Growth Differences of Growth Hormone Transgenic Female and Male Channel Catfish, *Ictalurus punctatus*, Grown in Earthen Ponds to Sexual Maturation

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Abstract
This study compared growth performance between female and male transgenic channel catfish, *Ictalurus punctatus*, containing channel catfish growth hormone full-length cDNA driven by the ocean pout antifreeze protein promoter, opAFP-ccGH, the rainbow trout metallothionein promoter, rtMT-ccGH, or both constructs, and their non-transgenic siblings in earthen ponds at 16 and 48 months of age. Body weight between the transgenic and their non-transgenic siblings differed (*P* < 0.001) at all ages. Transgenic F2 opAFP-ccGH grew 1.51- to 2.58-, F2 rtMT-ccGH grew 1.44- to 2.99- and F1 fish transgenic for both constructs grew 1.36- to 2.92- fold larger than their non-transgenic sibling controls, depending upon age and sex. Body weight of the transgenic GH males was significantly higher than those of the transgenic GH females at 16 months of age (*P* < 0.001). However, body weight of the transgenic GH females was significantly higher (*P* < 0.001) compared with those of the transgenic GH males at 48 months of age, but not for the double transgenics (*P* > 0.05). In the case of non-transgenic GH siblings, males were larger than females at both 16 and 48 months of age (*P* < 0.001). Sexually dimorphic responses to GH transgenes were the opposite after sexual maturation. When critically low dissolved oxygen levels were encountered, survival of transgenic male and female opAFP-ccGH channel catfish was lower than that of controls (*P* = 0.004), as well as rtMT-ccGH females (*P* = 0.11), which is not surprising since the largest fish are most likely to succumb during an oxygen depletion.

Keywords Transgenic channel catfish · Growth rate · Growth hormone (GH) cDNA · opAFP-ccGH · rtMT-ccGH · Sexual maturation

Introduction
More than 820 million people are suffering from chronic undernourishment in the world (FAO 2021a), and the vast majority of the world’s hungry people live in developing countries (FAO 2021b; World Bank 2021). The global population is expected to grow by 47% and reach 11.2 billion people by 2100 (United Nations 2017). As the world population grows, the global demand for food is growing rapidly (FAO 2018). Aquaculture products are an important source of animal protein and nutrition around the world (USSEC 2018). Genetic improvement of aquaculture species is an effective way to address the challenge of hunger.
Channel catfish, *Ictalurus punctatus*, is the primary aquaculture species in the United States, accounting for over 50% of all U.S. aquaculture production (Dong et al. 2017). Catfish are produced primarily in Mississippi, Alabama, Arkansas, and Texas (USDA 2019). Gene transfer is an efficient and fast technique to increase growth and aquaculture production (Devlin et al. 2004). Growth hormone (GH) transgenic technology has been used to produce various fast-growing transgenic fish species, such as channel catfish (*Ictalurus punctatus*) (Dunham et al. 1992), Atlantic salmon (*Salmo salar*) (Du et al. 1992; Cook et al. 2000), Nile tilapia (*Oreochromis niloticus*) (Rahman et al. 2001), mud loach (*Misgurnus mizolepis*) (Nam et al. 2002), common carp (*Cyprinus carpio*) (Dunham et al. 2002), coho salmon (*Oncorhynchus kisutch*) (Devlin et al. 2004), and rainbow trout (*Oncorhynchus mykiss*) (Crossin et al. 2015).

GH transgenesis can result in greatly increased growth rate in fish from 2- to an incredible 40-fold (Dunham et al. 1999; Abass et al. 2020). Depending on the family, transgenic common carp *Cyprinus carpio* expressing rainbow trout cDNA grew 3% to 37% and 0% to 49% faster than their non-transgenic (Dunham et al. 2002). Transgenic Atlantic salmon *Salmo salar* expressing a chinook salmon GH cDNA driven by the ocean pout antifreeze protein (opAFP) gene grew 2- to 6-fold larger than the non-transgenic control at one year of age, with the largest transgenic fish was 13 times larger than the average non-transgenic fish (Du et al. 1992). Transgenic tilapia *Oreochromis niloticus* expressing a chinook salmon GH cDNA driven by the opAFP grew 2.5- to 4- fold faster than non-transgenic (Rahman et al. 1998; Rahman and Maclean 1999; Caelers et al. 2005). The response to growth hormone transgene expression has been variable in channel catfish, *Ictalurus punctatus*, perhaps due to differences in the promoter, construct, genetic background, environment, stocking density or length of study. The growth rate of channel catfish was improved by 0–33% by transferring salmonid growth hormone transgenes driven by the RSV-LTR (Rous Sarcoma Virus Long Terminal Repeat) promoter when grown in ponds or tanks to 40–60 g with pelleted feed (Dunham et al. 1999). When only natural food was available (Dunham et al. 1999). Mone transgenes driven by the RSV-LTR (Rous Sarcoma Virus Long Terminal Repeat) promoter when grown in ponds or tanks to 40–60 g with pelleted feed (Dunham et al. 1999).

Commercial production of transgenic fish is slowly becoming relevant. Triploid Atlantic salmon (*Salmo salar*) containing a chinook salmon GH transgene driven by the ocean pout antifreeze promoter (opAFP-GHc2) was approved for commercial production for human consumption in the USA and Canada, and have been sold in Canada for the past three years (Democracy Now 2016; Coghlan 2017; Waltz 2017; FDA 2020). The first GH salmon grown in the United States are scheduled for harvest in the summer of 2021 (The Fish Site 2020), and all 5 tonnes are sold (Horton 2021). Internationally, field trials for GH salmon have been approved or are underway in Argentina, Brazil and China (Wulf and Frank 2020). If other trials and regulation approvals go forward, other transgenic aquatic organisms, including channel catfish, may eventually be in the marketplace.

The objectives of this study were to compare the growth performance and survival rate among female and male transgenic opAFP-ccGH, rtMT-ccGH, opAFP/rtMT-ccGH, and non-transgenic channel catfish fed pelleted feed in earthen pond at 16 and 48 months of age. This trial allows examination of the relationship between sexual maturity and growth for GH-transgenic and control channel catfish females and males.

**Materials and Methods**

**Broodstock Spawning, Fry Production and Rearing**

Two F₁ channel catfish males and one channel catfish female transgenic for channel catfish growth hormone (ccGH) full-length cDNA driven by the ocean pout *Zoarces americanus* antifreeze protein promoter (opAFP-ccGH), five F₁ channel catfish males transgenic for the rainbow trout *Oncorhynchus mykiss* metallothionein promoter (rtMT-ccGH) (Abass et al. 2021), and five pairs of control channel catfish were pond-spawned in an earthen pond (0.04-ha, 1.8 m deep) located at the Catfish Genetics Research Unit, E. W. Shell Research Center, Auburn University, AL, USA. This resulted in F₂ opAFP-ccGH, F₂ rtMT-ccGH, F₁ opAFP/rtMT-ccGH transgenic and non-transgenic channel catfish. Fry remained in the spawning pond and were reared with 1,550 kg of brood fish/ha.

The fish were fed to satiation one time per day with 32% crude protein floating catfish pellets. Dissolved oxygen slightly decreased at 5.0 and 7.5 ppt salinities (Abass et al. 2020). The opAFP-ccGH and rtMT-ccGH transgenic channel catfish with their respective full-sibling controls were grown from fry to approximately 10-g fingerlings in the tank environment. The opAFP-ccGH genotype grew approximately 50% larger than their non-transgenic full-siblings, and the rtMT-ccGH genotype grew approximately 58% larger than their non-transgenic full-siblings (Abass et al. 2021).
(DO) and water temperature were measured daily in the early morning using a YSI Pro20 Dissolved Oxygen Meter (Pentair Aquatic Eco-Systems, Inc., Apopka, FL), and aeration was provided during daytime only if the dissolved oxygen was critically low (<3 mg/L) during summer. Other water quality parameters such as pH, total ammonia (NH3), nitrite (NO2), hardness, and alkalinity were measured once a week during the culture period using a Fish Farm 9 Test Kit (Pentair Aquatic Eco-Systems, Inc., Apopka, FL). DO was maintained between 4.5 and 6.5 mg/L, pH was maintained between 7 and 7.2 mg/L, ammonia was at <1 mg/L, nitrite was maintained at 0.05 mg/L, hardness was maintained between 46 and 68 mg/L, and alkalinity maintained between 40 and 84 mg/L.

Fish were grown for 16 months, and a total of 218 progeny were harvested (both transgenic and non-transgenic), weighed, PIT-tagged to differentiate the genetic groups at final harvest, and stocked communally in another 0.04-ha earthen pond. Additionally, 83 non-transgenic channel catfish of similar genetic background from another pond were stocked, resulting in a stocking density of 7,600 fish/ha. The genomic DNA was isolated and analyzed from fin clips from each fish to determine the presence of channel catfish GH cDNA, opAFP, and rtMT promoter sequences by polymerase chain reaction (PCR) amplification (Abass et al. 2016, 2021). Fish culture procedures were identical to the first phase of culture.

When the fish were 25 months of age, a low dissolved oxygen event occurred, and 71 fish died. Fish were sexed by examination of the urogenital area. Fish then were harvested at 48 months of age to evaluate the growth of growth hormone-transgenic channel catfish by construct promoter type, survival, and sexual dimorphism at two ages. A total of 163 fish were used for the analysis of body weight gain from 16 to 48 months of age.

### Statistical Analysis

Data were collected for statistical analysis, including sex, body weight at 16 months (BW16) and body weight at 48 months (BW48). Body weight was expressed as mean ± SD, and subjected to one-way ANOVA, and significance of differences among different genetic groups were assessed using Duncan’s (1955) multiple comparison test at P < 0.05. The percent of body weight increase (％BW) was defined as = (BW48-BW16)/BW16 × 100. Then, a two-way ANOVA analysis was performed to explore the effects of sex, genotype and the interaction of sex-by-genotype on body weight at 16-month age, 48-month age, and the percent of body weight gain. The full-model was defined as $Y_{ijk} = \mu + \alpha + \beta_j + (\alpha\beta)_j + \epsilon_{ijk}$, where $\alpha$ was the sex effect with two levels $i = \text{female and male}$; $\beta$ was the genotype effect with four levels, $j = \text{opAFP-ccGH, rtMT-ccGH, opAFP/rtMT-ccGH,} \text{ and their non-transgenic sibling control}$; $k$ was the observations of total fish ($N = 218$ for body weight at 16-month; and $N = 163$ for BW at 48 month and for ％BW gain) in the study. Statistical analyses were conducted using IBM SPSS software, version 27. An online statistical calculator (http://www.biostathandbook.com/fishers.html) was used for the Fisher’s exact test to compare the percent survival of the transgenics and their non-transgenic controls after exposure to low dissolved oxygen.

### Results

#### Growth Rate

At 16 months of age after growth in an earthen pond, the largest transgenic channel catfish containing channel catfish Ictalurus punctatus growth hormone (ccGH) cDNA driven by the ocean pout Zoarces americanus antifreeze protein promoter (opAFP), opAFP-ccGH, was 5.2 times larger, the largest transgenic channel catfish containing channel catfish growth hormone (ccGH) cDNA driven by the rainbow trout Oncorhynchus mykiss metallothionein promoter (rtMT), rtMT-ccGH was 3.3 times larger, and the largest transgenic channel catfish containing both opAFP and rtMT, opAFP/rtMT-ccGH, was 1.7 times larger than the mean of the non-transgenic siblings. The mean body weights of transgenic opAFP-ccGH, rtMT-ccGH, opAFP/rtMT-ccGH and non-transgenic siblings are summarized in Table 1.

At 48 months of age, the largest transgenic opAFP-ccGH was 5.3 times larger, the largest transgenic rtMT-ccGH was 4.3 times larger, and the opAFP/rtMT-ccGH was 3.4 times larger than the average non-transgenic sibling, with all transgenic types larger than the controls ($P < 0.001$) (Fig. 1b and Table 1). There was a significant difference between mean body weights of transgenic channel catfish and their non-transgenic siblings at all ages ($P < 0.001$) (Fig. 1 and Table 1).

The mean body weights of female and male transgenic opAFP-ccGH, rtMT-ccGH, opAFP/rtMT-ccGH and non-transgenic siblings are summarized in Table 2, with the transgenic genotypes being larger than the control ($P < 0.001$). Significant differences in body weight between transgenic males and females compared to their controls were observed for the opAFP-ccGH and rtMT-ccGH ($P < 0.001$) (Fig. 2a and b and Table 2). However, both male and female opAFP/rtMT genotypes had body weight similar to that of male control siblings ($P < 0.001$) (Fig. 2c and Table 2). Significant differences in body weight between the sexes were found at both 16- and 48-months of age ($P < 0.001$), with significant effects of sex by genotype at 48 months of age ($P < 0.001$) (Fig. 2 and Table 2). In the case of transgenic GH males, mean body weight was
significantly higher compared than of the females at age 16 months ($P < 0.001$). However, mean body weight of transgenic females was significantly higher than those of the males at age 48 months ($P < 0.001$) (Fig. 2). The body weight of 16-month-old transgenic opAFP-ccGH males was 1.28 times higher than that of the females, rtMT-ccGH males 1.21 times higher and opAFP/rtMT males 1.19 times higher than that of the females ($P > 0.05$) (Table 2). The body weight of 48-month-old transgenic opAFP-ccGH females was 1.32 times higher than that of the males ($P < 0.001$), and for transgenic rtMT-ccGH females was 1.38 times higher than that for males ($P = 0.004$). However, opAFP/rtMT females were not significantly larger ($P = 0.70$), just 1.07 times larger than their corresponding transgenic males.

In the case of non-transgenic siblings, males grew faster than females at both 16 and 48 months of age ($P < 0.001$) (Fig. 2), but growth rate of control males and females from 16 until 48 months was not different ($P > 0.05$). Apparently, elevation of growth hormone GH levels or associated epistasis alters sexually dimorphic growth after sexual maturation. The body weight of 16-month-old non-transgenic control males was 1.66 times higher than that of the females. The body weight of 48-month-old non-transgenic males was 1.76 times higher than that of the females. The body weights of females and males of transgenic fish were compared with their non-transgenic control counterparts (Figs. 3 and 4).

### Table 1

| Genotype         | 16 Months | 48 Months | % BW gain |
|------------------|-----------|-----------|-----------|
|                  | N BW (g)± SD (CV) | N BW (g)± SD (CV) | %BW gain± SD (CV) Range |
| opAFP-ccGH       | 50 713.0± 360.7a (50.6) | 24 1876.3 ± 827.54 (44.1) | 164.8± 80.8a (49.0) 55.1–387.2 |
| rtMT-ccGH        | 41 680.5± 251.0a (36.9) | 30 2173.3 ± 588.0a (27.1) | 221.0± 94.6a (42.8) 46.5–400.0 |
| opAFP/rtMT-ccGH  | 7 644.3± 89.2a (13.8) | 6 2121.7 ± 406.3a (19.1) | 227.9± 53.1a (23.3) 164.7–290.6 |
| Control          | 120 473.8± 237.3a (50.1) | 103 726.6 ± 395.2a (54.4) | 67.4± 56.0a (83.1) 1.9–290.0 |

*aopAFP-ccGH channel catfish, Ictalurus punctatus, transgenic for channel catfish growth hormone (ccGH) cDNA driven by the ocean pout Zoarces americanus antifreeze protein promoter (opAFP), rtMT-ccGH Channel catfish transgenic for channel catfish growth hormone (ccGH) cDNA driven by the rainbow trout Oncorhynchus mykiss metallothionein promoter (rtMT), opAFP/rtMT-ccGH channel catfish transgenic both opAFP-ccGH and rtMT-ccGH, and Control channel catfish non-transgenic (sibling). Means that do not differ at the $P=0.05$ are followed by the same superscript (Duncan’s multiple range test). Means followed by different superscripts among different genetic groups at the same age are different ($P<0.001$).

b, c Means that do not differ at the $P=0.05$ are followed by the same superscript (Duncan’s multiple range test). Means followed by different superscripts among different genetic groups at the same age are different ($P<0.001$).
Generally, it is easier for a small fish to double its body weight compared to a larger fish. Even though the transgenic fish were larger than controls at 16 months, their rate of growth was even more rapid ($P < 0.001$) than controls from 16 to 48 months, as the mean percentage body weight gain of GH transgenic was 164.8–227.9% and the control was 67.4% (Table 1). The percentage body-weight gain of GH transgenic females was 202.4–269.0%, GH transgenic males

| Genotype | Sex | N (16 Months) | % BW Gain (16–48) | N (48 Months) | % BW Gain (48–48) |
|----------|-----|---------------|-------------------|---------------|-------------------|
| opAFP-ccGH | Female | 4 | 595.0±64.5% (10.8) | 219.7±49.1% (22.4) | 2136.7±968.0% (45.3) |
| rtMT-ccGH | Female | 4 | 595.0±64.5% (10.8) | 219.7±49.1% (22.4) | 2136.7±968.0% (45.3) |
| Control | Female | 68 | 369.0±162.4% (44.0) | 555.4±167.2% (30.1) | 73.1±63.1% (86.2) |
| opAFP-ccGH | Male | 3 | 710.0±250.7% (41.0) | 2046.7±393.1% (19.2) | 59.1±43.3% (73.2) |
| rtMT-ccGH | Male | 13 | 771.5±386.5% (50.1) | 1715.6±585.8% (34.1) | 124.0±75.6% (61.0) |
| Control | Male | 52 | 611.0±250.7% (41.0) | 975.1±490.6% (50.3) | 59.1±43.3% (73.2) |
| opAFP/rtMT-ccGH | Female | 4 | 595.0±64.5% (10.8) | 219.7±49.1% (22.4) | 2136.7±968.0% (45.3) |
| rtMT-ccGH | Male | 13 | 771.5±386.5% (50.1) | 1715.6±585.8% (34.1) | 124.0±75.6% (61.0) |
| Control | Male | 52 | 611.0±250.7% (41.0) | 975.1±490.6% (50.3) | 59.1±43.3% (73.2) |

**% BW Gain**

Generally, it is easier for a small fish to double its body weight compared to a larger fish. Even though the transgenic fish were larger than controls at 16 months, their rate of growth was even more rapid ($P < 0.001$) than controls from 16 to 48 months, as the mean percentage body weight gain of GH transgenic was 164.8–227.9% and the control was 67.4% (Table 1). The percentage body-weight gain of GH transgenic females was 202.4–269.0%, GH transgenic males

**Fig. 2** Mean body weight (BW, g) of the female and male F2 generation of opAFP-ccGH and rtMT-ccGH transgenic, F1 opAFP/rtMT transgenic and non-transgenic sibling control channel catfish Ictalurus punctatus grown in earthen pond at 7600 fish/ha at 16 and 48 months of age. a opAFP-ccGH, b rtMT-ccGH and, c opAFP/rtMT-ccGH. opAFP-ccGH channel catfish, Ictalurus punctatus, transgenic for channel catfish growth hormone (ccGH) cDNA driven by the ocean pout Zoarces americanus antifreeze protein promoter (opAFP), rtMT-ccGH channel catfish transgenic for channel catfish growth hormone (ccGH) cDNA driven by the rainbow trout Oncorhynchus mykiss metallothionein promoter (rtMT), opAFP/rtMT-ccGH channel catfish transgenic both opAFP-ccGH and rtMT-ccGH, T transgenic channel catfish, and N non-transgenic sibling control channel catfish. Means that do not differ at the $P = 0.05$ are followed by the same superscript (Duncan’s multiple range test). Means followed by different superscripts among different genetic groups at the same age are different ($P < 0.001$)
124.0–186.8%, control females 73.1%, and control males 59.1% (Table 2). The effects of genotype, sex and the interaction of genotype by sex on % BW gain were significant \((P < 0.001)\). All the transgenic GH females had significantly larger body weight gain than both sexes of non-transgenic controls \((P < 0.001)\) (Table 2). The opAFP/rtMT-ccGH males had gained more body weight than both sexes of non-transgenic controls \((P < 0.001)\). Males of both opAFP-ccGH and rtMT-ccGH were larger than non-transgenic male controls \((P < 0.001)\).

**Survival Rate when Exposed to Low Dissolved Oxygen**

An unexpected low oxygen event occurred in the pond, causing high mortality. Survival of transgenic male and female opAFP-ccGH channel catfish was lower than controls \((P = 0.0004–0.004)\), as well as rtMT-ccGH females \((P = 0.107)\) (Table 3), which is not surprising since the largest fish are most likely to succumb during an oxygen depletion.

**Discussion**

This is the first report comparing the growth performance between female and male GH-transgenic channel catfish and their non-transgenic siblings in earthen ponds at commercial densities to food size and sexual maturity. There was a significant difference between mean body weights of transgenic and their non-transgenic siblings at all ages.

GH plays an important and critical role in regulating mRNA expression for growth-related genes (Ji et al. 2011). Response to GH transgenesis is complex and variable in fish, but almost always positive from a growth standpoint. GH gene insertion can result in a 25–40% growth enhancement upwards to greatly increased growth rate from 2 to an incredible 40- fold or even higher (Dunham et al. 1999). The differences of growth rate increase might be caused by different types of gene constructs used (Devlin et al. 2004), site of transgene integration in the host chromosome, copy number of transgenes, the activity and type of promoter used (Moav et al. 1992; Leggatt et al. 2012), different methods and rearing time (Kurdianto et al. 2016), strain (Devlin et al. 2004; Leggatt et al. 2017), genetic background (Chan et al. 2021), and genotype \(\times\) environment interactions (Vandersteen et al. 2019; Abass et al. 2020).

It has been hypothesized that GH transgene insertion has more dramatic results in wild fish, and with increasing domestication and approach to theoretical growth limitations, the effects are less extraordinary. Devlin et al. (2001) found that salmonid GH-gene constructs that had a dramatic effect on growth in wild rainbow trout strains (with naturally low growth rates) had little or no effect in strains where growth rate has been enhanced by long-term...
selection. These wild transgenic rainbow trout grew at the same rate as fast-growing non-transgenic, domestic rainbow trout. However, additional data on transgenic rainbow trout (Devlin et al. 2001) refute this hypothesis of the effect of wild and domestic genetic backgrounds on response to GH transgene insertion. A transgenic wild × domestic crossbreed was by far the largest genotype, 18 times larger than the non-transgenic wild parent, 13 times larger than the non-transgenic wild × domestic crossbreed, 9 times larger than the non-transgenic domestic parent and more than 2.5 times larger than the wild F77 transgenic parent in this experiment (Devlin et al. 2001). The transgenic with 50% of its heritage from domestic sources was much larger than a wild transgenic, so great response from some domestic genotypes is possible. Additionally, Gaffney et al. (2020) reported that hatchery and domestic coho salmon usually had similar responses to GH transgene, but in their 2020 experiment the wild transgenics had by far the best response. However, this “wild” transgenic strain had been gone through several generations of domestication, and thus, technically, was not wild. Abass et al. (2021) and Zhong et al. (2012) reported that gene transfer coupled with family selection might results in larger fish than GH transgenesis alone.

Another hypothesis is that very slow growing species have the greatest response to GH insertion, such as the 30X increase in growth of the GH transgenic mud loach, Misgurnus mizolepis, (Nam et al. 2001). However, GH zebrafish, Danio rerio, grew 2.5X faster than normal (Figueiredo et al. 2007), showing that this hyper-response to GH gene insertion is not universal among species with small body weights and slow growth.

Sexual dimorphism in growth has been observed in many fish species of commercial interest (Malison et al. 1985).
Table 3 Percent survival after exposure to low dissolved oxygen, and mean body weight (BW) (g ± standard deviation (SD), coefficient of variation (CV) and range for survivors and mortalities of female and male F2 generation of the transgenic and non-transgenic sibling control channel catfish Ictalurus punctatus, and F1 opAFP/rtMT transgenic channel catfish grown in earthen pond at 7,600 fish/ha

| Genotypea | Sex  | N   | Survival (%) | Pb | Survivorsc | Mortalitiesd |
|-----------|------|-----|-------------|----|------------|--------------|
|           |      |     |             |    | BW (g ± SD (CV)) | Range | BW (g ± SD (CV)) | Range |
| opAFP-ccGH | Female | 24  | 50.0 | 0.0004 | 692.5 ± 157.3b (22.7) | 540–1,120 | 554.1 ± 106.2c (19.2) | 370–680 |
| rtMT-ccGH  | 28   | 75.0 | 0.107 | 665.2 ± 154.5b (23.2) | 500–1,200 | 557.1 ± 71.3c (12.8) | 500–640 |
| opAFP/rtMT-ccGH | 4  | 75.0 | 0.38 | 590.0 ± 78.1c (13.2) | 500–640 | 610.0 ± NAa (NA) | NA |
| Control    | 68   | 89.7 | NA   | - | - | - |
| opAFP-ccGH | Male  | 26  | 46.2 | 0.004 | 364.1 ± 165.5a (45.5) | 100–680 | 411.4 ± 134.1a (32.6) | 130–500 |
| rtMT-ccGH  | 13   | 69.2 | 0.54 | 843.3 ± 436.5a (51.8) | 390–1,570 | 610.0 ± 197.3a (32.3) | 390–870 |
| opAFP/rtMT-ccGH | 3  | 100.0 | 1.00 | 710.0 ± 79.4a (11.2) | 650–800 | - | - |
| Control    | 52   | 80.8 | NA   | - | - | - |

Means that do not differ at the Pb=0.05 are followed by the same superscript (Duncan’s multiple range test). Means followed by different superscripts among different genetic groups are different (P<0.001 for survivors and P=0.099 for mortalities).

*aopAFP-ccGH CHANNEL catfish, Ictalurus punctatus, transgenic for channel catfish growth hormone (ccGH) cDNA driven by the ocean pout Zoarces americanus antifreeze protein promoter (opAFP), rtMT-ccGH channel catfish transgenic for channel catfish growth hormone (ccGH) cDNA driven by the rainbow trout Oncorhynchus mykiss metallothionein promoter (rtMT), opAFP/rtMT-ccGH channel catfish transgenic both opAFP-ccGH and rtMT-ccGH, and Control channel catfish non-transgenic (sibling) *bPb=Probability of a survival difference between a transgenic genotype and corresponding control when exposed to critically low dissolve oxygen (Fisher’s Exact Test) *cInitial mean body weight (BW), coefficient of variation (CV) and range at 16 months of age of survivor of catfish genotypes after exposure to low dissolved oxygen *dInitial mean body weight (BW), coefficient of variation (CV) and range at 16 months of age of loss of catfish genotypes after exposure to low dissolved oxygen

Channel catfish males grew faster than females when stocked together in ponds (Beaver et al. 1966; Dunham et al. 1985; Goudie et al. 1994; Davis et al. 2007). Goudie et al. (1994) reported that at 10 months of age, sex did not influence the growth rates of channel catfish weighing less than 50 g; however, Brooks et al. (1982) observed sexually dimorphic growth in channel catfish at 20–30 g. At 26 months of age, channel catfish males were 5–10% longer and 6–37% heavier than females (Simco et al. 1989). El-Ibiary et al. (1976) and Bondari et al. (1985) also reported significant differences in body weight between male and female channel catfish averaging 500–600 g; males channel catfish were 22.4% heavier and 7.4% longer than females. Sex had an increasing influence on growth rate with increasing age and size in channel catfish (El-Ibiary et al. 1976).

The ccGH cDNA constructs altered both the growth rate and the relationship of sexually dimorphic growth of channel catfish. The body weight of the F1 and F2 transgenic GH and non-transgenic siblings was significantly different between the sexes at both 16 and 48 months of age. Transgenic GH males were larger than transgenic GH females at 16 months of age when males and females were all immature. However, transgenic GH females were larger than transgenic GH males at 48 months of age after sexual maturation. Non-transgenic males were larger than non-transgenic females at both 16 and 48 months of age. Transgenic GH males were 19–28% heavier than transgenic GH females at 16 months of age for fish averaging 644–713 g. However, transgenic GH females were 7–38% heavier than transgenic GH males at 48 months of age for fish averaging 1876–2173 g. Even at the younger age of 16 months, the male size advantage was reduced by expression of the ccGH transgene, as non-transgenic males were 65% heavier than non-transgenic females compared to 19–28% advantage of transgenic males over transgenic females. The control males continued their size advantage over control females, 76%, at 48 months of age, while the transgenic males lost their growth advantage over transgenic females. Generally, the relative growth difference between rapidly growing genotypes and slow-growing genotypes decreases once the fish pass the fingerling stage (Dunham 2011). However, the relative growth rate of GH transgenic channel catfish was 2–3 times higher than the controls from 16 to 48 months of age, greatly expanding their relative growth advantage. Strong sexually dimorphic growth between female and male observed in the 5750A transgenic coho salmon strain with the females being larger as expected, a result similar to ours with channel catfish, but not in M77 strain harbouring the same GH gene construct (Chan et al. 2021).

The GH-transgenic channel catfish exhibited lower survival when an oxygen depletion occurred in the pond. This is not surprising, since the GH transgenic fish were larger than the controls, and low dissolved oxygen usually results in mortality...
of large fish before small fish (Dunham 2011). However, this relationship is not always constant and the size-hypoxia tolerance relationship in ictalurid catfishes is affected by relative size differences, life stage, genotype and genotype-environment interactions (Dunham et al. 2014; Wang et al. 2017). In the current experiment, the very largest individual died during the oxygen depletion, but many of the other large fish survived. Although results were variable, the smaller size of the controls appears to have allowed them higher survival at critically low oxygen levels. Physiological studies would be needed to clarify the mechanism underlying mortality differences between the GH transgenic and non-transgenic control channel catfish.

Conclusions

Expression of the opAFP-ccGH and rtMT-ccGH transgenes greatly increased growth rates in the $F_1$ and $F_2$ GH-transgenic male and female channel catfish. These constructs disrupted normal sexual dimorphic growth relationships, decreasing the degree of sexual dimorphism and reversing the relationship, with GH females reaching a larger size than males at 48 months of age. This increased growth and size made the GH transgenic channel catfish more vulnerable to low oxygen levels. This risk should be mitigated by ensuring that emergency aeration equipment is always functioning and possibly by combining genetic enhancement programs in the future to better simultaneously address improvement of growth and hypoxia tolerance.

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Author Contribution

NA and RD conducted and designed the experiment, wrote the manuscript, and revised the final manuscript. NA identified fishes, checked water quality, collected samples, isolated DNA, administered the PCR analysis, performed the statistical analysis, analyzed the results, prepared tables and figures, and drafted the manuscript. BS and RD assisted in statistical analysis. BS, AA, AE, ZQ, HL, RO, and ZY assisted with execution of the experiment and data collection. All authors read and approved the final manuscript.

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Data Availability

The data sets used and analyzed during the current study are available from the corresponding author on reasonable request.

Code Availability

Not applicable.

Declarations

Ethical Approval

All experimental protocol used in this experiment were approved by the Auburn University Institutional Animal Care and Use Committee (AU-IACUC) before the experiment was initiated, and followed Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) protocols and guidelines.

Conflict of interests

We declare that we have no significant competing financial, professional, or personal interests that might have influenced the performance or presentation of the work described in this manuscript.

Consent to Participate

Not applicable.

Consent for Publication

The corresponding author confirms all authors agree with the content of the manuscript.

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