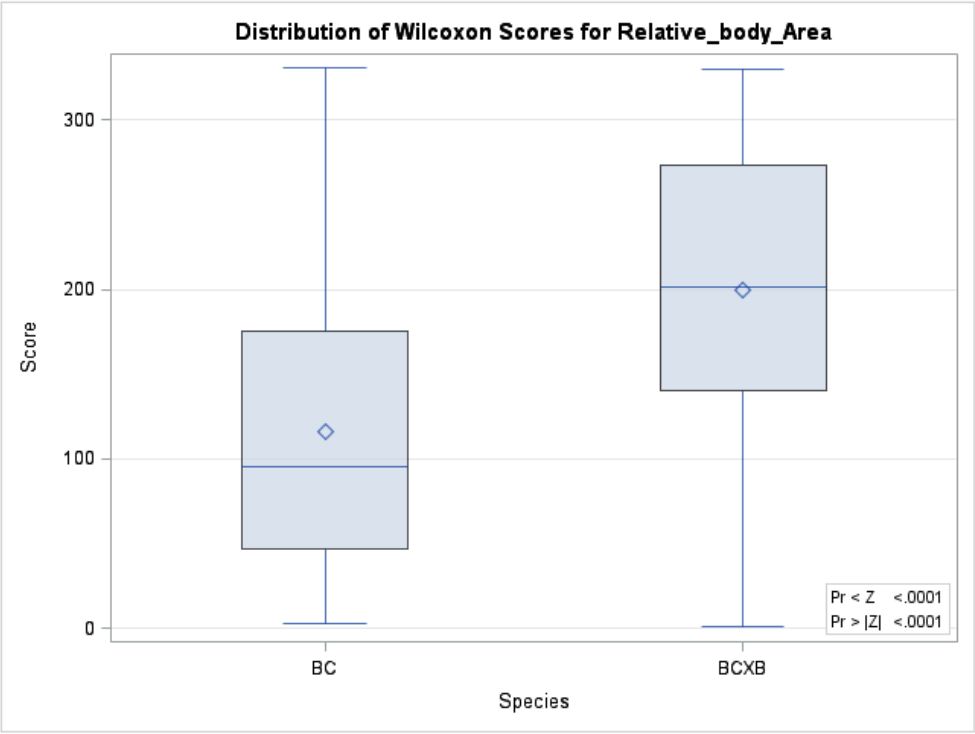
Fig. 1 Wilcoxon scores distribution of backcross catfish (BC) (derived from channel catfish (C), *Ictalurus punctatus*, and blue catfish (B), *I. furcatus*) and backcross catfish ♀ × blue catfish ♂ (BC × B) grown in 0.04-ha pond at 9550 fish / ha for 9 months[1]

[1]Higher score distribution means higher body weight distribution

Fig. 2 Wilcoxon scores distribution of body weight of backcross (derived from channel catfish (C), *Ictalurus punctatus*, and blue catfish (B), *I. furcatus*) catfish (BC) ♀ × blue catfish ♂ (BC × B) grow in 0.04-ha ponds at a density of 11,100 fish / ha for 9 months[1]

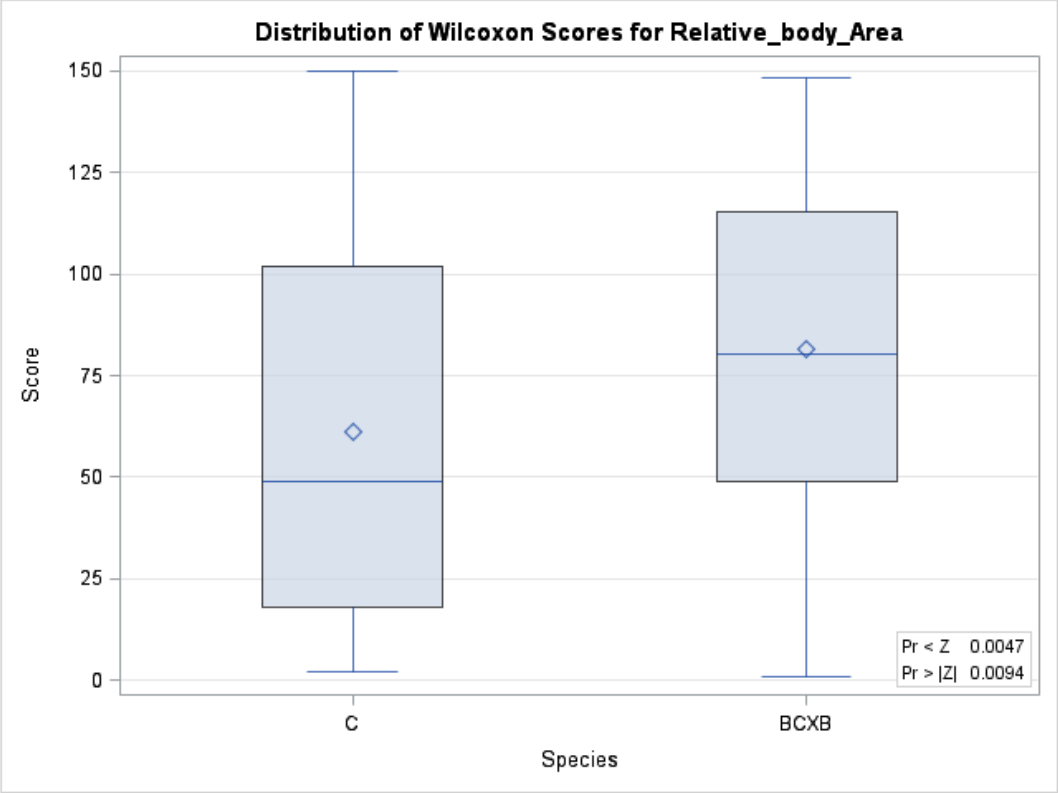
[1]Higher score distribution means higher body weight distribution

Fig. 3 Wilcoxon scores distribution of backcross catfish (BC) (derived from channel catfish (C), *Ictalurus punctatus*, and blue catfish (B), *I. furcatus*) and backcross catfish ♀ × blue catfish ♂ (BC × B) grown in 0.04-ha pond at 9550 fish / ha for 9 months[1]



[1]Higher score distribution means higher body weight distribution

Fig. 4 Wilcoxon scores distribution of backcross (derived from channel catfish (C), *Ictalurus punctatus*, and blue catfish (B), *I. furcatus*) catfish (BC) ♀ × blue catfish ♂ (BC × B) grow in 0.04-ha ponds at a density of 11,100 fish / ha for 9 months[1]



[1]Higher score distribution means higher body weight distribution