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Evaluation of growth, sex (male proportion; sexual dimorphism), and color segregation in four cross combinations of different strains of XX female and YY male Nile Tilapia

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Abstract

Four cross combinations of different YY male and female Nile Tilapia *Oreochromis niloticus* strains were evaluated for growth, sex, and color segregation. Red color parental strains included blotched phenotypes. The Genetically Improved Farmed Tilapia (GIFT) was the only dark (wild-type) color parental strain. Fish of the same age and cross were stocked in three replicate tanks for four crosses in one recirculating system for 167 days. Data recorded included feed consumed, body weight, total length, color, sex, and fillet weight. YY males crossed with GIFT females (Cross 2) exhibited superior growth that was significantly different ($p < .05$) to other three crosses. Male proportions were 79–100%. Only YY males crossed with the LSA female strain (Cross 4) yielded 100% males, but, Cross 4's productivity was inferior to that of Cross 2. Body weight advantage of males over females was 28.7–84.2%. Color segregation indicated that red color trait in Nile Tilapia is autosomal dominant, and black patch coverage was variable. This study showed that different parental strain combinations clearly impact productivity traits, and that YY male technology combined with crossbreeding provide the opportunity for genetic improvement and development of commercially beneficial superior traits in Nile Tilapia.

KEYWORDS
crossbreeding, Nile tilapia, YY male technology

1 | INTRODUCTION

Global tilapia production increased from 175, 260 t (valued at US$150 million) in 1984 to 5.88 million tonnes (US$11.03 billion) in 2017; Nile Tilapia *Oreochromis niloticus* dominated production during this period and reached 4.13 billion tonnes (US$7.61 billion) in 2017 (FAO, 2019). Tilapia production in the USA increased from 2,172 t (valued at US$8.46 million) in 1990 to 10,097 t (US$35.36 million) in 2009 and was 8,736 t (US$43.54 million) in 2017 (FAO, 2019). Meanwhile, US tilapia imports grew from 3,389 t in 1992 to 188,700 t in 2018 (NOAA, 2019). Although the causes of dependence on imported tilapia and seafood in general are multi-faceted, and although the United States has its unique set of aquaculture challenges, U.S. aquaculture may address this trade imbalance by continued efforts at diversification of local aquaculture with alternative aquaculture species such as Nile Tilapia, by focusing on developing strategies for sales in local markets, and by use of innovative technologies such as improved tilapia genetic resources.

Genetic improvement of Nile Tilapia *Oreochromis niloticus* through long-term selection programs includes several commercially desirable traits such as faster growth, body weight at harvest, fillet yield, and red color for whole-fish consumers (Bentsen et al., 2012; Eknath & Hulata, 2009; Gjerde, Mengistu, Ødegård, Johansen, & Altamirano, 2012; Khaw, Ponzoni, Yee, Aziz, & Bijma, 2016; Lago, Rezende, Dias, Freitas, & Hilsdorf, 2017; McAndrew, Roubal, Roberts, Bullock, & McEwen, 1988; Ponzoni et al., 2011; Thodesen et al., 2013). Tilapia (genus *Oreochromis*) is one of the most globally important groups of farmed food fish in which body color is of major economic significance (Colihueque & Araneda, 2014; Colihueque, Parraguez, Estay, & Diaz, 2011; Gomelsky, 2011). In comparison to fish with wild-type (dark) color, red colored tilapia are considered of high quality and highly attractive for whole-fish consumers in Asian and Latin American countries such as Thailand, Malaysia, and Mexico, and they are reported to have high market value (including in the United States) because of their similar appearance to marine species (Ng & Hanim, 2007; Pongthana, Nguyen, & Ponzoni, 2010; Popma & Masser, 1999; Ramírez-Paredes, Garduño-Lugo, & Muñoz-Córdova, 2012).

Another area of economic importance is sex regulation. Sex control is sought after in tilapia production primarily because males are larger and grow faster than females, and all-male production deters uncontrolled reproduction, a likely outcome of mixed-sex culture. Sex regulation is achieved in many countries through the use of 17α-methyltestosterone (MT), but MT is not approved and is still under investigation for use in tilapia by the U.S. Food and Drug Administration; and, countries such as India, Costa Rica, Ecuador, European Union countries, and some sub-Saharan countries have restricted the sale and culture of hormone-treated fish (Mlalila, Mahika, Kalombo, Swai, & Hilonga, 2015; Phelps, 2006). In contrast, genetic sex regulation (YY males) as a means of all-male production provides a direct alternative to the use of MT on fish destined for human consumption. Although high male proportions (95% and 98.5% to 99.6%) were reported in YY male offspring (Kamaruzzaman, Nguyen, Hamzah, & Ponzoni, 2009; Mair, Abucay, Beardmore, & Skibinski, 1995), male proportions need to be improved considering that a wider range of male proportions (72–100%) have been reported and attributed to parental, autosomal, polymorphic, or exogenous factors (Baroiller & D'Cotta, 2019; Beardmore, Mair, & Lewis, 2001; Tariq Ezaz, Myers, Powell, McAndrew, & Penman, 2004).

This study combined the use of YY male technology and crossbreeding of different strains of Nile Tilapia to obtain four crosses for comparative raising and evaluation of productivity traits. Crossbreeding for genetic improvement uses less resources and time than traditional selection programs, and it consists of testing inter-strain crosses to identify seed stock with superior traits based on the heterotic effect (Dunham, 2011; Gomelsky, 2011). Growth performance (body weight, daily growth rate, feed consumption, Feed Conversion Ratio, fillet yield), sex (male proportion; sexual dimorphism), and color segregation were evaluated, and one cross exhibited superior growth not usually reported.

2 | MATERIALS AND METHODS

2.1 | Study site, broodstock, spawning, and nursing

This research was conducted in clear-water, indoor, recirculating aquaculture systems (RAS) at the Aquaculture Research Center of Kentucky State University in Frankfort, KY. The strain designation, color (as described by vendor), vendor, and other characteristic details of the broodstock used in four cross combinations are listed in Table 1. The color phenotype of red broodstock strains in this study included fish with red body color without black spots or patches, and red body color with a small patch of black pigmentation (Table 1). Parental strains used for spawning were stocked at a ratio of 3–5 females to one male in four recirculating systems on March 6, 2017 (Table 1). Each spawning system included a 1893-L, flat-bottomed, circular tank (Polytank, Inc., Litchfield, MN); a biofilter, Model T400 (Waterco USA, Augusta, GA); a submersible pump, Danner Model 9.5B (Amazon.com LLC, Seattle, WA); a 1,000 W submersible heater and thermostat (Pentair Aquatic Eco-Systems, Inc, Apopka, FL); and, one 3000-Lumen LED work light (Utilitech, Romulus, MI). The water volume of each spawning tank was kept at 1184-L. A photoperiod of 12 h light/12 h dark and a water temperature of 28°C were maintained during spawning.

Broodfish were fed 4.8-mm tilapia feed (Triton 3606, Cargill Animal Nutrition, Albany, NY) at 0.5–1% body weight/day during spawning. Females in each spawning system were checked every 2 weeks for the presence of eggs in the oral cavity, but it was not until 8 weeks after initial stocking that eggs were collected from all four spawning systems on the same day (May 8, 2017). The eggs obtained from at least two fish from each of the four crosses were combined, and eggs from each of the four crosses were incubated separately in McDonald-type hatching jars (Pentair Aquatic Eco-Systems, Inc. Apopka, FL) in a recirculating trough system kept at 28°C. Swim-up fry were fed 0.3 to 0.4-mm feed (Aquaxcel 5014; Cargill Animal Nutrition, Albany, NY) four to six times/day up to 30 days post hatch (dph). Fry from each cross (185–195 fry/cross) were then moved to four 416-L tanks and kept separate in another recirculating system. They were fed 0.6- to 0.8-mm and 1.5-mm feed (Aquaxcel 5014 and Aquaxcel 4512; Cargill Animal Nutrition, Albany, NY) four times/day until they were 71 dph.

TABLE 1 Strain designation, color (as defined by vendor), vendor, sex, number of fish, and the mean (±SD) of the body weight (BW, g) and total length (TL, cm) of five Nile Tilapia *Oreochromis niloticus* parental strains used in four cross combinations were listed

Cross	Strain	Color	Vendor	Sex	No. fish	BW, g	TL, cm
1	Til-aqua YY	Red[a]	MAI	M	3	768 ± 97	34 ± 1.6
	Miami[b]	Red[a]	MAI	F	14	173 ± 41	20 ± 1.4
2	Til-aqua YY	Red[a]	MAI	M	3	931 ± 2	35 ± 1.6
	GIFT[c]	Dark	MAI	F	9	378 ± 1	26 ± 1.3
3	Til-aqua YY	Red[a]	MAI	M	2	805 ± 15	35 ± 0.3
	LSA	Blotched[d]	LSA	F	9	448 ± 135	27 ± 2.7
4	Fishgen YY	Red[a]	FGL	M	3	1,131 ± 254	38 ± 1.5
	LSA	Blotched[d]	LSA	F	9	402 ± 130	27 ± 2.8

Note: MIA: Miami Aqua-culture Inc. (Boynton Beach, FL); Til-Aqua YY strain was acquired by MIA from YY-Male producer, Til-Aqua International (Someren, Netherlands). LSA: Louisiana Specialty Aquaculture LLC, Robert, LA. FGL: Fishgen Ltd (Swansea, UK); Fishgen YY strain was acquired from this company.

[a]Although these strains were marketed and sold as 'red' color fish, they included either fish with red color without any black pigmentation, or red fish with minimal expression of blotched phenotype (presence of small black patches).

[b]Local Florida farm strain.

[c]Genetically Improved Farmed Tilapia; wild-type (dark) body color originally from Thailand.

[d]This strain was developed by LSA and all fish exhibited red color with large black patches (strong expression of blotched phenotype).

2.2 | Comparative raising

The recirculating system used for comparative raising included a 1,223-L bioreactor filter, Sweetwater Model LSB25 (Pentair Aquatic Eco-Systems, Inc, Apopka, FL); solids removal filter, Sweetwater 990 Model (Pentair Aquatic Eco-Systems, Inc, Apopka, FL); 6,000 W titanium inline heater (Pentair Aquatic Eco-Systems, Inc, Apopka, FL) to maintain water temperature at 28°C; and, 12 946-L culture tanks. Each culture tank was stocked with 50 fingerlings of the same age (71 dph) from the same cross, and each of the four crosses was randomly assigned three replicate tanks.

Feeding was started on day 1 (the day after stocking) when fish were 72 dph. Fish were fed different size floating tilapia feed during the comparative raising period: 1.5- and 2.2-mm Aquaxcel 4512; 3.2-mm Triton WW 4010 Transition; and 4.8-mm Triton 3606 (Cargill Animal Nutrition US-Aqua, Franklinton, LA). Feed size was changed based on direct observation of fish feeding activity, and three feeding guidelines: "Tilapia Feeding Guidelines" (John O'Rourke, Cargill Animal Nutrition, US-Aqua, personal communication), "Feeding Chart for Tilapia" (David Brock, Rangen Inc., personal communication), and "Suggested feed size and feeding" in Publication No. 282 of the Southern Regional Aquaculture Center (DeLong, Losordo, & Rakocy, 2009).

Feed sizes and feeding regime were: 1.5-mm three times/day (day 1–21); 2.2-mm three times/day (day 22–54); 1:1 mixture of 2.2 and 3.2-mm three times/day (day 55–65); 3.2-mm three times/day (day 66–76); 3.2-mm two times/day (day 77–106); 1:1 mixture of 3.2 and 4.8-mm two times/day (day 107–116); and, 4.8-mm two times/day (day 117–167). Fish were fed to apparent satiation for each feeding session. The minimum time between consecutive feeding sessions during the day was 4 hr to allow for gastric evacuation and appetite return (Riche, Haley, Oetker, Garbrecht, & Garling, 2004).

Water quality parameters measured during the comparative raising period were: temperature, dissolved oxygen, pH, and salinity (two to three times a week) using a ProDSS Multiparameter Meter (YSI Incorporated, Yellow Springs, OH); and total ammonia nitrogen, nitrite, and alkalinity (one to two times a week) using the DR3900 Spectrophotometer (Hach Company, Loveland, CO).

2.3 | Data collection

Feed was weighed in aliquots of 35, 30, 20, 15, 10, and 5 g in multiple-labeled weigh boats prior to each feeding session. Feed was given one aliquot at a time to each tank. As soon as one aliquot of feed was eaten (0–20 pellets remaining), another aliquot of equal or smaller weight was supplied. Fish actively ate larger aliquots (35, 30, and 20 g) more frequently in the first 30–40 min of feeding, and smaller feed portions (15, 10, or 5 g) were given thereafter until feeding activity ceased.

The body weight (g) and total length (cm) were measured from a random sample of fish for each tank on July 26, 2017 (day of stocking; 10 fish/tank); August 27, 2017 (day 33; 15 fish/tank); September 27, 2017 (day 64; 15 fish/tank); November 1, 2017 (day 98; 15 fish/tank); and, December 4, 2017 (day 131; 25 fish/tank). After 167 days, the fish were fasted for 24 hr, then harvested and euthanized for final data collection on January 11 and 12, 2018. Data recorded for each fish at the time of harvest included body weight (g), total length (cm), color, and sex identification by dissection and gross visual examination of the gonads. Ten fish from each tank (30 fish/cross) were processed to obtain the skinless fillet weight (g).

The color phenotype was recorded as either dark (wild-type), solid red (no black pigmentation on body surface), or as red-black (RB) for fish with red body color with presence of black pigmentation on the body surface (blotched phenotype). Fish exhibiting the blotched phenotype were assigned to one of three qualitative categories to characterize variability of black pigmentation: RB1 = red body color with presence of one small patch of black pigmentation; RB2 = red body color with presence of two or three small patches of black pigmentation in different areas; and RB3 = red body color with two or more large black patches that were distributed over large areas.

2.4 | Growth parameter calculations

Survival rate in each tank was calculated as a percentage for the number of fish collected during harvest from the number of fish stocked. The mean body weight in each tank was calculated for the day of stocking and for five subsequent sampling dates. Weight gain for 167 days in each tank was calculated as WG = $W_f - W_i$, where W_f = the final mean body weight and W_i = initial mean body weight. Daily growth rate (g/day) in each tank was calculated as W_G/t, where W_G = weight gain (g) at 167 days, and t = 167 days. The number of feed aliquots and their weights were recorded for each tank for each feeding session, and total feed consumed was calculated as the sum of aliquot weights fed to each tank for each feeding session during 167 days. The Feed Conversion Ratio (FCR) for the 167 days in each tank was calculated as F/WG, where F = total feed fed and WG = weight gain at 167 days. The body condition factor 'K' was calculated as $(W/TL^3) \times 100$, where W = body weight (g) and TL = the total length (cm) of each fish at harvest. Fillet yield (%) was calculated as fillet weight/total body weight × 100. The number of males and females for each cross were recorded for investigation of sex segregation, and their body weight (g) at harvest was used for evaluation of sexual dimorphism. The body weight advantage of males over females in crosses was calculated (as a percentage) as the difference between mean weight of males and females divided by the mean weight of females multiplied by 100.

2.5 | Statistical analysis

The SAS® University Edition software (SAS Institute Inc., Cary, NC) was used for statistical analysis. The dependent variables of daily growth rate (g/d), feed consumed, FCR, body condition factor, and fillet yield after 167 days of raising were analyzed as a response to the treatment of parental strain combination (hereafter cross) as either a linear model or a nonparametric one-way test, depending on the whether the residuals of the linear model were normally distributed, as determined by a Shapiro–Wilk test (PROC UNIVARIATE). Three models were constructed for each dependent variable: (1) the effect of cross, sampling day, and their interaction on body weight data for six sampling dates; (2) the effect of cross alone; and, (3) the effects of cross, sex, and their interaction on body weight obtained at harvest to evaluate sexual dimorphism. If the residuals were normally distributed, the linear model (PROC MIXED) was then interpreted with cross, sampling day, and sex of fish as fixed effects. No random variable was included, and this procedure was selected because it employs restricted estimation maximum likelihood (REML), which reduces heteroscedasticity. Post-hoc tests used Tukey–Kramer adjustment (PROC MIXED, LS MEANS/ADJUST = TUKEY). If the residuals were not normally distributed, the same statistical hypotheses were evaluated by nonparametric Kruskal-Wallis and Dwass-Steel-Critchlow-Fligner tests (PROC NPAR1WAY). Nonparametric tests were only used for fillet data set only. The significance threshold for all statistical analyses was $p < .05$.

3 | RESULTS

3.1 | Comparative raising and growth

Water quality measurements (mean ± SD) during the comparative raising period were: 27 ± 1°C water temperature; 6.0 ± 0.8 mg/L dissolved oxygen; 7.98 ± 0.29 pH; 0.23 ± 0.30 mg/L total ammonia nitrogen; 0.19 ± 0.15 mg/L nitrite; 120 ± 30 mg/L alkalinity; and, 2.4 ± 0.7 ppt salinity. Survival ranged from 99 to 100% (Table 2). Cross, sampling day, and the interaction of cross and sampling day had a significant ($p < .0001$) effect on body weight (Figure 1). Cross 2 (Til-Aqua YY males × GIFT females) had the highest mean body weight, and this was significantly different ($p < .0001$) to that of Cross 1 (Til-Aqua YY males × Miami females), Cross 3 (Til-Aqua YY males × LSA females), and Cross 4 (Fishgen YY males × LSA females) throughout 167 days of growth (Table 2 and Figure 1). Cross 2 grew 75% larger and 1.8 times faster than Cross 1, 62% larger and 1.7 times faster than Cross 3, and 57% larger

TABLE 2 Growth parameters (mean ± SD) of Nile Tilapia crosses measured for 167 days of comparative raising were: (i) initial body weight (BW_I, g), (ii) final body weight (BW_F, g), (iii) survival (%), (iv) daily growth rate (g/d), (v) feed consumed (kg), (vi) feed conversion ratio (FCR), (vii) body condition factor (K), and (viii) percent fillet yield (FY, %). Significant differences ($p < .05$) among crosses were indicated by different lowercase letters 'x, y, and z'

Cross	Survival (%)	BW_I (g)	BW_F (g)	g/d	Feed (kg)	FCR	K	FY (%)
1	99	21.10 ± 1.34 x	402 ± 93 z	2.28 ± 0.23 z	29.7 ± 3.5 y	1.57 ± 0.05 x	2.14 ± 0.2 y	31 ± 1.1 y
2	99	21.33 ± 0.19 x	702 ± 151 x	4.08 ± 0.04 x	49.7 ± 1.1 x	1.47 ± 0.02 y	2.49 ± 0.2 x	36 ± 1.0 x
3	100	18.59 ± 0.48 y	434 ± 127 yz	2.35 ± 0.12 yz	30.2 ± 0.8 y	1.46 ± 0.04 y	2.12 ± 0.2 y	32 ± 0.3 y
4	100	17.45 ± 1.09 y	447 ± 94 y	2.57 ± 0.05 y	31.8 ± 1.2 y	1.48 ± 0.03 y	2.13 ± 0.2 y	31 ± 0.5 y

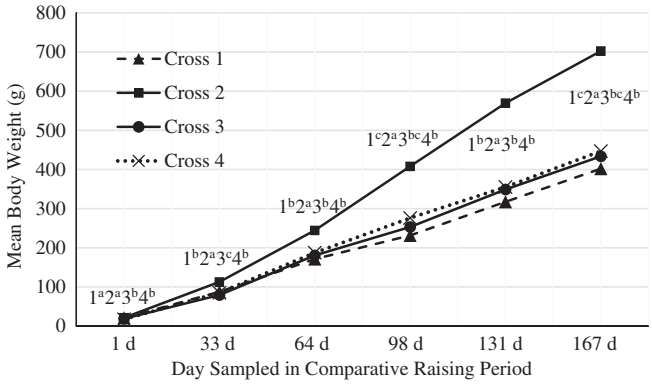

FIGURE 1 Growth of Nile Tilapia crosses during 167 d period. Significant differences ($p < .05$) in body weight among the crosses for six sampling days were indicated by different letter superscripts

and 1.6 times faster than Cross 4 as measured by final body weight and daily growth rate (Table 2). Cross 2 had the highest mean daily growth rate (4.08 g/d); this was significantly different ($p < .0001$) from that of the other crosses (2.28 g/d, Cross 1; 2.35 g/d, Cross 3; and 2.57 g/d, Cross 4) (Table 2).

The FCR (1.47) for Cross 2 was significantly different ($p = .0388$) from that of Cross 1 (1.57), but it was not significantly different ($p > .05$) from that of Cross 3 (1.46) and Cross 4 (1.48) (Table 2). The total quantity (mean ± SD) of feed consumed by Cross 2 was greater (49.7 kg ± 1.1) and significantly different ($p < .0001$) compared to the quantities consumed by each of the other crosses (Table 2). The body condition factor for Cross 2 was higher ($K = 2.49$) and significantly different ($p < .0001$) than that of Cross 1 ($K = 2.14$), Cross 3 ($K = 2.12$), and Cross 4 ($K = 2.13$) (Table 2). The fillet yield for Cross 2 (36%) was significantly different ($p = .0001$) from that of the other crosses (31–32%).

3.2 | Male proportion and sexual dimorphism

Data on sex segregation and harvest weight by sex are presented in Table 3. Only Cross 4 (Fishgen YY males × LSA females) comprised 100% males; and, Crosses 1, 2, and 3 (Til-Aqua YY males crossed with females from three distinct strains) were 79–85% males (Table 3). Although females were identified in these three crosses, no evidence of reproduction (presence of eggs or fry) was observed at any time. The effect of cross, sex, and the interaction of cross and sex on body weight at harvest were significant ($p < .05$). The mean body weight of males was larger and significantly different ($p < .0001$) compared to their female cohorts in Crosses 1, 2, and 3; and, the body weight advantage of males ranged from 28.7 to 84.2% (Table 3). Although females were significantly smaller than their male cohorts,

TABLE 3 Sex segregation (%) and sexual dimorphism in body weight (mean ± SD) of Nile Tilapia crosses. Significant differences ($p < .05$) in body weight were indicated by the letters "a and b" for males and female from the same cross

	No. fish	Sex segregation (%)		Body weight (g)		Body weight (%)
Cross	Analyzed	Male	Female	Male	Female	Advantage of males
1	149	79	21	422 ± 89 a	328 ± 69 b	28.7
2	149	79	21	762 ± 99 a	472 ± 71 b	61.4
3	150	85	15	466 ± 105 a	253 ± 76 b	84.2
4	150	100	0	447 ± 94	-	

TABLE 4 Color characteristics and proposed genotypes of parental fish and offspring in crosses with regard to major color-determining gene (*R*/*r*) and segregations of fish in crosses with regard to black blotching intensity[a]

	Color of parental fish		Proposed genotypes of parental fish				Segregation of all fish (%)		Segregation of blotched fish (%)		
Cross	Males	Females	Males	Females	Proposed genotypes of offspring	No. of fish analyzed	Solid red (R)	Blotched (RB1 + RB2 + RB3)	RB1	RB2	RB3
1	R and RB1	R and RB1	*RR* or *Rr*	*RR* or *Rr*	*RR* and *Rr*	149	48.3	51.7	84.4	11.7	3.9
2	R and RB1	Dark	*RR*	*Rr*	*Rr*	149	0	100	0	3.4	96.6
3	R and RB1	RB3	*RR*	*Rr*	*RR* and *Rr*	150	44.0	56.0	85.7	14.3	0
4	R and RB1	RB3	*RR*	*Rr*	*RR* and *Rr*	150	10.0	90.0	57.8	33.3	8.9

Note: R: red body color without black spots or patches. RB1: red body color with presence of a small patch of black pigmentation. RB2: red body color with two or three small patches of black pigmentation on skin in different areas. RB3: red body color with two or more large black patches distributed over large areas.

[a]Categories of black blotching intensity.

Cross 2 female mean body weight (472 g) was superior to that of males (422 g) and females (328 g) in Cross 1 and to females in Cross 3 (253 g) (Table 3); and, Cross 2 female mean body weight was similar to that of males in Cross 3 (466 g) and Cross 4 (447 g) (Table 3).

3.3 | Color segregation

Data on color segregation of the four parental strain combinations were presented in Table 4. No dark (wild-type) body color phenotype was observed in any fish in any of the four crosses. Two distinct red body color phenotypes were observed: (a) solid red (complete absence of black pigmentation on body surface); and (b) blotched (red body color with variable presence of black pigmentation) (Table 4). The solid red body color phenotype comprised 48.3% of Cross 1, 44.0% of Cross 3, and 10.0% of Cross 4. The remainder of Cross 1 and Cross 3 mostly exhibited the "RB1" blotched phenotype (a red body color with the presence of one small patch of black pigmentation), while in Cross 4 a higher number of fish exhibited the "RB2" blotched phenotype (red body color with presence of two or three small patches of black pigmentation) (Table 4). The blotched phenotype comprised 100% of Cross 2, with 96.6% of fish exhibiting the highest degree of dark pigmented coverage represented by the "RB3" phenotype (red body color with two or more large black patches distributed over large areas) (Table 4).

4 | DISCUSSION

4.1 | Comparative raising and growth

Water quality parameters were maintained within the optimal range for tilapia tank culture (DeLong et al., 2009). Survival in this study was similar to survival rates (97–100%) previously reported for other Nile Tilapia growth studies conducted in RAS (Arredondo-Figueroa, Núñez-García, Ponce-Palafox, & Ángeles Barriga-Sosa, 2015; Ridha, 2006a, 2006b). Multiple growth parameters evaluated strongly indicated that Til-Aqua YY males crossed with females from the Genetically Improved Farmed Tilapia (GIFT) strain (Cross 2) exhibited superior productivity traits compared to the other three crosses tested.

Cross 2 yielded a higher mean daily growth rate (4.08 g/day for 167 days; 50 fish/0.95 m^3) than that previously reported for different Nile Tilapia strains (Arredondo-Figueroa et al., 2015, Ridha, 2006a, 2006b). Daily growth rates for Crosses 1, 3, and 4 (2.28–2.57 g/day) were similar to those reported (2.26–2.51 g/day for 168 days; 200 fish/m^3) for genetically improved strains of Nile Tilapia (Ridha, 2006a). Other studies reported lower daily growth rates of 1.35 g/day for unimproved strains, and 2.01–2.19 g/day for improved strains (for 104 days; 125 fish/m^3) (Ridha, 2006b); and 0.4 g/day (for 63 days, 75 fish/m^3); 0.9 g/d (for 63 days, 30 fish/m^3); and 3.6 g/day (for 63 days; 10 fish/m^3) for an unidentified Nile Tilapia strain (Arredondo-Figueroa et al., 2015). More recently, a 7.67 g/day growth rate was calculated at the point of inflection (for 240 days) for genetically improved Nile Tilapia strains using the Gompertz exponential model (dos Santos, Silva, de Almeida, Mareco, & Salomão, 2019). Daily growth rates calculated based on their data for the first three successive growth intervals were 1.57 g/day (60 days; 120 fish/m^3), 5.06 g/day (60 days; 80 fish/m^3), and 7.23 g/day (60 days; 60 fish/m^3); and, the mean daily growth rate was 4.9 g/day for 180 days (dos Santos et al., 2019). In comparison to previous studies, Cross 2 exhibited a superior growth rate advantage not often reported, and the other three crosses exhibited growth rates that were similar to those previously reported for Nile Tilapia reared in RAS.

In terms of feed conversion efficiency, fish can achieve higher growth rates by (i) decreasing the amount of food consumed in relation to weight gain; (ii) increasing the quantity of feed consumed as a result of increased appetite; or (iii) by a combination of effective feed utilization and higher feed consumption (Gomelsky, 2011). The amount of feed consumed by Cross 2 was greater compared to the other crosses by 1.67 times more (Cross 1), 1.65 times more (Cross 3), and 1.56 times more (Cross 4). Thus, we can deduce that Cross 2's accelerated and larger growth was because of superior appetite and the higher quantity of feed consumed. The FCRs reported in this study (1.49–1.57) were similar to the higher end of the range for FCR previously reported (1.04–1.61) (Arredondo-Figueroa et al., 2015; dos Santos et al., 2019; Ridha, 2006a, 2006b).

Although the body condition factor has not frequently been reported in Nile Tilapia RAS-based growth studies, the values reported in this study (2.14–2.49) were higher than values in a nutrition study (1.83–2.01) on Nile Tilapia (Herath, Haga, & Satoh, 2016). The fillet yield obtained in Cross 2 (36%) was within the higher end of the range reported for Nile Tilapia strains raised in RAS (32% and 34 to 38%) (Garduño-Lugo, Granados-Alvarez, Olvera-Novoa, & Muñoz-Córdova, 2003; Rutten, Bovenhuis, & Komen, 2004).

4.2 | Male proportion and sexual dimorphism

Sexual size dimorphism in this study varied widely as shown in body weight advantage (29–84%) of males to females. The range of variation in sexual size dimorphism was similar to that previously reported for seven tilapia strains (Lind et al., 2015). Data on sexual dimorphism and growth parameters obtained in the present study showed that 100% male proportion (Cross 4) did not inherently result in optimal production, and that the effect of the parental strain combination used to produce Cross 2 was significant in yielding fast-growing and predominantly male tilapia.

4.3 | Color segregation

McAndrew et al. (1988) have performed a comprehensive investigation of inheritance and expression of red color in Nile tilapia. The results of that study showed that the red body color in this species is controlled by a dominant allele *R* of one gene (*R*/*r*). Fish homozygous for recessive allele (genotype *rr*) have dark (wild-type) color type while fish with genotypes *RR* and *Rr* can have either solid red (without black spots or patches) or red-black (blotched) body color. Blotching is a very variable trait; the black patches can cover up to about 25% of the fish surface. The degree of blotching is reduced with increase of the number dominant allele *R* in fish genotype. Fish with genotype *RR* have lower degree of blotching than heterozygotes *Rr*; however, the ranges of variability of this trait between *RR* and *Rr* fish are overlapping (McAndrew et al., 1988). In further studies, Mather, Lal, and Wilson (2001), Garduño-Lugo, Muñoz-Córdova, and Olvera-Novoa (2004) and Thodesen et al. (2013) reported a decrease in intensity of black blotching in red tilapia by selection applied in several consecutive generations while Hilsdorf, Penman, Farias, and McAndrew (2002), Rajaee (2011) and Lago et al. (2019) have described development and quantification of black pigmentation in red tilapia.

As mentioned above, the Til-Aqua YY, Fishgen YY and Miami parental strains were marketed and acquired as 'red' fish for the present study. In reality, fish from these strains were either red (R category—fish with red body color with no black pigmentation) or had minor expression of black patches (RB1 category) (Table 4). The presence of black spots on an otherwise basic red phenotype is a common characteristic of red tilapia stocks (Mather et al., 2001). No dark (wild type) fish were recorded in Cross 2, which was obtained by crossing Til-Aqua males (R and RB1 categories) with dark (wild type) GIFT females. This shows that in Nile tilapia used in the present study, the same as in experiments by McAndrew et al. (1988), the red color is controlled by a dominant mutation. The absence of dark fish in Cross 2 showed also that parental Til-Aqua males were homozygous for dominant allele (genotype *RR*) but not heterozygous (*Rr*) (see Table 4). No solid red (R category) and blotched fish with minor development of black patches (RB1 category) were present in Cross 2 while 96.6% of blotched fish in this cross belonged to RB3 category with highest level of black patches development. This is in agreement with an observation by McAndrew et al. (1988) that fish, which are originated from crosses of wild-type fish, have most intense blotch patterns. As mentioned above, the LSA parental strain was originated from crossing of red fish with wild-type (dark) fish and was heavily blotched. This indicates that LSA females used for production of Crosses 3 and 4 were obviously heterozygous for red color gene (genotype *Rr*). Because no dark (wild type) fish were observed in Crosses 3 and 4 it can be suggested that parental YY males for these crosses (Til-Aqua and Fishgen YY males, respectively) were homozygous for dominant allele (genotype *RR*) but not heterozygous (genotype *Rr*) (see Table 4). In Crosses 1, 3, and 4 the offspring could have genotypes *RR* and *Rr* (Table 4); however, it is impossible to determine exactly the genotype of every fish in crosses because, as was shown by McAndrew et al. (1988), the rate of blotching in fish with these genotypes overlaps. Segregations of fish with regard to red color development in Crosses 1 and 3 were similar. The proportions of solid red fish (R category) were 48.3 and 44.0% while proportions of fish with minimal degree of blotching development (category RB1) were 84.4 and 85.7% in Crosses 1 and 3, respectively. Cross 4 was characterized by more intensive rate of blotching: proportions of fish of categories R and RB1 in this cross were 10.0 and 57.8%, respectively.

4.4 | Genetic differentiation of parental strains

Recently, Delomas, Gomelsky, Vu, Campbell, and Novelo (2019) reported data on genetic differentiation of eight Nile Tilapia strains including those that were used as broodstock in this study. Nile Tilapia strains were analyzed based on biallelic single-nucleotide polymorphisms (SNPs), and genetic differentiation was measured by pairwise fixation index (F_{ST}) in which values increase with the increase in genetic distance between strains (Delomas et al., 2019). The highest value of pairwise F_{ST} (0.24) was observed between parental strains used for production of Cross 1 (Til-Aqua YY males x Miami females), the intermediate value of F_{ST} (0.15) was detected between strains used for production of Cross 2 (Til-Aqua YY males × GIFT females), and the smallest level of genetic differentiation (F_{ST} = 0.09) was

observed between parental strains used for production of Cross 3 (Til-Aqua YY males × LSA females) and Cross 4 (Fishgen YY males × LSA females) (Delomas et al., 2019). The results of the present study showed that Cross 2 had highest productivity, Cross 1 was the least productive, and Crosses 3 and 4 demonstrated intermediate productivity. The data obtained in these two studies indicate that performance of the tested inter-strain crosses may not depend on the level of genetic differentiation between parental strains. The absence of dependence between pairwise F_{ST} values for the strains and the performance data obtained in the present study contradicts the suggestion that the rate of heterosis increases with increase of genetic distance between strains used in crosses (Shikano & Taniguchi, 2002). However, the results of the present study support the proposition that the effect of heterosis vary between different strains and the possibility of heterosis needs to be determined on a case-by-case basis (Gjedrem & Robinson, 2014).

4.5 | Practical implications and recommendations

This study was designed to test the combination of YY male technology and crossbreeding for identifying potential gains in growth and for investigating sex and color. The results provided strong evidence that the genetic contribution of different parental strain combinations clearly impact commercially important productivity traits, and that crossing YY males with females from genetically improved tilapia strains such as the GIFT strain provide the opportunity to produce predominantly male, fast-growing fish of superior size. This shows the importance of the genetic material (species and strain) used to obtain highly beneficial crosses for commercial use; thus, it is strongly advisable that in order to improve tilapia production and profitability, suppliers, and farmers should ascertain the genetic background and productivity of fish they supply or purchase for hatchery (seedstock) and grow-out purposes.

The ability to raise tilapia such as those obtained from Cross 2 is of high potential benefit for different commercial production systems such as in RAS for maximum control of culture parameters, and such as in cage culture in ponds to maximize use of pond resources in temperate climate regions such as Kentucky that have a limited time window for growth (110–120 days) during summer (Danaher, Tidwell, Coyle, Dasgupta, & Zimba, 2007). It is important to note that though no reproduction was observed during rearing of these four crosses in this study, reproduction is possible in unconfined stocking in ponds; thus, tilapia such as Cross 2 that may be predominantly but not 100% male should be raised in culture environments (such as in cages in ponds) that inhibit reproduction (Bentsen et al., 2012; Danaher et al., 2007; Lind et al., 2015; Tidwell, Coyle, & Bright, 2010).

Studies on red color tilapia report the high cultural, aesthetic, and commercial value of red tilapia (Ng & Hanim, 2007; Pongthana et al., 2010; Ramírez-Paredes et al., 2012), and the negative effect on appearance of the blotched phenotype (black patches) on red body color of tilapia that, by implication, has negative market appeal (Garduño-Lugo et al., 2004; Mather et al., 2001; McAndrew et al., 1988; Thodesen et al., 2013). Based on these suggestions on red color tilapia marketing trends, solid red fish and fish with minimal expression of blotching (e.g., RB1 and RB2) such as in Crosses 1, 3, and 4 may be more desirable for marketing as live or dress-out (gilled, gutted and scaled) products, while Cross 2 may be considered more appropriate as a fillet product. However, marketing and sales data on tilapia is insufficient (and not readily available, or reported), and researchers, farmers, and consumers would benefit from studies investigating acceptance and willingness to pay for red color morphs (complete red coverage; and, red color fish with RB1, RB2, and RB3 type blotched phenotypes), and wild-type color (dark) fish in whole fish markets. In addition, future studies should continue efforts at genetic improvement of tilapia by combining practical genetic approaches such as YY male technology and crossbreeding, and by investigating the productivity of crosses between dark (wild-type) color YY males and dark color females from genetically improved fish such as the GIFT strain.

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REFERENCES

Arredondo-Figueroa, J., Núñez-García, L., Ponce-Palafox, J., & Ángeles Barriga-Sosa, I. (2015). Performance of brooders, fry and growth of the Nile tilapia (*Oreochromis niloticus*) cultured in an experimental recirculating aquaculture system. *Agricultural Sciences*, *6*, 1014–1022.

Baroiller, J., & D'Cotta, H. (2019). Sex control in tilapias. In H.-P. Wang, F. Piferrer, & S.-L. Chen (Eds.), *Sex control in aquaculture* (Vol. 1, pp. 191–222). New Jersey, USA: John Wiley & Sons, Inc..

Beardmore, J. A., Mair, G. C., & Lewis, R. I. (2001). Monosex male production in finfish as exemplified by tilapia: Applications, problems, and prospects. *Aquaculture*, *197*, 283–301.

Bentsen, H. B., Gjerde, B., Nguyen, N. H., Rye, M., Ponzoni, R. W., Palada de Vera, M. S., … Eknath, A. E. (2012). Genetic improvement of farmed tilapias: Genetic parameters for body weight at harvest in Nile tilapia (*Oreochromis niloticus*) during five generations of testing in multiple environments. *Aquaculture*, *338-341*, 56–65.

Colihueque, N., & Araneda, C. (2014). Appearance traits in fish farming: Progress from classical genetics to genomics, providing insight into current and potential genetic improvement. *Frontiers in Genetics*, *5*, 251–251.

Colihueque, N., Parraguez, M., Estay, F. J., & Diaz, N. F. (2011). Skin color characterization in rainbow trout by use of computer-based image analysis. *North American Journal of Aquaculture*, *73*, 249–258.

Danaher, J. J., Tidwell, J. H., Coyle, S. D., Dasgupta, S., & Zimba, P. V. (2007). Effects of two densities of caged monosex Nile tilapia, *Oreochromis niloticus*, on water quality, phytoplankton populations, and production when polycultured with *Macrobrachium rosenbergii* in temperate ponds. *Journal of the World Aquaculture Society*, *38*, 367–382.

Delomas, T. A., Gomelsky, B., Vu, N., Campbell, M. R., & Novelo, N. D. (2019). Single-nucleotide polymorphism discovery and genetic variation in YY-male and mixed-sex strains of Nile tilapia available in the United States. *North American Journal of Aquaculture*, *81*, 183–188.

DeLong, D. P., Losordo, T. M., & Rakocy, J. E. (2009). *Tank culture of tilapia*. stoneville, MS: Southern Regional Aquaculture Center. https://srac.tamu.edu/viewCategory/11

dos Santos, V. B., Silva, V. V., de Almeida, M. V., Mareco, E. A., & Salomão, R. A. S. (2019). Performance of Nile tilapia *Oreochromis niloticus* strains in Brazil: A comparison with Philippine strain. *Journal of Applied Animal Research*, *47*, 72–78.

Dunham, R. A. (2011). Intraspecific crossbreeding. In *Aquaculture and fisheries biotechnology*: *Genetic approaches* (pp. 58–64). Wallingford, England: CABI Publishing, Wallingford, United Kingdom.

Eknath, A. E., & Hulata, G. (2009). Use and exchange of genetic resources of Nile tilapia (*Oreochromis niloticus*). *Reviews in Aquaculture*, *1*, 197–213.

FAO. (2019). *Fishery and aquaculture statistics. Global aquaculture production 1950-2017 (FishstatJ)*. Rome: FAO Fisheries and Aquaculture Department. www.fao.org/fishery/statistics/software/fishstatj/en

Garduño-Lugo, M., Granados-Alvarez, I., Olvera-Novoa, M. A., & Muñoz-Córdova, G. (2003). Comparison of growth, fillet yield and proximate composition between Stirling Nile tilapia (wild type) (*Oreochromis niloticus*, Linnaeus) and red hybrid tilapia (Florida red tilapia × Stirling red *O. niloticus*) males. *Aquaculture Research*, *34*, 1023–1028.

Garduño-Lugo, M., Muñoz-Córdova, G., & Olvera-Novoa, M. Á. (2004). Mass selection for red colour in *Oreochromis niloticus* (Linnaeus 1758). *Aquaculture Research*, *35*, 340–344.

Gjedrem, T., & Robinson, N. (2014). Advances by selective breeding for aquatic species: A review. *Agricultural Sciences*, *5*, 1152–1158.

Gjerde, B., Mengistu, S. B., Ødegård, J., Johansen, H., & Altamirano, D. S. (2012). Quantitative genetics of body weight, fillet weight and fillet yield in Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, *342-343*, 117–124.

Gomelsky, B. (2011). *Fish genetics: Theory and practice*. Saarbrücken, Germany: VDM Verlag Dr. Mueller GmbH & Co. KG.

Herath, S. S., Haga, Y., & Satoh, S. (2016). Effects of long-term feeding of corn co-product-based diets on growth, fillet color, and fatty acid and amino acid composition of Nile tilapia, *Oreochromis niloticus*. *Aquaculture*, *464*, 205–212.

Hilsdorf, A. W. S., Penman, D. J., Farias, E. C., & McAndrew, B. (2002). Melanophore appearance in wild and red tilapia embryos. *Pigment Cell Research*, *15*, 57–61.

Kamaruzzaman, N., Nguyen, N. H., Hamzah, A., & Ponzoni, R. W. (2009). Growth performance of mixed sex, hormonally sex reversed and progeny of YY male tilapia of the GIFT strain, *Oreochromis niloticus*. *Aquaculture Research*, *40*, 720–728.

Khaw, H. L., Ponzoni, R. W., Yee, H. Y., Aziz, M. A. b., & Bijma, P. (2016). Genetic and non-genetic indirect effects for harvest weight in the GIFT strain of Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, *450*, 154–161.

Lago, A. A., Reis-Neto, R. V., Rezende, T. T., da Silva Ribeiro, M. C., de Freitas, R. T. F. & Hilsdorf, A. W. S. 2019. Quantitative analysis of black blotching in a crossbred red tilapia and its effects on performance traits via a path analysis methodology. *Journal of Applied Genetics*, *60*, 393–400.

Lago, A. A., Rezende, T. T., Dias, M. A. D., Freitas, R. T. F., & Hilsdorf, A. W. S. (2017). The development of genetically improved red tilapia lines through the backcross breeding of two *Oreochromis niloticus* strains. *Aquaculture*, *472*(Supplement 1), 17–22.

Lind, C. E., Safari, A., Agyakwah, S. K., Attipoe, F. Y. K., El-Naggar, G. O., Hamzah, A., … Ponzoni, R. W. (2015). Differences in sexual size dimorphism among farmed tilapia species and strains undergoing genetic improvement for body weight. *Aquaculture Reports*, *1*, 20–27.

Mair, G. C., Abucay, J. S., Beardmore, J. A., & Skibinski, D. O. F. (1995). Growth performance trials of genetically male tilapia (GMT) derived from YY-males in *Oreochromis niloticus* L.: On station comparisons with mixed sex and sex reversed male populations. *Aquaculture*, *137*, 313–323.

Mather, P. B., Lal, S. N., & Wilson, J. (2001). Experimental evaluation of mass selection to improve red body colour in Fijian hybrid tilapia (*Oreochromis niloticus* × *Oreochromis mossambicus*). *Aquaculture Research*, *32*, 329–336.

McAndrew, B. J., Roubal, F. R., Roberts, R. J., Bullock, A. M., & McEwen, I. M. (1988). The genetics and histology of red, blond and associated colour variants in *Oreochromis niloticus*. *Genetica*, *76*, 127–137.

Mlalila, N., Mahika, C., Kalombo, L., Swai, H., & Hilonga, A. (2015). Human food safety and environmental hazards associated with the use of methyltestosterone and other steroids in production of all-male tilapia. *Environmental Science and Pollution Research*, *22*, 4922–4931.

Ng, W., & Hanim, R. (2007). Performance of genetically improved Nile tilapia compared with red hybrid tilapia fed diets containing two protein levels. *Aquaculture Research*, *38*, 965–972.

NOAA. (2019). National Oceanic and Atmospheric Administration, Office of Science and Technology. Commercial Fishery Statistics on Foreign Trade. Database of Annual Trade Data by Product, Country/Association. Tilapia Imports into the US 1992-2018. Retrieved from: https://www.st.nmfs.noaa.gov/commercial-fisheries/foreign-trade/.

Phelps, R. P. (2006). Hormone manipulaton of sex. In C. E. Lim & C. D. Webster (Eds.), *Tilapia: Biology, culture, and nutrition* (pp. 211–252). Binghamton, NY: The Haworth Press, Inc..

Pongthana, N., Nguyen, N. H., & Ponzoni, R. W. (2010). Comparative performance of four red tilapia strains and their crosses in fresh- and saline water environments. *Aquaculture*, *308*, S109–S114.

Ponzoni, R. W., Nguyen, N. H., Khaw, H. L., Hamzah, A., Bakar, K. R. A., & Yee, H. Y. (2011). Genetic improvement of Nile tilapia (*Oreochromis niloticus*) with special reference to the work conducted by the WorldFish center with the GIFT strain. *Reviews in Aquaculture*, *3*, 27–41.

Popma, T., & Masser, M. (1999). *Tilapia life history and biology*. stoneville, MS: Southern Regional Aquculture Center. https://srac.tamu.edu/viewCategory/11

Rajaee A. H. (2011). Genetic approaches to the analysis of body colouration in Nile tilapia (*Oreochromis niloticus*). PhD Thesis. University of Stirling, Scotland, UK.

Ramírez-Paredes, J. G., Garduño-Lugo, M., & Muñoz-Córdova, G. (2012). Productive performance of a new synthetic red tilapia population 'Pargo-UNAM' compared with that of wild-type Nile tilapia (*Oreochromis niloticus* L.). *Aquaculture Research*, *43*, 870–878.

Riche, M., Haley, D. I., Oetker, M., Garbrecht, S., & Garling, D. L. (2004). Effect of feeding frequency on gastric evacuation and the return of appetite in tilapia *Oreochromis niloticus* (L.). *Aquaculture*, *234*, 657–673.

Ridha, M. T. (2006a). Comparative study of growth performance of three strains of Nile tilapia, *Oreochromis niloticus*, L. at two stocking densities. *Aquaculture Research*, *37*, 172–179.

Ridha, M. T. (2006b). Evaluation of growth performance of nonimproved and improved strains of the Nile tilapia (L.), *Oreochromis niloticus*. *Journal of the World Aquaculture Society*, *37*, 218–223.

Rutten, M. J. M., Bovenhuis, H., & Komen, H. (2004). Modeling fillet traits based on body measurements in three Nile tilapia strains (*Oreochromis niloticus* L.). *Aquaculture*, *231*, 113–122.

Shikano, T., & Taniguchi, N. (2002). Using microsatellite and RAPD markers to estimate the amount of heterosis in various strain combinations in the guppy (*Poecilia reticulata*) as a fish model. *Aquaculture*, *204*, 271–281.

Tariq Ezaz, M., Myers, J. M., Powell, S. F., McAndrew, B. J., & Penman, D. J. (2004). Sex ratios in the progeny of androgenetic and gynogenetic YY male Nile tilapia, *Oreochromis niloticus* L. *Aquaculture*, *232*, 205–214.

Thodesen, J., Rye, M., Wang, Y.-X., Li, S.-J., Bentsen, H. B., Yazdi, M. H., & Gjedrem, T. (2013). Genetic improvement of tilapias in China: Genetic parameters and selection responses in growth, survival and external color traits of red tilapia (*Oreochromis* spp.) after four generations of multi-trait selection. *Aquaculture*, *416-417*, 354–366.

Tidwell, J. H., Coyle, S. D., & Bright, L. A. (2010). Polyculture of Nile tilapia, *Oreochromis niloticus*, either confined in cages or unconfined in freshwater prawn, *Macrobrachium rosenbergii*, ponds. *Journal of the World Aquaculture Society*, *41*, 616–625.

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