

# Hybridization and Growth Performance of Progeny from Crosses between *Clarias gariepinus* and *Heterobranchus* sp.

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## Abstract

Mixed hybridisation trials involving *Clarias gariepinus* (CG), *Heterobranchus longifilis* (HL), and *Heterobranchus bidorsalis* (HB) were carried out with success using Ovulin as inducing agent. The crossing was followed by an early growth trial using post-yolk sac larvae that lasted ten days. Latency periods in the two catfishes differ, with 11 hours in crosses involving female CG and 12 hours in crosses involving female HL. The fertilisation rates of eggs from the females of the two species indicate that there is no significant discernment in fertilisation ( $p > 0.05$ ), with values ranging from 68.38% (CG×HB) to 80.94% (HL×CG). However, rates <80% indicate egg quality problems. The hatching rate was significantly higher ( $p < 0.05$ ) in both *C. gariepinus* (62.12%) and *Heterobranchus* sp. (47.75%) line crosses than the hybrids. There is an additive genetic effect due to the combination of pure-line male and female gametes that elicit better growth as against survival in crosses involving *C. gariepinus*. The genotype for better survival is in the maternal line of *H. longifilis*. These culminate into heterosis for growth in hybrid CG×HB and survival in hybrid HL×CG.

## Introduction

In Nigeria, nine species of catfish are being cultured, with *Clarias gariepinus* being the most popular (Williams et al., 2008). The production of *C. gariepinus* exceeds tilapia in Nigeria, with the catfish species accounting for more than 60% of total freshwater fish output (WorldFish, 2018). Production and hatchery technology of species within the family Clariidae are well-developed (Dauda et al., 2018). Growth in *C. gariepinus* is superior to *C. anguillaris* (Dada et al., 1999). This informs the popularity of the species *C. gariepinus* in Nigerian aquaculture. The efforts at multiplying seeds and disseminating seeds of this important aquaculture species have been a private

sector effort with mixed reports of successes and failures among farmers.

Hybridisation is a genetic tool that can be used to improve economic traits in fish since it is a technique that involves mating between species that have a common phylogenetic ancestry in the evolutionary ladder (Bondoc, 2008). Earlier reports of hybridization between four common catfishes: *C. gariepinus*, *C. anguillaris*, *Heterobranchus bidorsalis*, and *Heterobranchus longifilis* (Dada et al., 1999) have shown promising results for growth among the progeny but without report of heterosis. Recent reports of hybridization include hybridization between *C. gariepinus* and *Heterobranchus longifilis* (Ataguba et al., 2010) and between *C. gariepinus* and *H. bidorsalis*

(Ndimele et al., 2011). However, no report on hybridization involves crosses between *C. gariepinus*, *H. longifilis*, and *H. bidorsalis*.

Heterosis or hybrid vigour occurs when genes combine and interact within gene pools. Distinct populations, inbred lines, or species contribute to the different alleles. According to Birchler et al. (2006), the level of heterozygosity determines heterosis. On the other hand, non-additive genetic effects determine improvement (Varona et al., 2018). Hybrid vigour in future crosses is usually lost with further crossing beyond F<sub>1</sub> (Grant & Grant, 1994). Still, backcrossing can be used to fix the economic trait that was gained in the hybridization of species (Bondoc, 2008).

Knowledge of heterosis and growth performance among the progeny of hybridization trials form the base for developing new breeding programmes to develop specialized lines that can overcome the drawbacks in hybrid progeny (Šimková et al., 2022). It has been shown that F<sub>1</sub> hybrid progeny are known to exhibit reduced fertility or no fertility at all (Bondoc, 2008; Dunham & Argue, 2000). Therefore, a goal in an improvement programme could be the development of hybrid lines with improved reproductive capacity (high fertility and hatchability) and survival. Hybrid vigour in fertilisation, hatching, and survival has been reported in Cameroon crosses between *C. gariepinus* and *C. jaensis* (Tiogué et al., 2020). Similarly, hybrid vigour in hatching and survival has been reported for crosses involving *C. gariepinus* and *H. bidorsalis* (Owodeinde et al., 2010).

Moreover, there has been a report of increased egg weight relative to parental lines in the F<sub>1</sub> hybrid cross between *C. gariepinus* and *H. longifilis* (Nwadukwe, 2015). These clearly show that hybrid vigour can be produced due to hybridization between *C. gariepinus* and *Heterobranchus* sp. According to a report by Nguenga et al. (2000), progeny from crossings of *H. longifilis* strains exhibited hybrid vigor. Similar improvements in hatchability and survival of crosses between the same species were found by Olufeagba and Okomoda (2015). There are no reports on the heterosis of crosses involving the three species: *C. gariepinus*, *H. bidorsalis*, and *H. longifilis*. Therefore, the present research intends to determine the effects of hybridization on fertilisation, hatching, early growth, and progeny survival from crosses that involve three

African catfish species: *C. gariepinus*, *H. bidorsalis*, and *H. longifilis*.

## Materials and Methods

### Sample Collection and Broodstock Selection

Samples of *C. gariepinus* (CG:♂,♀) were obtained from one fish farm while *Heterobranchus bidorsalis* (HB:♂) and *Heterobranchus longifilis* (HL:♀) were collected from a different farm both within Makurdi (7°43'55.92"N and 8°32'20.76"E), Benue state, Nigeria. The farms maintained pure lines of these broodstock. The mature females were selected based on age and size (Ataguba et al., 2012; Ibiwoye, 2017) as well as reproductive readiness (swollen, well-distended soft abdomen, rounded genital opening for females, and reddish pointed genital papillae for males) (de Graaf & Janssen, 1996). The mean weight of the broodstock (Table 1) was 1085.83g and 450g for CG and HL females, respectively, while male broodstock had a mean weight of 1044.67g (CG) and 455.83g (HB). Samples were collected in September 2021.

### Experimental Design

Twelve (12) spawning trials were carried out, making four (4) treatments with three replicates for each treatment. The crossing was based on the available broodstock; hence reciprocal crosses were not carried out. All female broodstock (CG and HL) were crossed (Table 2) with all the male broodstock (CG and HB).

### Artificial Fertilisation

The broodstock were transferred to the Teaching and Research Hatchery, Department of Fisheries and Aquaculture, Joseph Sarwuan Tarka University, Makurdi (University of Agriculture Makurdi), Benue State, and acclimatized for three days in concrete tanks with separate tanks for each species and sex. They were fed with Coppens® feed at 3% body weight twice a day before the commencement of the experiment.

The fish were injected based on their weight using a synthetic hormone - Ovulin. Ovulin was administered intramuscularly at 0.5 ml per kg of female fish (Table 1).

**Table 1.** Broodstock weight and hormone (Ovulin) administered.

S/No.	Female				Male	
	CG	Hormone (ml)	HL	Hormone (ml)	CG	HB
1	1045	0.52	450	0.23	1120	500
2	970	0.49	470	0.24	1160	450
3	1180	0.59	420	0.21	998	460
4	1200	0.6	420	0.21	970	450
5	1150	0.58	460	0.23	1010	425
6	970	0.49	480	0.24	1010	450
Mean	1085.83		450.00		1044.67	455.83
SE	42.60		10.30		31.20	10.00

\*Single intramuscular injection at 20:00 hours

After the injection, the fish were maintained in concrete tanks with a water temperature of 26.5°C for the latency duration. The latency period was monitored for each group of females beginning from the time of injection.

After the latency period, the male broodstock were immersed in 100L of water dosed with clove oil at 0.1ml/l following Fernandes et al. (2016) and observed until opercular movement ceased. Each male broodstock was then removed one after the other for the surgical procedure, as Bart (1988) described. The male broodstock was placed on an aluminum sheet on a table with the ventral surface upwards before an incision was made on the abdomen along the vent line but not opening the vent. A single lobe of the testes (the left lobe) was removed and kept in labeled Petri dishes. The eggs stripped from the females were weighed and then fertilised using milt from the testes after being incised with a surgical blade and squeezed gently to release the milt. The fecundity was determined using a sample of 1g of eggs counted in triplicates to give an average of 710 eggs per gram.

**Determination of Fertilisation Rate**

Fertilisation rate was determined using a random sample of 50 eggs from each replicate of the crosses. The eggs were observed under the microscope (Leica DM1000) within 12 hours of fertilisation to determine fertilisation rates. The eggs without a neural fold that marks the beginning of somitogenesis (Schmidt et al., 2013) were declared unfertilised (Bart & Dunham, 1996; Dunham et al., 1999). The number of eggs was estimated using the gravimetric method (number of eggs/g). The eggs were fertilised to generate four mating combinations (generic crosses) replicated three times in a completely randomized design (CRD).

**Hatchability**

Eggs were incubated in plastic aquaria with a water volume of 40L and mosquito mesh as substrate.

Aeration was provided to all tanks during the period of incubation. Percentage hatchability was estimated 24 hours after hatching was completed. Hatchability was estimated using the volumetric method. The incubation bowl was stirred gently to disperse the larvae evenly in the water. A beaker (100ml) was used to collect water from the bowl, with the dispersed larvae swimming freely inside. The number of larvae in the volume of water was counted. This procedure was repeated three times, and the average number was taken. A mathematical relationship between the value obtained and the total water volume served to estimate the hatchling population. The hatching rate was determined using a modified version of the formula provided by Adebayo and Popoola (2008):

$$Hatching\ Rate = \frac{Total\ Number\ of\ Hatched\ Eggs}{Total\ Number\ of\ Incubated\ Eggs} \times 100$$

**Determination of Growth Rate of Larvae**

The growth of the progeny from the crosses of the three African catfishes from the first feeding stage up to day 14 after hatching was determined using a sample of 150 larvae from each genetic group stocked separately in a triplicate 60L plastic bowl with constant aeration. The water was changed every four days. *Ad libitum* feeding using dried decapsulated *Artemia* sp. cysts were employed within the first seven days. Afterward, larvae were introduced gradually to an artificial dry diet of coppens (200 – 300 µm) catfish feed. *Artemia* and the artificial dry diet were offered alternately to the larvae during the weaning period, which lasted for four days.

All larvae were collected from each aquarium every two days using a fine mosquito mesh-size net to determine bulk weight. These were weighed on a sensitive balance while they remained in a plastic bowl containing water whose weight had been pre-determined. At the end of the trial, the bulk weight and the number of surviving fry were determined. Parameters determined include: Initial weight ( $W_0$  mg),

**Table 2.** Punnett’s square for crosses of *C.gariepinus* and *Heterobranchus* sp.

♂ ♀	CG	HB
CG	CG × CG	CG × HB
HL	HL × CG	HL × HB

HB= *Heterobranchus bidorsalis*  
 HL= *Heterobranchus longifilis*  
 CG= *Clarias gariepinus*

**Table 3.** Egg and breeding parameters of crosses between *C. gariepinus* and *Heterobranchus* sp.

Treatment	Egg Weight (g)	Latency Period (Hours)	Fertilisation Rate (%)	Hatchability (%)
CG×CG	287±100 <sup>b</sup>	11.0±0.0 <sup>a</sup>	77.78±4.35	62.12±5.8 <sup>b</sup>
CG×HB	270±66.00 <sup>b</sup>	11.0±0.0 <sup>a</sup>	68.38±5.07	41.84±2.07 <sup>ab</sup>
HL×CG	8.35±0.85 <sup>a</sup>	12.0±0.0 <sup>b</sup>	80.94±5.27	32.12±2.4 <sup>a</sup>
HL×HB	8.30±0.20 <sup>a</sup>	12.0±0.0 <sup>b</sup>	75.76±4.67	47.75±3.8 <sup>ab</sup>
p-value	0.048	<2.0×10 <sup>-16</sup>	0.414	0.022

final weight ( $W_1$  mg), Weight gain,  $W_1 - W_0$  (mg), daily growth rate:  $W_1 - W_0 / t$  and the specific growth rate (SGR): which was derived following a two step procedure described by Crane et al. (2019) with the first step being the determination of a dimensionless variable  $g$ :

$$g = \ln W_1 - \ln W_0 / t$$

The second step involved the normalization of the variable to yield the dimension of  $\% \cdot \text{day}^{-1}$  using:

$$\text{SGR} = 100(e^g - 1)$$

Where:  $W_0$  = mean initial body weight,  
 $W_1$  = mean final body weight  
 $t$  = time (days)  
 $\ln$  = Natural Logarithm  
 $e$  = Euler's number

### Survival

The survival rate of larvae was estimated on day 14 post-hatching. A physical count was done for each tank using small aliquots of water in a beaker (500ml) until all surviving fish were counted.

### Heterosis

Heterosis was estimated for final weight, SGR, DGR, and survival rate using a formula adapted from Lynch and Walsh (1998) and Walsh and Lynch (2018):

$$H_T = \sum_{i=1}^n \frac{O_i - \frac{P_1 + P_2}{2}}{\frac{P_1 + P_2}{2}} \div n$$

Where  $H_T$  = Heterosis for trait T,  $O_i$  = Trait value for  $i^{\text{th}}$  replicate,  $P_1$  = Trait value for parental line 1 (CG×CG),  $P_2$  = Trait value for parental line 2 (HL×HB),  $n$  = number of replicates

### Water quality

Dissolved oxygen was determined using a Hanna Instruments HI9143 handheld DO meter, while pH, temperature, conductivity, and TDS were determined using a Eutech Instruments Cyberscan PC300. The mean pH across all tanks was  $7.55 \pm 0.03$ , and electrical conductivity was  $124.5 \pm 5.87 \mu\text{S} \cdot \text{cm}^{-1}$ . Total dissolved solids (TDS) was  $26.31 \pm 0.22 \text{mg} \cdot \text{l}^{-1}$  and dissolved oxygen was  $4.51 \pm 0.07 \text{mg} \cdot \text{l}^{-1}$ . The temperature was observed to be  $26.31 \pm 0.22^\circ\text{C}$ .

### Data Analysis

Data were analysed using R version 4.0.0 (R Core Team, 2020). Differences in the hatching, growth, and survival rates across the treatments were determined using one-way ANOVA in R

### Results

#### Total Fecundity, Fertilisation Rates, and Hatching Rate

Total fecundity was generally high in *Clarias gariepinus* than in *Heterobranchus longifilis* (Figure 1). Fecundity was highly variable, even among *C. gariepinus*, which exhibited high fecundity. The female *C. gariepinus* crossed with male *H. bidorsalis* had a higher fecundity than that crossed with the male *C. gariepinus*.

The breeding performance of the crosses (Table 3) shows that the weight of eggs stripped from each female for each cross was significantly different ( $p < 0.05$ ) and also indicative of the fecundity, as shown in figure 1. The latency period of *H. longifilis* was longer (12 hours) compared to 11 hours for *C. gariepinus*. Fertilisation rates did not differ across the treatments ( $p > 0.05$ ). Hatchability, on the other hand, differed significantly ( $p < 0.05$ ). The pure line cross between *C. gariepinus* yielded the highest hatchability (62.12%), followed by the ♀HL × ♂HB cross with 47.75%.

**Table 4.** Growth performance and survival of progeny from crosses of three species of African catfishes.

Cross	MIW (mg)	MFW (mg)	SGR ( $\% \cdot \text{day}^{-1}$ )	DGR ( $\text{mg} \cdot \text{day}^{-1}$ )	SR (%)
CG×CG	17.50±0.64	122.67±9.62 <sup>c</sup>	21.45±1.35	10.52±1.01 <sup>b</sup>	73.34±0.61 <sup>a</sup>
CG×HB	16.57±0.46	108.40±4.47 <sup>bc</sup>	20.65±0.27	9.18±0.41 <sup>ab</sup>	71.39±0.23 <sup>a</sup>
HL×CG	16.30±0.44	90.87±6.85 <sup>ab</sup>	18.69±0.97	7.46±0.69 <sup>a</sup>	87.05±0.95 <sup>c</sup>
HL×HB	15.17±0.52	79.33±1.13 <sup>a</sup>	18.01±0.56	6.42±0.16 <sup>a</sup>	77.66±0.27 <sup>b</sup>
p-value	0.075	0.006	0.078	0.009	0.000

Significant differences ( $p < 0.05$ ) are indicated by different superscripts in the columns

**Table 5.** Heterosis for growth and survival traits in crosses between three African catfishes.

Hybrid	Heterosis			
	Final Weight	SGR	DGR	Survival Rate
CG×HB	0.07	0.05	0.08	-0.05
HL×CG	-0.10	-0.05	-0.12	0.15
Overall	-0.03	-0.01	-0.03	0.10

**Early Growth and Survival Rates of Progeny**

At the point of yolk sac absorption and the start of first feeding (Day 4), all larvae appeared equal in size (Figure 2), with the progeny from the cross CG×CG taking the lead on day six but declined on day eight and later peaked from day 10 to day 14. The cross CG×HB followed the parental *C. gariepinus* line closely with the hybrid cross HL×CG following during the parental *Heterobranchus* sp. Cross HL×HB lagged.

Initial progeny weights utilized in the second part of this experiment (Table 4) were not significantly different across the genetic groups or crosses. However, there was a significant difference in the final weights ( $p < 0.05$ ) with the *Heterobranchus* sp. The parental line (HL×HB) had the least MFW, while the progeny from the cross CG×CG had the highest MFW. The pattern of specific growth rate was similar to the MFW, although it was not significantly different. The daily growth rate was significantly different ( $p < 0.05$ ) and followed the pattern of MFW. Survival was higher in the groups that involved the female *H. longifilis*, with significant differences recorded ( $p < 0.05$ ).

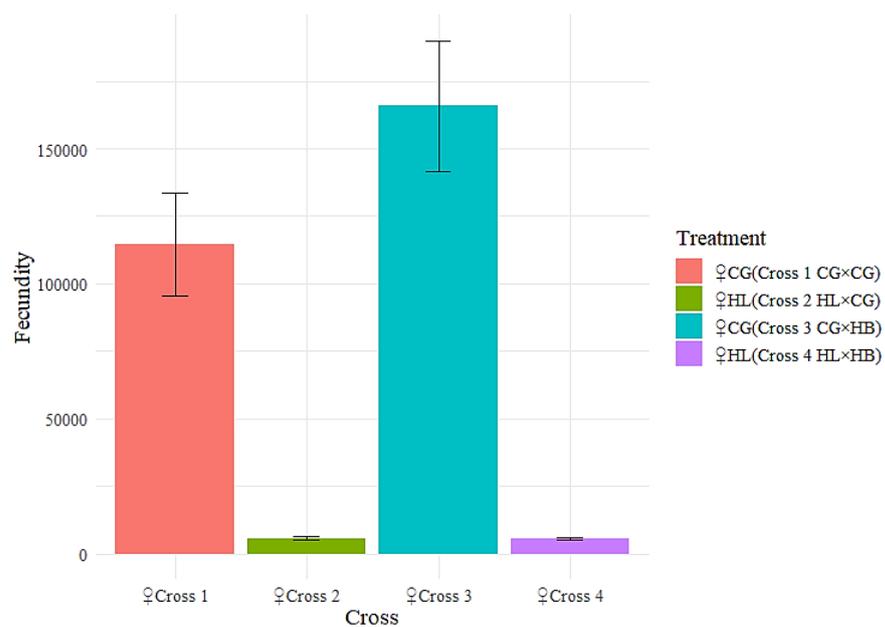
**Heterosis**

A positive heterosis for final weight (14 days post hatching), SGR, and DGR, as well as a negative heterosis for survival, were documented by the cross CG×HB (Table 5). On the other hand, the cross HL×CG showed negative heterosis for final weight, SGR, and DGR but had a positive heterosis for survival rate. Due to the negative heterosis from HL×CG, the overall heterosis of hybrids for final weight, SGR and DGR was negative while survival rate was positive (0.10).

**Discussion**

The latency periods were maternal-specific. As much as the latency period can be species-specific, it can also be determined by other factors, including hormone type, dosage, and water temperature. The temperature recorded during the broodstock holding period through to stripping in this trial was 0.5°C less than that recorded by Olaniyi and Omitogun (2014). Still, it produced a lesser latency period than the 14 hours the authors reported for *H. bidorsalis*. The use of gonadotropin-releasing hormone (GnRH) as standalone hormonal administration seems to elicit longer latency periods in these catfish species than other hormonal administrations like HCG (Legendre & Otémé, 1995) and even amphibian pituitary extract (Nwadukwe, 1993). At 26.5°C, *C. gariepinus* induced using ovaprim ovulated faster than *H. longifilis*. The underlying endocrinology behind this has not been investigated. However, Abolagba (1999) has shown that temperature reduces the latency period in *H. longifilis* more than in *C. gariepinus*.

The fertilisation rates recorded here are comparable to those generally observed in Clariidae induced and spawned artificially, including 80 to 95% in *C. gariepinus* (Oellermann, 1995), 87.1 to 95.2% in *H. longifilis* (Nguenga et al., 2000), and 79.25 to 87.57% in *C. jaensis* (Zango et al., 2016). However, the hybrid cross CG×HB had the lowest fertilisation rate (68.38%). That notwithstanding, the fertilization rate is determined mainly by egg quality, a phenotypic trait that is difficult to quantify (Bobe, 2015). However, gene expression has been proven effective in gilthead sea bream (Georgiou et al., 2022). Still, machine learning has been used to overcome this in Salmon (Cardona et al., 2021), but



**Figure 1.** Total fecundity of broodstock of two clariid species stripped under each treatment.

fertilization rates between 80-90% indicate the overwhelming presence of good quality eggs (Dettlaff et al., 1993). In a previous report, Ataguba et al., (2009) recorded a higher fertilization rate (88.57%) between female *C. gariepinus* and male *Heterobranchus longifilis*. According to previous reports, hybridization failures are possible and could account for the current reduction in fertilization rate (Cacot, 2006; Zango et al., 2016). The success of embryonic development is often used as a yardstick to determine fertilization success hence the long duration, usually 10 to 12 hours in quantifying fertilization rate. However, it is important to note that fertilization occurs between 2 seconds to 30 seconds after the introduction of sperm with visible alterations to the cortical layer around the micropyle of the egg (Dettlaff et al., 1993). Several factors can affect the fertilization of eggs in fish, including in-vivo mechanisms within the female fish (Mohagheghi Samarin et al., 2015) and external environmental conditions (Legendre et al., 2000; Luo et al., 2017). The fertilized eggs may have lost fertility before stripping, failed to come in contact with sperm, or died at the early stage of embryogenesis (Dettlaff et al., 1993). Physical injury to the eggs during mixing with milt cannot be ruled out as a cause of egg infertility. Eggs that do not come in contact with sperm after contact with water tend to die faster than those that fail embryogenesis. This phenomenon occurs because the glycoprotein outer layer of the egg hydrates and causes an influx of water into the egg due to osmotic pressure, which eventually leads to the death of the eggs (Fyhn et al., 1999; Rizzo & Bazzoli, 2020).

The hatching rates were lower in all genetic types (41.84% to 62.12%) compared to those obtained in the crosses between *C. gariepinus* and *H. longifilis* (84.16% to 87.43%) by Ataguba et al. (2009). Higher values (90.37 to 92.96%) were reported for *H. longifilis* breeding by

Agnès et al. (1995), and those from 68.07 to 72.82% reported in a closed loop by Tiogué et al. (2018) Tiogué et al., (2018) in *C. gariepinus* induced by 6ertili. This should be partially due to the pH levels in the tanks, an index for ammonia contamination since higher pH as observed in the tank bearing the cross CG×HB (7.60) is indicative of toxic ammonia (Wurts, 2003). This process is also affected by temperature and probably explains why the cross HL× CG (pH=7.45) has the least hatchability considering the higher temperature in the tank (26.35°C). Hormonal-related differences in hatchability can be deduced from previous reports using various hormones. The current hatching rates are close to 51.64 to 65.58% recorded by Tiogué et al. (2018) in *C. gariepinus* induced by hypophysation under closed or static conditions. Poor hatching (1.10 to 19.38%) has been reported in *C. jaensis* induced with hCG and homoplastic pituitary extract (Zango et al., 2016). Hormone type could account for differences in hatching rates as much as species differences, and water temperature can also be blamed.

GAs observed in the current study, growth performance depends on the survival rate, an index akin to stocking density (Oguguah et al., 2011). The progeny from the pure line CG×CG cross outperformed the other progenies of female *H. longifilis* crosses at a similar survival rate to the hybrid cross CG×HB. These two crosses had the best growth rates and the lowest survival rates. The final weight attained by progeny from the pure line cross of *C. gariepinus* as well as that of progeny from the hybrid CG×HB indicates that there is an additive genetic effect as a result of the combination of pure line male and female gametes that elicit better growth as against survival. The effect of this genotype can be seen clearly from the CG×HB cross that outperformed the HL×CG and HL×HB crosses (Ndimele

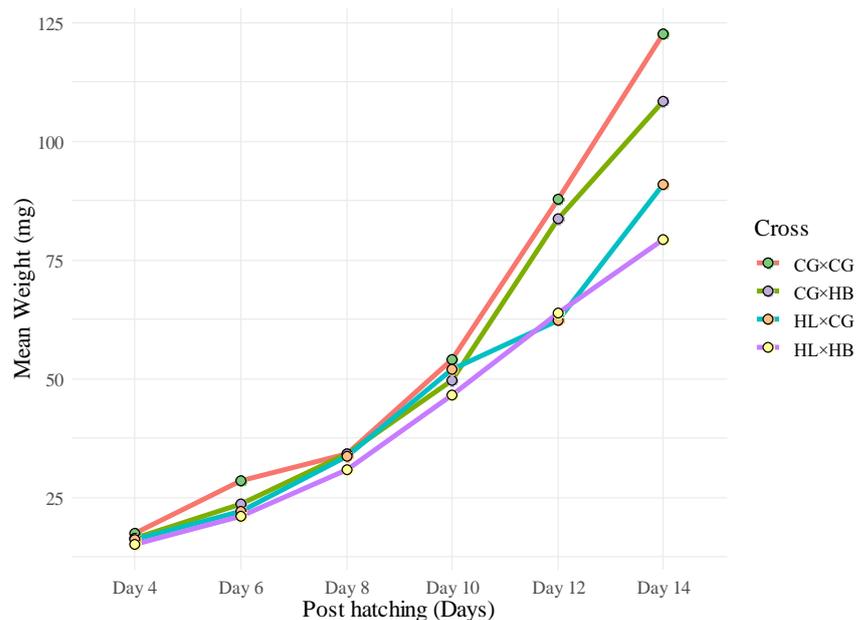


Figure 2. Temporal progression of body weight of progeny from crosses of three African Catfishes.

et al., 2011). The specific growth rate as derived in this report, is about the first for the African catfish after Crane et al. (2019) report detailing the inaccuracy of the formula routinely used for SGR. The values obtained here are higher than those earlier reported (Ataguba et al., 2009) for crosses of *C. gariepinus* and *H. longifilis*, even after adjustment with the suggested formula by Crane et al. (2019). This result suggests that environment and gene interactions effectively shape phenotypes (Nguyen et al., 2017). Early growth in fish is usually exponential; hence, the SGR at this stage is very high compared to SGR obtained over longer periods. Also, the age of fish at the start of any growth trial determines their SGR. Values of SGR between  $1.67\% \cdot \text{day}^{-1}$  and  $1.9\% \cdot \text{day}^{-1}$  were reported for crosses of *C. gariepinus*, and *H. longifilis* stocked at the age of 53 days (Legendre et al., 1992) while values greater than  $7\% \cdot \text{day}^{-1}$  have been reported for crosses of *C. gariepinus* and *H. bidorsalis* stocked at the age of 14 days (Ndimele et al., 2011). The fry used in the current study were four days old at stocking, and exponential growth commenced at eight days of age for all crosses. This time gap explains why the daily weight gain was higher in this report than that of Ndimele et al. (2011).

The survival rate of larvae at the end of the 10 days of rearing was variable among the progeny. Survival rates for the crosses CG×CG, CG×HB, and HL×HB were quite below 80% but are well above previous reports in the literature. Survival rates among hybrids of *Heterobranchus* sp. (♀) and *Clarias* sp. (♂) have been reported to show better survival (Dada et al., 1999) with a similar result in the present study. This result implies a greater environmental and stress tolerance in *Heterobranchus* sp. than in *Clarias* sp. Moreover, the survival rate of the cross involving both species of *Heterobranchus* was higher and significantly better than those of pure *Clarias gariepinus* or the hybrid cross between *C. gariepinus* and *H. bidorsalis*. Only the cross HL×CG recorded a survival rate >80%. In addition, the genotype for better survival seems to be in the maternal line as it was conferred on progeny from crosses with *H. longifilis* as dams. At the same time, the crossing of *C. gariepinus* with *H. bidorsalis* negatively impacts progeny survival.

Heterosis for growth was positive in the hybrid CG×HB but negative in the hybrid HL×CG. An inverse scenario supports this for the survival rate in both crosses. The crossing of *Heterobranchus* (♀) and *Clarias* (♂) has been shown to produce better survival than the reciprocal cross (Legendre et al., 1992). The implication is that in exploiting heterosis for growth, it is essential to factor in survival to determine where the trade-off between growth and survival will yield maximum biomass. This metric is of high commercial interest. Heterosis per cross, as reported, brings the potential of each cross to the fore. If we used the mid-hybrid and mid-parent version of the formula for heterosis, the conclusion would show that heterosis is only in favour of survival.

## Conclusion

Latency periods in the two catfishes differ, with greater time being required for *Heterobranchus* sp. to reach ovulation after injection with Ovulin. The fertilization rates of eggs from the females of the two species indicate an underlying egg quality problem since the rates in three crosses were less than 80%. Hatching rate was higher in both *C. gariepinus* and *Heterobranchus* sp. line crosses than in the hybrids. There is an additive genetic effect due to the combination of pure-line male and female gametes that elicit better growth as against survival in crosses involving *C. gariepinus*. The genotype for better survival is in the maternal line of *H. longifilis*. These culminate into heterosis for growth in hybrid CG×HB and survival in hybrid HL×CG.

## Ethical Statement

This research was conducted based on the enabling legal provisions captured in the Constitution of the Federal Republic of Nigeria, Criminal Code Act. Cap C38 LFN 2004, Animal Diseases (Control) Act. Cap A17 LFN, 2004 and the Veterinary Surgeon Act Cap V3 LFN 2004. The protocol was approved by the Animal use and care committee of the College of Veterinary Medicine, Federal University of Agriculture, Makurdi, Nigeria via document No. nvriAUCC F001/15. Surgical Procedure was carried out on fish after anaesthesia using clove oil (0.5ml/l). After surgery, cuts were sutured and fish were administered 20mg/Kg body weight of Amoxicilin before being revived and released into the ponds.

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## Author Contribution

Conceptualization: GAA, AA; Data Curation: GAA; Formal Analysis: GAA, AA; Fund acquisition: GAA, AA; Investigation: GAA, AA; Methodology: GAA; Supervision: GAA; Validation: GAA; Writing (Draft): AA, GAA; Writing (Review): GAA

## Conflict of Interest

The authors have no conflict of interest to declare.

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