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Can Tho University Journal of Science

website: ctujs.ctu.edu.vn

DOI: 10.22144/ctu.jen.2021.034

Growth and survival performance of G2 crossbreeds from two strains of bighead catfish (*Clarias macrocephalus*)

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Article info.

Received 22 Aug 2020 Revised 31 Jan 2021 Accepted 20 Nov 2021

Keywords

Bighead catfish, crossbreeding, genetic improvement, growth, larval rearing

ABSTRACT

The development of bighead catfish (Clarias macrocephalus) aquaculture in Viet Nam has been constrained by the slow growth performance in the domesticated strain. Crossbreeding can be a solution to improve fish production. This study was aimed to evaluate the growth and survival performance of the second generation (G2) fingerlings crossbreeds (CMCT and CTCM) from two bighead catfish strains (wild Ca Mau, CM, and domesticated Can Tho, CT) compared to offspring from their original strains (G0-CT and G0-CM) and G1 crossbreeds ($\bigcirc CM \times \bigcirc CT$ and $\bigcirc CT$ $\times \mathcal{CM}$). The larvae were reared in 50-L tanks (3 to 5 replications) with the density of 1,000 individuals/tank and fed with Moina and artificial feed (40% protein). After 40 days of rearing, the fish grew differently among crosses (P < 0.05). In which, the two best-growth groups included CM×CT crossbreeds (body weight of 1,114 mg and length of 4.55 cm) and pure crossbred line CTCM (1,050 mg and 4.51cm, respectively). The CTCM group was recorded the highest survival rate (37.8%) while G0-CT was the lowest (27.2%); however, the difference in survival was not significant (P>0.05). The performance of these crossbreeds should be investigated further at the grow-out stage to see whether crossbreeding between wild and cultured strains of bighead catfish may be used in practice to increase bighead catfish production.

1. INTRODUCTION

Catfishes in the genus *Clarias* are widely distributed in subtropical and tropical areas of Asia and Africa (Teugels & Adriaens, 2003). In Viet Nam for instance, *Clarias* catfish is one of the commercially important aquaculture fish groups consisting of *C. macrocephalus*, *C. gariepinus*, *C. fuscus*, *C. batrachus*, and one hybrid *C. macrocephalus* (*Cm*) x *C. gariepinus* (*Cg*) (Lenonnand et al., 1998). Among the four *Clarias* species, bighead catfish (*C. macrocephalus*) is a popular species with a high economic value (Hien et al., 2018). It has long been an important target for aquaculture in Southeast Asia (Carreon et al., 1976; Na-Nakorn et al., 1995).

Bighead catfish is however not so popular in intensive farming due to low disease resistance and a slow growth rate (FAO, 2010). While its hybrid catfish ($Cm \ge Cg$) that exhibits fast growth rate, high tolerances to environmental conditions, and resistance to diseases has been cultured in many provinces in Viet Nam (Duong et al., 2017). Native bighead catfish remains to be the most preferred

choice by consumers amongst the *Clarias* catfishes because of its better flesh quality. Therefore, bighead catfish has higher market prices compared to the hybrid catfish.

To cope with the above drawbacks, genetic improvement of the native bighead catfish through crossbreeding of different populations is a potential choice. In which, intraspecific crossbreeding often increases the growth rate of aquaculture species, although specific heterotic combinations need to be experimentally identified (Hulata, 2001). It also has the advantage of simplicity and the often immediate and visible impact on animal performance. Various intraspecific crossbreeding cases have been reported (Dunham, 2011). For example, the Vietnamese \times Hungarian common carp has fast growth and high survival rates under different production conditions and for this reason, the crossbreed is very popular in both the northern and southern regions of Viet Nam (Thien & Trong, 1995). Similarly, crosses of six different strains of silver barbs in Viet Nam have shown heterosis for growth rate (Bentsen et al., 1996).

In this study, the survival, and growth performances of crossbreeds created through intra-specific crossbreeding across two generations of C. *macrocephalus* were investigated. The findings for this undertaking would shed light on the possibilities of improving the growth performance of bighead catfish through intraspecific crossbreeding.

2. MATERIALS AND METHODS

2.1. Source of broodstock

The original broodstock (called G0) of *C. macrocephalus* adults were collected from U Minh Ha National Park in Ca Mau province (wild, CM), Lung Ngoc Hoang conservation in Hau Giang province (wild, HG) and from a hatchery in Can Tho province (cultured, CT) in March 2019. They were cultured in a recirculating system at College of Aquaculture and Fisheries (CAF) and were abbreviated as G0-CM, G0-CT, and G0-HG.

Nine diallel crosses including three pure and six reciprocal crosses (\bigcirc CM x \bigcirc CT, \bigcirc CT x \bigcirc CM, \bigcirc HG x \bigcirc CT, \bigcirc CT x \bigcirc HG, \bigcirc HG x \bigcirc CM, and \mathcal{Q} CM x \mathcal{O} HG) from the three strains were produced and evaluated their growth from May 2019 to December 2019. At the market stage, two crossbreeds \mathcal{Q} CM x \mathcal{O} CT and \mathcal{Q} CT x \mathcal{O} CM showed better growth performance among six crossbreeds (Duong et al., 2022) and they were chosen for the present experiment. They were marked as G1-CMCT and G1-CTCM. By May 2020 these crossbreeds had become mature and 15-20% larger adults were chosen to produce offspring (G2-CMCT and G2-CTCM). These offspring were compared with those produced by G1 crosses between CM and CT strains (G1-CM x G1-CT and their reciprocals G1-CT x G1-CM) and by two pure G0 (\bigcirc CM x $\partial^{\circ}CM$ and $\mathcal{Q}CT \times \partial^{\circ}CT$). In sum, six offspring treatments were evaluated in this study (Table 1).

Parents producing the offspring	Notes on parent conditions	Label for offspring treatments
♀G1-CMCTx ♂ G1-CMCT	These C1 second more and head	1. CMCT
♀GI-CTCMx♂G1-CTCM	These G1 groups were produced	2. CTCM
♀G1- CMx♂G1- CT	in May, 2019 and cultured in the same conditions	3. CMxCT
♀G1- CTx♂G1-CM	same conditions	4. CTxCM
ĢG0- CMx♂G0-CM	G0 is the generation producing	5. G0-CM
♀G0- CTx♂G0- CT	G1 above	6. G0-CT

Table 1. Experimental crosses/treatments

2.2. Artificial propagation, fertilization and incubation

Five to seven pairs of mature breeders from each group (Table 1) were injected with human chorionic gonadotropin hormone (HCG) and pituitary gland (PG) hormone (as described in Tran & Duong, 2021). After the 12 h latency period, the milt was obtained from the testes of freshly - killed male breeders and was pooled from several individuals. Milt was mixed with eggs using dry fertilization method. Fertilized eggs were removed adhesiveness

by urea solution (3 g urea + 4 g NaCl in 1 L of distilled water) and then 1.5 ppt tannin solution was added for 10 seconds to remove the stickiness of the eggs. Finally, clean water was used to wash out all the chemical residues from the eggs before incubation. Incubation and hatching of eggs were carried in the jar incubating system. The water temperature in the incubator fluctuated between 28– $34^{\circ}C$.

2.3. Design of larval rearing experiment

The rearing system included 50-L rectangular tanks (containing 40 L of water) equipped with aeration. The three-day-old larvae were pooled by each cross and randomly stocked in tanks at a density of 1000 larvae per tank. Each cross had 5 replications except for CMxCT and CTxCM which were replicated 4 and 3 times, respectively, due to the inadequate number of larvae.

The larvae were fed twice daily (8 a.m. & 5 p.m.) with Moina for 10 days, starting from the 3rd day of hatching. Thereafter, commercial feed (40% protein) was added to combine with Moina for the weaning of fry from the 9th day to the 15th day when they could eat 100% artificial feed. At this point, they were fed four times a day (8 a.m., 11 a.m., 2 p.m. and 5 p.m.). Uneaten artificial feed and waste were siphoned and water was exchanged 30 to 50% every day. The water temperature over the rearing period fluctuated between 26-34°C while the water pH shifted between 6.8 and 7.6.

2.4. Sampling and data collection

The experiment lasted for 40 days. Before stocking, 30 larvae from each cross were measured of their initial weight and length. At the middle (20th day) and at the end of the experiment (40th day), 30 to 40 larvae from each tank were randomly sampled and recorded their weight and length. These data were used to determine the survival rate (SR) and growth performance parameters (daily weight gain, DWG; daily length gain, DLG; specific growth rate in weight, SGRW; and specific growth rate in length, SGRL) as follows:

DWG (g/day)

= Final body weight (g) – Initial body weight(g) Rearing period in days

DLG (mm/day)

= Final body length(mm) – Initial body length(mm) Rearing period in days

$$SGRW(\%/day) = \frac{\text{Ln final weight} - \text{Ln initial weight}}{\text{Rearing period in days}} \times 100$$

SGRL (%/day) = $\frac{\text{Ln final length} - \text{Ln initial lenght}}{\text{Rearing period in days}} \times 100$

SR (%) =
$$\frac{\text{Number of survival fish}}{\text{Number of fish stocked}} \times 100$$

Coefficients of variation (CV, %)

 $= \frac{\text{Standard deviation}}{\text{mean weight}} \times 100$

2.5. Statistical analysis

Survival rates and coefficient of variance were transformed to natural logarithm before statistical analysis (Warton & Hui, 2011). These transformed data and growth parameters were compared among fish treatments using one-way analysis of variance (ANOVA), followed by Duncan's multiple range tests. The significant difference was evaluated at the level of 0.05. SPSS version 20.0 was used for statistical analysis.

3. RESULTS

3.1. Growth in weight of six crosses

The growth trend in weight of six larval groups during 40 days of experiment is shown in Figure 1. Overall, the weight of the larvae in all the treatments demonstrated an upward trajectory over the experimental period. However, the growth trend differed remarkably among fish groups as evidenced by the slope of the growth lines, especially in the last 20 days. CMxCT and CTCM exhibited best parallel growth while G0-CT, CMCT, and G0-CM demonstrated a moderate increase. Contrastingly, a slow growth line was manifested in CTxCM.

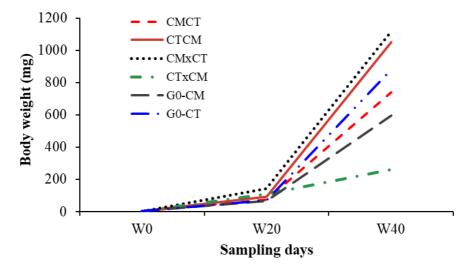


Figure 1. Larval body weight trend during 40 rearing days of six experimental crosses

Analysis of variance showed significant differences (P<0.05) in all growth weight parameters (Table 2). At the beginning of the experiment, the three-days

old larvae weighed from 2.10 (CMxCT) to 3.24 mg (G0-CT). CTCM was the second large fish (3.04 mg), while the other groups had similar initial weights (from 2.55 to 2.72 mg).

Table 2. Means±SD of initial weight, mid-weight, final weight, daily weight gain (DWG) and specific growth rate in weight (SGRW) of the six experimental crosses

Crosses	W0 (mg)	W20 (mg)	W40 (mg)	DWG (mg/day)	SGRW (%/day)
CMCT	2.60	73.7±11.5ª	740.2±139.8 ^{bc}	18.4 ± 3.5^{bc}	14.1 ± 0.4^{bc}
CTCM	3.04	$93.4{\pm}23.0^{ab}$	$1,050\pm148^{cd}$	26.2 ± 3.7^{cd}	14.6 ± 0.4^{cd}
CMxCT	2.10	141.8±39.6°	$1,114\pm353^{d}$	27.8 ± 8.8^{d}	15.6 ± 0.8^{d}
CTxCM	2.55	110.7 ± 8.3^{b}	262.5 ± 77.4^{a}	6.5 ± 1.9^{a}	11.5 ± 0.8^{a}
G1-CM	2.72	67.9 ± 15.0^{a}	594.1±267.1 ^{ab}	14.8 ± 6.7^{ab}	13.3 ± 1.2^{b}
G1-CT	3.24	71.2 ± 7.8^{a}	878.8 ± 334.8^{bcd}	21.9 ± 8.4^{bcd}	13.8±1.0 ^{bc}

Means in the same column having the same superscript letter are not significantly different (P>0.05)

After 20 days of larval rearing, fish growth was significantly separated into three groups. The CMxCT group showed the highest weight (141.8±39.6 mg), significantly different from the other groups (P<0.05). It was followed by its reciprocal CTxCM and CTCM (110.7±8.3 and 93.4±23.0 mg, respectively). Contrastingly, the other three groups G0-CM, G0-CT, and CMCT had the lowest weights. At the end of the experiment (40th day), CMxCT and CTCM groups recorded the highest weights of 1,050±148 mg and 1,114±353 mg, respectively. CTxCM group did not maintain its growth trend that was observed during the midsampling, and instead, it ranked the last in the final sampling with a mean weight of 262.53±77.40 mg (Table 2).

The daily weight gain (DWG) and the specific growth rate in weight (SGRW) among all the

treatments were statistically different (P<0.05). CTxCM group performed exemplary well in both variables at 27.8 \pm 8.8 mg/day and 15.6 \pm 0.8 %/day, respectively. This was closely followed by CTCM (26.2 \pm 3.7 mg/day and 14.6 \pm 0.4 %/day, respectively). The poorest DWG and SGRW was recorded in CTxCM with 6.5 \pm 1.9 mg/day and 11.5 \pm 0.8 %/day accordingly.

3.2. Growth in length

Figure 2 presents the trend in length gain of the larvae at three sampling periods (at the beginning, 20th and 40th day). Generally, the growth trend of body length was similar to that observed in the body weight, showing different slopes among fish groups and between two sampling periods. CMxCT and CTCM exhibited an outstanding length gain while on the other hand, CTxCM revealed a slow length gain.

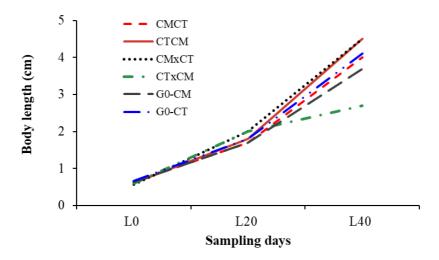


Figure 2. Larval body length trend for the six experimental crosses

In Table 3, the means for initial length (L0), midlength (L20), final length (L40), DLG, and SGRL were significantly different among fish crosses (P<0.05) between the crosses. Generally, the body length exhibited a similar trend of differences among treatments to that was manifested in the body weight. The highest body length at the 20th day (2.0 ± 0.2 cm was observed in the intraspecific cross CMxCT and its reciprocal CTxCM. Towards the end of the rearing period, CMxCT and CTCM groups had the highest body length (mean of 4.5 cm), insignificantly followed by G0-CT and CMCT (P>0.05), while G0-CM had the lowest final length (2.7±0.2 cm). Other growth parameters including DLG and SGRL showed similar trend as that of final length.

 Table 3. Initial length, mid-length, final length, daily length gain (DLG) and specific growth rate in length (SGRL) of the six experimental crosses

Cross	L0 (cm)	L20 (cm)	L40 (cm)	DLG (cm/day)	SGRL (%/day)
CMCT	0.63 ± 0.04^{bc}	1.7±0.1ª	4.0 ± 0.2^{bc}	$0.10{\pm}0.01^{bc}$	4.6 ± 0.2^{bc}
CTCM	0.61 ± 0.03^{b}	$1.8{\pm}0.2^{ab}$	4.5±0.3°	$0.10{\pm}0.01^{\circ}$	5.0 ± 0.2^{cd}
CMxCT	$0.57{\pm}0.04^{a}$	$2.0{\pm}0.2^{b}$	$4.5 \pm 0.6^{\circ}$	$0.10{\pm}0.01^{\circ}$	5.2±0.3°
CTxCM	$0.58{\pm}0.03^{a}$	$2.0{\pm}0.0^{b}$	$2.7{\pm}0.2^{a}$	0.05±0.00 a	$3.9{\pm}0.2^{a}$
G0-CM	0.64 ± 0.02^{cd}	$1.7{\pm}0.2^{a}$	3.7 ± 0.7^{b}	0.08 ± 0.02^{b}	4.3 ± 0.5^{b}
G0-CT	0.66 ± 0.02^{d}	$1.8{\pm}0.1^{ab}$	4.1 ± 0.6^{bc}	0.09 ± 0.01^{bc}	4.6 ± 0.3^{bc}

*Means in the same column having the same superscript letter are not significantly different (P>0.05)

3.3. Survival rates and growth differentiation

There was no significant difference (P>0.05) in the survival rates among fish treatments, ranging from 27.2% (G0-CT) to 37.8% (CTCM) (Table 4).

Bighead catfish in all treatments showed high variation in individual growth. The higher the coefficient of variance (CV) in the body weight and length indicated a higher level of heterogeneity in sizes. Coefficient of variance in weight determined at the end of the larval rearing experiment (Table 4) was much higher (ranging between 60.8% and 94.6%) compared to CV in length (21.7-26.7%). The CTxCM cross showed the highest CVs (94.69 \pm 9.5% in weight and 26.7 \pm 1.5% in length) whereas CTCM was recorded the least values (55.8 \pm 14.3% and 20.3 \pm 3.4%, respectively). However, differences in CV were not significant in weight (P>0.05) but in length (P<0.05).

Treatment	CV in weight (%)	CV in length (%)	Survival rate (%)
CMCT	60.8±11.4ª	21.7±2.6ª	35.6±10.2ª
CTCM	55.8±14.3ª	20.3±3.4ª	37.8±2.9ª
CMxCT	70.5±24.2ª	24.5 ± 4.0^{ab}	28.1±11.9 ^a
CTxCM	94.6±9.5ª	26.7 ± 1.5^{b}	$34.4{\pm}3.0^{a}$
G0-CM	88.3±51.3ª	23.3 ± 3.3^{ab}	38.3 ± 9.8^{a}
G0-CT	71.3±18.1ª	23.0±3.0 ^{ab}	27.2 ± 5.5^{a}

Table 4. Coefficients of variation (CV) and survival rates in the six treatments

*Means in the same column having the same superscript letter are not significantly different (P>0.05).

The distribution of final weight values within and between fish treatments is shown in Figure 3. The highest ratios were distributed between 0.3 and 2.0g.

The frequencies of the weight values between treatments didn't conform to the common trend.

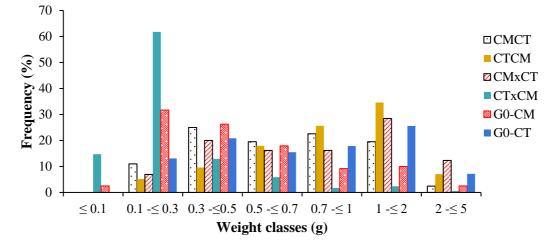


Figure 3. Growth differentiation of the larvae among the treatments on the 40th day

In the CTxCM and G0-CM groups, the frequencies dropped gradually from small to large sizes, with the highest ratio being recorded in the weight class of 0.1 to 0.3 g (61% and 29%, respectively) and the lowest in the class of 2.0 to 5.0 g. An opposite tendency was recorded in the CTCM cross, whereby the rate of the observation raised steadily from small to large weight classes. The frequencies in CMCT, CMxCT, and G0-CT cross oscillated between low and high within the range between 0.3 and 5.0 g.

4. DISCUSSION

The present study confirmed that G2 of CTxCM crossbreed (CTCM) exhibited over-performance compared to other crosses. They had a significantly faster growth rate, (insignificantly) higher survival rate and lower heterogeneity (lower CVs) than the other groups. Growth rate is considered a trait of great economic importance for all species used in aquaculture, and thus it is a trait of interest for most breeding programs (Tave, 1993; Dunham, 2011). The results of this study show that crossbreeding

between bighead catfish strains can produce better growth of progenies.

In other studies, crossbreeding between wild broodstock strains (CM, HG or Long An, LA) and a cultured strain in CT showed that the reciprocal crossbreeds CMxCT and CTxCM had better growth performance than other crossbreeds (Duong et al., 2020; Duong et al., 2022). Similar results were observed in the present study when CMxCT crossbred had the highest growth rate. Differently, its reciprocal CTxCM grew fast at first but was stunted in the later stage. It is common that the performance of reciprocal crossbreds can be different due to maternal effects (R. A. Dunham & Smitherman, 1983). More importantly, this study indicated that CMxCT crossbred can be used for grow-out (when CMxCT had better growth) and/or used as breeders when their offspring (CMCT and CTCM) exhibited outstanding performance compared to the original pure sources CM and CT.

Survival is among the most important traits determining production capacity in aquaculture. From the results of this study, the survival rates among all mating combinations were not different (Table 4). The outcomes of the study also indicated that the survival rates were relatively low in all treatments (ranging from 27 to 38%). The low survival could in part be linked to cannibalism due to uniformity in the larvae sizes (Figure 3). Clarias catfish are known for their cannibalistic behavior especially in their young stage in the case of C. macrocephalus. Legendre et al. (1992) suggested that size segregation is necessary for Clariid catfish culture to avoid cannibalism. In this study, size segregation was not done which might have increased the chances of cannibalism consequently resulting in poor survival performance. Similarly, Opiyo et al. (2017) reported a low survival rate of 23.28% in African catfish, which was linked to heterogeneity in sizes (high value of the coefficient of variation, CV), consequently resulting in cannibalism. In this study the CV in weight was high, ranging between 60.8 and 94.6%, thus confirming that indeed cannibalism contributed to the poor survival performance. Another possible factor pointing out to the low survival could be management and handling stress. Iwalewa et al. (2017) found the performance of C. gariepinus fingerlings in an intraspecific study to be associated with good management and adequate feeding. The daily siphoning of uneaten feed from the tanks

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followed by partial daily water exchange may have caused stress to the larvae and eventually leading to fatalities.

5. CONCLUSION AND RECOMMENDATIONS

G2 pure crosses of CTCM and CMCT and crossbred CMx(CT) generated from wild (CM) and cultured (CT) strains strains of bighead catfish showed the outstanding growth and survival at the fingerling stage.

The performance of the experimental crosses used in the study should be continuously evaluated in the grow-out stage to come up with a factual answer to whether crossbreeding between wild and cultured strains of bighead catfish can be applied in practice to improve production of bighead catfish. The good performance of CTCM and CMCT can be further maximized by applying selection in further generations.

ACKNOWLEDGMENTS

This study is funded by the Can Tho University Improvement Project VN14-P6, supported by a Japanese ODA loan. Our special thanks are sent to our colleagues and students, working in Department of Freshwater Aquaculture, College of Aquaculture and Fisheries, for their technical support during this experiment including but not limited to fish propagation and larval sampling.

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