EVALUATION OF REPRODUCTIVE PERFORMANCE GROWTH AND SURVIVAL IN WILD *CLARIA GARIEPINUS* (BURCHELL 1822) THROUGH INTRA-SPECIFIC HYBRIDIZATION OF TWO STRAINS FROM NIGERIA

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Abstract
Intra-specific hybridization experiments were carried out in wild C. gariepinus with the aim of evaluating the reproductive performance, (in terms of percentage fertility and hatchability) growth and survival. Live samples of wild C. gariepinus collected from River Argungu, Kebbi State and Eleyele Dam, Oyo State, Nigeria were crossbred and mated in pure parental and reciprocal crosses generating four mating combinations (generic crosses) replicated three times in Completely Randomized Design (CRD) manner. The F_1 generations were reared indoor for 56 days.The hybrid crosses showed intermediate characters between the pure crosses in Mean Weight Gain (MWG), Specific Growth Rate (SGR), Mean Final Length (MFL) and survival of fingerlings. However, the hybrids revealed lower characters compared to the progeny of pure parental groups in fertilisation rate, hatching and survival of larvae. On the other hand, fertilisation and hatching rate among the hybrids and the pure crosses were statistically significant ($P<0.05$). Similarly, significant difference ($P<0.05$) was observed between the two intra-specific hybrids. Though the crosses of pure Oyo species displayed significantly higher value in MWG, SGR, and MFL than other groups, fingerling survival was found to be lowest. The growth parameters like MWG, SGR and MFL of the hybrids of Oyo were found to be higher than pure Kebbi crosses, while survival of hybrid of Kebbi fingerlings was higher than pure Oyo. Therefore, this is considered as heterosis (hybrid vigour) for the hybrids in that, they have achieved better traits either one of the pure groups. Intra-specific cross of female local C. gariepinus from Kebbi and male wild C. gariepinus from Oyo (KB♀ × OY♂) be practised for optimum performance used in commercial production. This will ensure high fertility, hatchability, growth and survival rate.

Keywords: Evaluation, reproductive performance, growth, survival, intra-specific hybridization, *Clarias gariepinus,*

Introduction

Fish production is viewed as an important tool to close the gap between supply and demand of animal protein in Nigeria. National demand stands at 2.1 million metric tonnes per annum while national production from both capture and aquaculture stand at 800,000 tonnes (AIFP, 2014). In Nigeria about 1.3 million metric tonnes of fish are imported to meet the annual demand (FMA, 2015). Production from natural fisheries is estimated to be at a maximum sustainable level. Fish farming is the only available means to achieve a significant improvement in fish production. The aquaculture system in Nigeria is largely dependent on Clariidcatfishes (70 percent), tilapia, with smaller contributions from mullets and carps to total production (FAO, 2012).

Crossbreeding and hybridization are applied to utilize heterosis or complementary effect. Crossbreeding is used to evaluate performance and achieve improved traits (heterosis), minimize inbreeding and obtain better hybrids (Jothilakshmanan and Marx, 2013). Hybridization has also been successful in evaluating and improving reproductive traits, growth rate like daily weight gain, specific growth rate, survival rate (Hassan *et al.,* 2011 and Um-EKalsoom *et al.,* 2009).

Generally, hyridization simply refers to generating a new form of plant or animal either naturally or by human intervention by combining the genes of two different species or subspecies. Similarly, fish hybridization is when two different species, genera or families can be crossed to give the hybrid of desired qualities. Hybridization may involve combining different strains of a species (that is members of the same species with different characteristics). This is referred to as intraspecific hybridization. It is a hybridization exercise carried out between fish that belongs to the same species; for example between strains within a species. A strain within a species is a population with a common origin and history that possesses a unique trait that distinguished it from other strains (Dunham, 1995). Fish hybridization is one of the genetic techniques which help to remove undesirable

traits while retaining only the desirable ones. Fish production through hybridization is an age long practice in Africa. Hybridization in African catfishes *Clarias gariepinus, Clarias anguillaris, Heterobranchus bidorsalisand Heterobranchus longifilis* has been in practice in Africa (Adah *et al.,* 2014).

Therefore, evaluation of reproductive performance, survival and growth of wild *C. gariepinus* would be helpful in this direction.Evaluation of reproductive performance growth and survival in wild *Claria gariepinus* (Burchell 1822) through intra-specific hybridization of two strains from Nigeria is the focus of this study.

Materials and Methods

Sample Collection and Broodstock Selection

Live samples of wild *C. gariepinus* were collected from River Argungu, Kebbi State (within latitude 12°45'0.00"N and longitude 4°30'60.00"E) and Eleyele Dam, Oyo State (within latitude 7°31'0.00"N and longitude 3°33'60.00"E). The samples were obtained from catches of fishermen at landing site of each sampling location. *Clarias gariepinus* was identified in the field using the shape and the size of the vomerine toothplate, form of the teeth situated at the upper jaw and the absence of black spots on caudal fin or any other part of the body following identification keys of Reed *et al* (1967) and Teugels, (2003).The fishes obtained were transported in 50 litres plastic jerrycan to Fisheries and Hydrobiology Lab., Biology Department, Ahmadu Bello University, Zaria, Kaduna State. The broodstocks were acclimatized in concrete tanks for 2 months. They were fed with coppens feed at 3% body weight twice a day before the commencement of the experiment.

The matured males and mature gravid females fishes were selected, sexed and separated into males and females based on examination of the genital papillae (Teugels 1986).They were kept separately in four labeled plastic bowls containing water. The male and female fishes were weighed separately using a weighing balance (Model Sartorius AG, Gottinger CP 8201). Total and Standard Lengths were measured using meter rule.

Artificial fertilisation

The fishes were injected based on their weight using synthetic hormone (Ovaprim). Ovaprim was administered intramuscularly (above the lateral line, towards the tail) at the recommended rate of 0.5 ml per kg of female fish, and 0.25 ml per kg of male fish (Oyeleye and Omitogun, 2007).

After the 10 hours latency period, the milt was collected by sacrificing the male. The two testes lobes of the males were removed, well cleaned with tissue paper and kept in a labeled Petri dish. The abdomen of the females were well cleaned with tissue paper to avoid contact between the eggs and water, and then stripped of its eggs by a gentle application of pressure on the abdomen to release the eggs. The eggs were covered in the dry, labeled Petri dish and kept with labels.

The testes of the male were cut open using scissors and the milt was squeezed out and then 0.9% saline solution (NaCl) was added to the milt to facilitate fertilisation after which the milt was used to fertilize the already stripped eggs. Fertilisation rate was determined using 750 eggs from each strain, the number of eggs was estimated using the gravimetric method (number of eggs/g). The translucent eggs containing embryonic eyes at the time of polar cap formation 10 - 20 minutes after fertilisation were considered fertilised and counted to estimate fertilisation rate (De Graaf *et al.* 1995). The eggs were fertilised to generate four mating combinations (generic crosses) replicated three times in Complete Randomised Design (CRD) manner.

Pure Parental crosses

Female Kebbi × Male Kebbi (KB ♀ x KB ♂)
and Female Oyo × Male Oyo (OY ♀ x OY ♂)

Reciprocal crosses

Female Kebbi × Male Oyo (KB ♀ x OY ♂)
and Female Oyo × Male Kebbi (OY ♀ x KB ♂)

Percentage fertility of each cross was calculated using the formula:

% Fertility = (No. of fertilised eggs / No. of Extruded eggs) X 100% (Adebayo, 2006).

Incubation and hatching of eggs

Incubation and hatching of eggs were carried in six (6) aerated tanks containing clean kakabans (substrate attachment of eggs). Both the parental and the intra- specific crosses were replicated three times. The fertilised eggs were evenly spread on the kakaban in the tank at temperatures between 26–27°C. The percentage hatchability was determined by identifying the healthy developing eggs (fertilised eggs) which were transparent green brownish in colour

(Coppens, 2007) while the dead eggs (unfertilised eggs) which became white in colour were also estimated:

% Hatchability = (Total no. of fertilised eggs- Total no. of unfertilised eggs) X 100%

Total no. of fertilised eggs

Setting of indoor experiment and daily survival of hatchlings

The experiment was set up for 56 days indoor.The life cycle of the development begins with the fertilized egg to the fingerling stages at which the rearing period indoor ends. The larvae are considered after yolk sac absorption i.e. on 4thday to 14 days (Two weeks). The fry is considered from day 15 - 28 and the fingerling is considered from day 29 – day 56. Uneaten feeds were siphoned from the base of the aquaria every day before feeding to prevent fouling.About 25% of the culture water was always replaced every morning in order to eliminate shock and enhance survival of cultural organisms (Peter, 1987). The water quality parameters were recorded daily.The survival of larvae, fry and fingerlings in each bowl per treatment were taken on a aily basis for 56 days. Survivability evaluation was done for each stage of development.

Determinations of Growth Performance and Survival Rate

The growth performance of the larvae, fry, and fingerlings were determined in terms of Mean Weight Gain (MWG), Specific Growth Rate (SGR) and Mean Length Gain (MLG) parameters. Measurements were carried out fortnightly for weight (to the nearest g) with an electric balance and Total Length TL (to the nearest mm) for larvae, fry and fingerlings from each treatment (breed). Length gain, weight gain and specific growth rate (SGR) were determined by formula adopted from Adebayo (2006):

Weight gain = Mean final body weight (MFW) – mean initial body weight (MIW);

Length gain (MLG) = Mean final length (MFL) – mean initial length (MIL);

SGR = {(In W2 final weight–In W1 initial weight)/culture period} × 100

Where W1 is the initial fish weight (g) at time T1 (day) and W2 is the final fish weight at time T2 (day).

The rate of survival at each stage (two weeks for larvae and two weeks for fry and one month for fingerlings) was determined by counting and recording the mortality at the beginning and end of each stage. It was calculated by the formula adopted in Adebayo (2006) as follows:

Survival Rate (%) = No. of final alive at each stage X 100

Total No. counted at each stage

Data analyses for hybridization experiment

Data collected during the study were analysed using Minitab 14 software for descriptive statistics and Genstat Discovery edition 4 for analysis of variance (ANOVA). Reproductive and growth performance were analysed using one way ANOVA followed by Duncan's multiple range tests to determine significant differences among means ($p < 0.05$), and to rank significantly different means, respectively.

Results

Fertilisation and Hatching Rate

The hybrids showed intermediate values between the two parental line crosses for fertilisation. The maximum value for mean fertilisation rate (88.400±0.130%) was recorded in pure Kebbi strain of *C. gariepinus* (KB ♀ x KB ♂), whereas the minimum value (71.470±0.270%) was observed in pure Oyo strain (OY ♀ x OY ♂) (Table 1.). The data analysis showed significant difference ($P<0.05$) among all the crosses. The hybrids of female Kebbi (KB ♀ x OY ♂) recorded greater value (84.00±0.270%) for fertilisation compared to 78.270±0.14% that of its reciprocal hybrids of female Oyo (OY ♀ x KB ♂).

Table 1: Mean Percentage of Fertilisation and Hatching in pure parental line of *C. gariepinus* from Kebbi (KB♀ x KB♂) and Oyo (OY♀ x OY♂) and their reciprocal hybrids (KB♀ x OY♂) and (OY ♀x KB♂)

Combination of Crosses	Fertilisation (%)	Hatching (%)
KB♀ x KB♂	88.400±0.130^{a}	80.995±0.025^{a}
OY♀ x OY♂	71.4650±0.265^{b}	64.555±0.615^{b}

KB♀ x OY♂	84.000±0.270[c]	77.305±0.405[c]
OY ♀x KB♂	78.265±0.135[d]	72.235±0.465[d]

Values represent mean ± SE;
Mean values in a column under each parameter bearing different superscripts (a, b, c and d) differ significantly (P<0.05);
N.B: In any combination of crosses, the first set is for females and the second is for males throughout the text.

Growth rate

Fishes produced from all the breeding trails increased in length during the rearing period. The corresponding growth curve for each cross is presented in Figure 1. The hybrids showed intermediate values between the two parental line crosses for length parameters. The Mean Final Length (MFL) was 79.333± 0.333 mm for the hybrids of female Kebbi (KB ♀ x OY♂) and 79.000±0.000 mm for female Oyo (OY ♀ x KB ♂) and the mean length gain (MLG) was 70.967±0.285 mm for the hybrids of female Kebbi (KB ♀ x OY♂) and 70.900±0.058 mm for female Oyo (OY ♀ x KB ♂). The maximum values for MFL (80.667±0.333 mm) and MLG (70.967±0.384 mm) were recorded in pure Oyo (OY ♀ x OY ♂), whereas the minimum size for MFL (50.000±0.000 mm) and MLG (42.233±0.067mm) were observed in pure Kebbi (KB ♀ x KB ♂) (Figure 1.). However, values recorded in both hybrids for MLG show no statistical significant difference (P>0.05) between the hybrids and the pure Oyo strain indicating that length increase of the hybrids was similar to that of the fast growing pure Oyo fingerlings. The hybrids of female Kebbi (KB ♀ x OY♂) recorded greater values for both MFL and MLG compared to that of its reciprocal hybrids of female Oyo fingerlings (OY♀ x KB ♂).

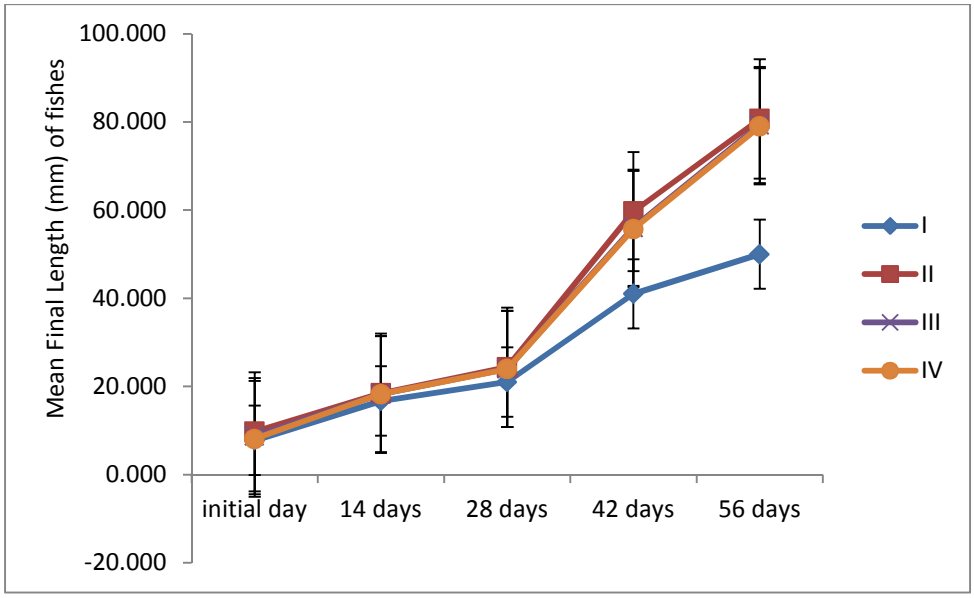

Figure 1: Growth curve showing length increase (mm) for a rearing period of 56 days in the year 2017 to 2018. (I- KB♀ x KB♂; II-OY♀ x OY♂; III-KB♀X OY♂ and IV-OY ♀x KB♂)

The mean specific growth rate (SGR) was also high (10.319±0.51 g) in pure Oyo (OY ♀ x OY ♂) followed (10.260±0.023 g) by the hybrids of female Kebbi (KB ♀ x OY ♂) fingerlings. The lowest value (8.131±0.092 g) was recorded in the pure Kebbi (KB ♀ x KB ♂) showing significant difference compared to the other crosses (P<0.05). It is clear from the result that the hybrids of female Kebbi performed better than its reciprocal hybrids of female Oyo fingerlings. Also, the statistical analysis did not show significant difference (P>0.05) between the hybrids and the pure *C. gariepinus* from Oyo indicating that Specific Growth Rates of the hybrids were similar to that of the fast growing pure *C. gariepinus* fingerlings from Oyo (Table 2.)

Table 2:Growth performance of fingerlings of the four crosses in terms of weight for a rearing period of 56 days (indoor).

	Combination of Crosses			
Growth	KB♀ x KB♂	OY♀ x OY♂	KB♀ x OY♂	OY ♀x KB♂

Parameters				
MIW(g)	0.013±0.000[a]	0.017±0.000[b]	0.016±0.000[b]	0.016±0.001[b]
MFW(g)	1.267±0.033[a]	5.600±0.058[b]	4.900±0.058[c]	4.900±0.000[c]
MWG(g)	1.253±0.034[a]	5.583±0.058[b]	4.884±0.057[c]	4.884±0.001[c]
SGR (g)	8.131±0.092[a]	10.319±0.051[b]	10.260±0.023[b]	10.231±0.130[b]

Values represent mean ± SE; Mean values in a row under each parameter bearing different superscripts differ significantly (P<0.05).

Survival rate

During the indoor rearing period, survival rate for all combinations of crosses of KB ♀ x KB ♂, OY ♀ x OY ♂, KB ♀ x OY ♂ and OY ♀ x KB ♂ from larval to fingerling stages are given in Table 3. Hybrids at their larval stage of development recorded the lowest survival and showed significant difference (P<0.05) from the parental crosses. During this period the highest survival was in pure Oyo followed by pure Kebbi. Although percentage survival of hybrid fry was higher 75.476±0.858% and 75.900±0.297%, it recorded lower than pure parental groups 79.389±1.507% and 76.723±0.100%. But in the subsequent developmental phase of the fingerlings, survival performance of the hybrids was much achieved and exceeded over the control groups significantly (P<0.05). In this stage, the highest (100±0.000%) was observed in hybrids of female Kebbi crosses (KB♀ x OY♂) followed (98.840±0.581%) by pure Kebbi (KB♀ x KB♂) (Table 3.). Moreover, in both the hybrids, there was no significant difference (P>0.05) in survival of fingerlings.

Table 3: Percentage Survival of Kebbi, Oyo strains, hybrids of female Kebbi and hybrid of female Oyo from larval to fingerling stage

	Survival %			
Culture stage	KB♀ x KB♂	OY♀ x OY♂	KB♀ x OY♂	OY ♀x KB♂
Larvae	74.333±0.882[c]	77.333±0.333[d]	70.667±0.667[b]	66.667±0.667[a]
Fry	79.389±1.507[b]	76.723±0.100[ab]	75.476±0.858[a]	75.900±0.297[a]
Fingerling	98.840±0.581[b]	73.371±0.157[a]	100.000±0.000[b]	98.582±1.418[b]

Values represent mean ± SE;
Mean values in a row under each parameter bearing different superscripts differ significantly (P<0.05)

Water Quality Parameters

Water temperature, dissolved oxygen and pH were monitored every weekly to maintain the quality of water. Water temperatures ranged between 26-30 ^{o}C, dissolved oxygen 6.3-7.6 mgL $^{-1}$, pH 6.8-7.5 throughout the experimental period (indoor) from larval to fingerling stages, and the values were within the recommended range for rearing catfishes (Madu et al, 1984; Ayokanmi, 1999;).

Pecentage Heterosis

In the case of heterosis in terms of weight gain (+7.898%), Specific Growth Rate SGR (+1.261%) was positive and survival performance (-9.450%) for larvae was negative for intraspecific hybrids compared with parental crosses while for growth in terms of weight gain (+42.891%), SGR (+11.062%) and performance in survival of fingerlings (+15.313%) at the end of the rearing period of 56 days (indoor) was positive for intraspecific hybrids as shown in Table 4. In addition, hybrids at the fingerlings showed superior performance of growth rate and survival in female Kebbi than female Oyo (Tables 3 and 4.) indicating that the acquiring of hybrid vigor for such traits appears to exist better in advanced (later) development stage rather than in earlier stage (larvae).

Table 4: Estimates of percentage heterosis (%H) of F_1 hybrids (KB♀ x OY♂, OY ♀ x KB♂) for reproductive, growth and survival traits during indoor rearing period of 56 days

Age(days)	Crossbred	Trait				
		Fertility	Hatchability	MWG	SGR	Survival

0	KB x OY(%H)	+1.50	+2.741	-	-	-
14	KB x OY(%H)	-	-	+7.898	+1.261	-9.450
28	KB x OY(%H)	-	-	+10.397	+0.081	-2.972
42	KB x OY(%H)	-	-	-0.636	+4.128	-1.126
56	KB x OY(%H)	-	-	+42.891	+11.062	+15.313
OVERALL	KB x OY(%H)	+1.50	+2.741	+15.138±9.548	+4.133±2.461	+0.441±5.269

MWG, SGR represent Mean Weight Gain, Specific Growth Rate, respectively.

Discussion

The achievements on the reproductive performances, growth and survival of the hybrids over the control groups were investigated in this research. Although the experiment of hybridization may succeed or fail, the success of the intraspecific hybridization between Kebbi and Oyo strains of *C.gariepinus* was demonstrated in this study. This was earlier evident in the same species as reported by Omeji *et al.* (2013) and Ochokwu, *et al*. (2016) in Nigeria. Also, the success of interspecific hybridization between *C. batrachus* and *C. gariepinus* was reported by Tilahun *et al.* (2016) in India which corresponds with the reports in the same species by Sahoo *et al*. (2003) in India and Khan *et al.* (2002) in Bangladesh and in some other countries as well.

Although fertilisation rate achieved in the hybrids was high (84.000± 0.270% and 78.27±0.140%) for KB♀ x OY♂ and OY♀ x KB ♂, respectively.It recorded intermediate value between the parental crosses. The hatching rate also followed the same trend as fertilisation rate recorded the intermediate value in the hybrids (Table 3.). Similar variations between fertilisation and hatching rates in hybrids and pure parental crosses were also made by various authors (Alikunhi and Chaudhuri, 1959; Chaudhuri, 1961; Adebayo, 2006). Similar report was given by Ndimele and Owodeinde (2012), who described that the pure breeds tolerate more stress than the hybrids.

The results of growth in both hybrids for MWG, SGR and MLG were intermediate between the two parental line groups and showed superiority over Kebbi crosses. This was similar with earlier studies which reported an intermediate growth performance of the parents for F_1 hybrids and its reciprocal crosses. Jothilakshmanan and Marx (2013) reported intermediate growth for hybrids of *Hetropneustes longifilis* and *Clarias batrachus*.

In this study, the values of heterosis for growth and in terms of weight gain was positive(+7.898%) for the intraspecific hybrids. Heterosis for SGR was also recorded positive (+1.261%). Similarly, Akinwande *et al.* (2011) reported positive heterosis for the interspecific hybrids of *Clarias gariepinus* and *Clarias anguillaris*. On the other hand, Ataguba *et al.* (2010) reported negative heterosis for growth (-42%) in the hybrids of *C. gariepinus* and *Heterobranchus longifilis* after 56 days of larval to fingerling rearing. In our observation, the values for pure Oyo (OY ♀ x OY ♂) were significantly ($P<0.05$) higher than the other crosses and this result was in agreement with other studies (Adewolu *et al.,* 2008; Ataguba *et al.*, 2009, 2010; Ndimele and Owodeinde, 2011).

In the current study, a significantly higher survival was achieved in both hybrids during fingerling stages over the parental groups which could be attributed to improved hybrid vigour. This is in agreement with the findings of earlier reports which indicated that hybrids in most cases were superior to the parental strains (Bakos, 1982; Jensen *et. al.,* 1983; Madu and Ita, 1990 and Salami *et. al.,* 1993). In the present study, heterosis for survival of fingerlings in the hybrids was positive (+15.313%). On the other hand, survival of larvae recorded was low in both the hybrid crosses and varied significantly ($P<0.05$). The effect of hybridization to lower performance of survival is also claimed by the report of other researchers (Sahoo *et al.,* 2003; Ndimele and Owodeinde, 2012). Similarly, Jothilakshmanan and Marx (2013) in the hybrids of *Hetropneustes longifilis* and *Clarias batrachus* reported reduced survival (0.8 and 0.9%) due to high rate of mortality of the hatchlings when the transition from endogenous to exogenous feeding took place.

During the study period, the pure Kebbi progenies which were poor in survival at initial stages significantly improved and progressed in its survival performance better than pure Oyo and showed similarity to the hybrids without significant variation at the end of the experiment. This might have been related to its improved adaptability. This is supported by the improved survival

percentage recorded as each group of progenies passed through the successive developmental stages. This finding is in agreement with the report of Tilahun *et al.,* (2016) for the hybrids of female *C. batrachus* (Cb♀ x Cg♂) that achieved better than the pure batrachus in India.

Conclusions and Recommendations

Intra-specific hybridization experiments were carried out in wild *C. gariepinus* with the aim of evaluating the reproductive performance (in terms of percentage fertility, hatchability, survival rate) and growth performance. It is concluded that pure *C. gariepinus* strain from Kebbi (KB♀ × KB♂) performed better in fertility, hatchability and survival but shows lower growth while pure *C. gariepinus* strain from Oyo (OY♀ × OY♂) have better growth but lower fertility, hatchability and survival. The study revealed that the hybrids achieved better than the pure parental groups in reproductive traits (fertility and hatchabilty) and in growth and survival in indoor experiment except the low survival performance of the larvae recorded with a negative percentage of heterosis. It is concluded that the intra-specific hybrids achieved combined improved traits in reproductive (fertility, hatchability), growth and survival with higher combine improved traits performances in hybrids of female Kebbi and Male Oyo, (KB♀ × OY♂).

Intra-specific cross of female local *C. gariepinus* from Kebbi and male local *C. gariepinus* from Oyo (KB♀ × OY♂) be practised for optimum performance. This will ensure high fertility, hatchability, growth and survival rate. This result should, therefore, be used as baseline information that is extended to hatchery operators and growth out farm. Further improvement for the poor survival of the larvae is required as to produce sufficient seed for grow-out culture to exploit the potential of the hybrids in aquaculture. Finally, application of selective breeding for the genetic improvement of this wild *C. gariepinus* utilizing female Kebbi and Male Oyo, (KB♀ × OY♂) is also recommended.

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