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Growth Responses of Hybrid Vigour in Four Reciprocal Crosses of Three Farmed Catfishes

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ABSTRACT

This study looked at the expression of hybrid in four reciprocal crosses between three African catfish species: *Clarias gariepinus*, *Heterobranchus bidorsalis*, and *Heterobranchus longifilis*. The goal was to use growth performance and reproductive efficiency as indicators of hybrid vigor across reciprocal combinations. The reciprocal crosses included T_1: ♀*C. gariepinus* × ♂*H. bidorsalis*, T_2: ♂*C. gariepinus* × ♂*H. bidorsalis*, T_3: ♀*C. gariepinus* × ♂*H. longifilis*, and T_4: ♂*C. gariepinus* × ♀*H. longifilis*. A totally randomized design with three replicates per treatment was used for a 120-day feeding trial under controlled hatchery and grow-out conditions. Growth performance parameters such as weight gain, specific growth rate (SGR), average daily gain (ADG), and mid-parent weight gain (MWG) were assessed alongside reproductive indices such as fertilization and hatchability. T_2, also known as *Heteroclarias*, outperformed other crossings with a weight gain of 426.56g, SGR of 5.63%, and ADG of 36.40g/day (P<0.05). T_1 had the lowest weight gain (286.60g) and MWG (93.60%), but had the highest fertilization rate (85.40%). T_3 had the highest MWG (106.10%) while having the lowest SGR (3.91%) and ADG (22.10g/day), showing excessive weight growth compared to parental averages. Hatchability was best in T_3 (77.0%) and lowest in T_4 (72.87%). The results demonstrate that reciprocal hybridization has a considerable impact on both growth and reproductive features, with maternal impacts determining performance outcomes. T_2 is the most suited cross for commercial aquaculture where rapid development is desired, although T_1 and T_3 have the potential to improve hatchery efficiency and early survival. These findings highlight the significance of deliberate hybrid selection based on specific production goals in order to maximize catfish farming outcomes.

Keywords: Hybrid, Reciprocal cross, Catfish, Growth, Fertilization, Hatchability.

INTRODUCTION

The expansion of aquaculture production systems in Sub-Saharan Africa has demanded the creation of fish strains that are not only fast-growing but also resistant to environmental and disease challenges (Ajayi et al. 2022; Ogunji and Wuertz 2023). Catfishes, especially *Clarias gariepinus*, *Heterobranchus bidorsalis*, and *Heterobranchus longifilis*, are of significant commercial importance among freshwater finfish species due to their high market demand, tolerance to a wide range of environmental conditions, and adaptability to intensive culture systems (Lenient et al. 2008; Oyegbile et al. 2017; Bawa 2024).

Despite their promising culture potential, mono-species farming of these catfishes sometimes encounters genetic restrictions such as inbreeding depression and decreased growth performance throughout generations (Areola et al. 2024). Hybridization, particularly interspecific and reciprocal crosses, has attracted attention as a potential approach for improving aquaculture performance attributes, including as growth rate, feed

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efficiency, reproductive success, and survival (Rahman et al. 2013; Ochokwu et al. 2016; Rahman et al. 2018). Hybrid vigor, also known as heterosis, occurs when hybrid offspring outperform their purebred parents in terms of attributes, which is generally due to complementary gene interactions (Schnable and Springer 2013; Getahun et al. 2019).

The combination of *Clarias* and *Heterobranchus* species in reciprocal crosses has been explored in several studies (Ataguba et al. 2010; Ataguba and Angela 2024; Ntsoli et al. 2024), with variable outcomes depending on the direction of the cross (♀ × ♂). Reciprocal differences may stem from maternal effects, cytoplasmic inheritance, or sex-linked gene expression (Gonzalo et al. 2007; Bosland and Barchenger 2024). For instance, hybrids of ♀*C. gariepinus* × ♂*H. bidorsalis* which is commonly referred to as "Heteroclarias" has often been reported to exhibit faster growth and better feed utilization compared to other hybrid combinations (Meye and Omoruwou 2025; Shaibu et al. 2025). However, success in hybrid catfish production also depends on hatchability, fertilization efficiency, and survival rate, all of which may be influenced by environmental conditions and broodstock quality (Legendre et al. 2000; Bobe 2015).

Despite the existing literature, few studies have examined the development and reproductive success of reciprocal crosses between three parent species under identical rearing conditions. This study attempts to close that gap by assessing growth parameters (for example; weight gain, specific growth rate, average daily gain) and reproductive indices (e.g., fertilization rate, hatchability) as indicators of hybrid vigor in four reciprocal crosses of *C. gariepinus*, *H. bidorsalis*, and *H. longifilis*. Understanding these performance metrics is critical for selecting ideal hybrid combinations to increase production efficiency in commercial catfish farming operations. The aim of this study is to determine the extent of hybrid vigor expressed in four reciprocal crosses of *C. gariepinus*, *H. bidorsalis*, and *H. longifilis* by comparing their growth responses, feed utilization, and reproductive performance under controlled hatchery and rearing conditions.

MATERIALS AND METHODS

Study area

Department of Fisheries and Aquaculture Technology, Federal Owerri Nigeria was used for the study. The study area, Owerri is located in South – Eastern Nigeria and falls within the tropical rain forest zone of West Africa. It lies between $5^0 23^1 6876^{11}$ and $5^0 23^1 5.6150^{11}$ North of equator and longitude 6^0 59 ^{1} 10.55 04 ^{11} and 6^0 59^1 17.5800^{11} East of Greenwich. The area has a mean annual rainfall of 2.250–2500 millimeters and annual temperature range of 28-35% the relative humidity varies from 57.37% to 99.99% with the mean value of 88.44%.

Experimental design and procedure

The study which is designed as a completely randomized experiment (CRD) with 3 replicates involved three independent and simultaneous experiments.

Reciprocal crosses of parents for F_1hybrid

The parent hybrids were bred at the Departmental Laboratory under an intensive system. This is aimed at producing the F_1 progenies of hybrids of parent fishes which are of the same size and age as the pure line progenies.

The reciprocal crosses, which serve as treatments, are as follows:

T_1 = *C. gariepinus* (♀) × *H. bidorsalis* (♂)
T_2 = *C. gariepinus* (♂) × *H. bidorsalis* (♀)
T_3 = *C. gariepinus* (♀) × *H. longifilis* (♂)
T_4 = *C. gariepinus* (♂) × *H. longifilis* (♀)

Four spawning/hatchery tanks, each measuring 2m×1m×1.5m, were used for this phase of the experiment and replicated 3 times. Consequently, a total of 4×3=12 experimental units were utilized for this phase of the trial.

The rearing experiment was designed to commence two weeks after hatching, when the egg yolk must have been all absorbed, during which the survival rate was monitored. The fingerlings, which now average about 4.0cm in total length and 10.50g in weight, were stocked at the rate of 5 fingerlings/m^2 (Njoku et al. 2007). Consequently, each rearing tank will be stocked with 125 fingerlings of the various F_1 progenitors, which were of the same size and weight at commencement. The tanks were covered/lined with vinyl synthetic netting to prevent pests and predators. Tanks were filled with water to a depth of 1.0m and allowed to stand for two weeks before stocking. This was to allow for the development of zooplankton, the initial starter food for the fry (Njoku et al. 2007). The fryers were fed to satiation with hatched *Artemiasa lina*.

Hypophysation and artificial hybridization

The selected gravid females were weighed and injected intramuscularly with ovaprim synthetic hormone at a dosage of 0.5mL/kg of body weight, while the male was given half the dosage of that of the females. The brood fish head was covered with dry towel to restrain them from struggling, because fish generally remain calm when their eyes are covered. The recipient fish was injected in the dorsal muscle above the lateral line towards the dorsal section. The thumb was used to rub in the injection area to ensure thorough circulation of the hormone throughout the region and avoid backflow of the hormone. They were placed in separate bowls or ponds for latency period of 12h.

Stripping of eggs and collection of milt

The eggs from females were collected in dry plastic bowl, by stripping. Slight pressure was exerted on the belly towards the genital papilla to make the eggs flow out freely. This process continued until blood appeared which is a sign indicating the emptiness of the ovary. The males were sacrificed by killing them. Razor blade was used to dissect the male fish and the testes removed and cut into two lobes. The milt was pressed into the plastic bowl containing the eggs.

Fertilization and incubation

A clean feather was used to mix the eggs and the milt properly; normal saline was added to aid fertilization. The mixture was then stirred gently with a plastic spoon for one minute. The content was then poured onto a kaka'ban immersed in water. The eggs hatched in about 18-24h after fertilization.

Water quality parameters

Physico-chemical parameters evaluated include water temperature, pH, dissolved oxygen, turbidity, total dissolved solids, hardness, alkalinity, ammonia and phosphates.

Hybrid vigour (heterosis) parameters

Indices of hybrid vigour or heterosis consist of parameters that include growth rate, survival rate, hatchability, and average table size of fish. Parameters were determined as follows:

Data on feed consumed and weight gain were collected weekly for each unit from which the following growth performance parameters were evaluated.

Weight gain (g)

Weight gain (g) is defined as the total weight obtained at the end of rearing period less the initial weight of fish as follows:

Weight gain (g) = Final weight of fish obtained – Initial weight of fish

Percentage weight gain (PWG) %

$$(\%WG) = \frac{\text{Weight gain}}{\text{Initial Weght}} \times 100$$ (Sepahdar et al. 2009)

Specific growth rate (SGR) % =

= 100 (log W_2- log W_1) / (T_2-T_1) (Árnason et al. 2009)

Where W1 and W2 are the weights at time T1 and T2, respectively.

Where: T_1 and T_2 are time of experiment in days.

Relative growth rate (RGR) % $= \frac{\text{Final weight} - \text{initia weight}}{\text{Initial weight}}$ (Adesina et al. 2013)

Mean weight gain (MWG) (g) = Final mean weight – Initial mean weight (Adesina et al. 2013)

Average daily gains (ADG) $= \frac{\text{Final weight} - \text{initia weight}}{\text{Number of days}}$ (Adesina et al. 2013)

Fertilization rate (%): The percentage of eggs that are fertilized.

$$\text{Fertilization rate } (\%) = \frac{\text{Number of fertilized eggs}}{\text{Total number of eggs}} \times 100$$

Hatchability

The percentage hatchability is the total number of eggs hatched divided by the total number of fertilized eggs, multiplied by hundred.

$$\%\text{ Hatchability} = \frac{\text{Total number of Hatched eggs}}{\text{Total number of fertilized eggs}} \text{ x } 100$$

Survival performance

The method proposed by Adebayo (2008) was used to deduce the survival rates of the catfish at various stages, namely, larvae, fry, and fingerlings. Thus;

$$\text{Survival rate } \% = \frac{\text{Number of hatchlings alive at each stage}}{\text{Total numer of hatchlings}} \text{ x } 100$$

Statistical analysis

The growth responses of the four reciprocal crosses were compared with those of the parent progenies using the one-way analysis of variance (ANOVA), the trial having been given a completely randomized design (CRD). The difference in mean growth and survival was separated using Duncan's multiple range test (DMRT) or the least significant difference (LSD) methods at 5% significance level.

RESULTS

The result in Table 1 showed that T_2 had the highest weight gain (426.56±0.03) and it is significantly different (P<0.05) from others with the lowest weight gain in T_1. This also is reflected in the %weight gain where the lowest (98.41±0.03%) was recorded in T_1 and the highest in T_2 and T_3 with the value of 98.92±0.02% and 98.92±0.03% respectively. On the other hand T_3 had the lowest value (3.91±0.03%) in the specific growth rate (SGR) and it is significantly (P<0.05) different from other treatment while T_2 was the highest (5.63±0.02) significantly (P<0.05).

Table 1: Weight gain, %weight gain and ADG comparative analysis of relative hybrid vigor (Kurtosis) in reciprocal crosses of three farmed catfishes, *Clarias gariepinus, Heterobranchus bidorsalis and H. longifilis* during 120 days feeding trial

Reciprocal crosses	Weight gain (g)	% Weight gain	SGR (%)
T1	286.60±0.10[d]	98.41±0.03[c]	4.20±0.20[b]
T2	426.56±0.03[a]	98.92±0.02[a]	5.63±0.02[a]
T3	413.83±0.03[b]	98.72±0.02[b]	3.91±0.03[c]
T4	406.35±0.04[c]	98.92±0.03[a]	4.25±0.02[b]

Values (mean+SD) with different superscripts within the same column differ significantly (P<0.05).

In the RDR, as stated in Table 2, T_1 and T_2 were not significantly (P<0.05) different from each other in RGR; likewise, T_3 and T_4. The case is different with MWG, where T_3 was significantly high in the MWG with the value of 106.10±0.01% and T_1 with the lowest value of 93.60±0.36%. There was a significant reduction in ADG of T_3 (22.10±0.10 g/day) compared to others, with T_2 as the highest (36.40±0.20g/day), with a significant difference (P<0.05).

Table 2: RGR, MWG, and ADG comparative analysis of relative hybrid vigor (Kurtosis) in reciprocal crosses of three farmed catfishes, *Clarias gariepinus, Heterobranchus bidorsalis and H. longifilis* during 120 days feeding trial

Reciprocal crosses	RGR (%)	MWG (g)	ADG (g/day)
T1	2.24±0.04[b]	93.60±0.36[d]	30.30±0.20[b]
T2	2.25±0.02[b]	98.40±0.30[b]	36.40±0.20[a]
T3	2.44±0.05[a]	106.10±0.01[a]	22.10±0.10[d]
T4	2.40±0.03[a]	96.12±0.02[c]	22.40±0.02[c]

Values (mean+SD) with different superscripts within the same column differ significantly (P<0.05).

Table 3 also showed the results at the end of the experimental period where the fertilization rate was very high at T_1 (85.40±0.40%) and T_2 had the lowest (70.00±0.30%) in the group which was significantly different (P<0.05). But the hatchability rate was at its lowest at T_4 (72.87±0.06%) with the highest in T_3 (77.00±0.30%). With the survival rate, T_2 had the highest while T4 was the lowest.

Table 3: Fertilization (%), Hatchability rate (%) and Survival rate (%) comparative analysis of relative hybrid vigor (Kurtosis) in reciprocal crosses of three farmed catfishes, *Clarias gariepinus, Heterobranchus bidorsalis and H. longifilis* during 120 days feeding trial

Reciprocal crosses	Fertilization (%)	Hatchability rate (%)	Survival rate (%)
T1	85.40±0.40[a]	76.20±0.27[b]	75.00±0.10[b]
T2	70.00±0.30[c]	74.00±0.30[c]	77.05±0.05[a]
T3	76.50±0.40[b]	77.00±0.30[a]	74.73±0.21[c]
T4	75.30±0.02[b]	72.87±0.06[d]	73.30±0.30[d]

Values (mean+SD) with different superscripts within the same column differ significantly (P<0.05).

DISCUSSION

The examined hybrid vigor of in this research is reciprocal crosses of three farmed catfish species which were *C. gariepinus*, *H. bidorsalis* and *H. longifilis* by determining the growth responses and nutrient utilization of the experimental fishes across a 120-day feeding trial. In the determination of the weight gain across all the treatments, T_2 recorded the highest weight gain (426.56g) and was significantly different from other treatment. T_2 happen to be referred to as *Heteroclarias* following the report of experiment by Oyeyemi et al. (2020) that *Heteroclarias* has the potential to respond better to weight gain than other hybrids of the same species. Earlier studies have reported that *Heteroclarias* hybrid exhibit superior growth, improved survival and general hardiness than true breed of either *C. gariepinus* or *H. bidorsalis* (Meye and Omoruwou 2025; Shaibu et al. 2025). On the other hand, this was not the same with the observation of Olaniyi and Omitogun (2020) where after 28 and 84 days of experiment there was no significant difference in the weight gain of ♀*C. gariepinus* × ♂*C. gariepinus,* ♀*C. gariepinus* × ♂ *H. bidorsalis*, ♀*H. bidorsalis* × ♂*C. gariepinus,* and ♀*H. bidorsalis* × ♂ *H. bidorsalis*. Therefore, T_2 cross may be considered good for fast growth and productivity in commercial farming, where rapid biomass gain is desired.

The specific growth rate observed revealed that the progenies of T_1, T_3 and T_4 exhibited slower growth compared to its hybrid ♀*C. gariepinus* X ♂*H. bidorsalis*. The hybrid ♀*C. gariepinus* X ♂*H. bidorsalis* outperformed other species in terms of SGR performance. This results was the same as the report of Olaniyi and Omitogun (2020) after 4th and 12th weeks of experiment where hybrids between crosses of *C. gariepinus* and *H. bidorsalis* were observed. The results was different from the observation of Onyia et al. (2021) where the performance of hybrid ♀*C. gariepinus* × ♂*H. bidorsalis* was significantly higher (P<0.05) than its reciprocal ♀H. bidorsalis × ♂*C. gariepinus*. Relative growth rate (RGR) is an important indicator that measures how quickly the weight of fish increases relative to its initial weight within a period, provided there is a consistent method for comparing growth across fish sizes or experimental groups. The value of RGR was observed to be significantly higher at T_3 and T_4 than at T_1 and T_2 in this experiment.

Average daily gain (ADG) was significantly higher, outperforming the other groups (P<0.05), which suggests strong hybrid vigor in this T_2. This performance can be related to the report of Oyeyemi et al. (2020), which stated the fast growth rate potential of *Heteroclarias*. T_1 had the lowest weight gain and MWG, indicating the least favorable performance. T_3 and T_4 had intermediate weight gains, though T_3 had the lowest SGR (3.91%) and ADG (22.10g/day), yet showed exceptionally high MWG (106.10%), suggesting it grew beyond parental expectations in proportion, even if slower.

Fertilization rate at T_2 was the lowest, and T_1 showed the highest, indicating that T_1 may be suitable for hatching efficiency, although it is the least in nutrient utilization. In addition, it has better hatchability rates to prove its hatching efficiency potential, which confirms the report of Kolawole et al. (2022) that the hybrid (*Heteroclarias*) had the best fertilization, hatching, and survival rates. This is contrary to the earlier report by Ataguba et al. (2009) which had a higher fertilization rate (88.57%) between female *C. gariepinus* and male *H. longifilis*. Also, the case was not the same following the observation of (Ataguba and Angela 2024) that recorded 68.38% fertilization rate of the hybrid cross *C. gariepinus* and *H. bidorsalis*. Meanwhile, Wen et al. (2022) explained that the fertilization rate is determined mainly by egg quality, a phenotypic trait that is difficult to quantify.

According to previous reports, hybridization failures or successes can vary depending on the various factors present during the process of fertilization (Goswami, 2023; Naczk et al. 2025) and this could account for the current increase in fertilization rate in T1. Several factors can influence egg fertilization in fish, including internal 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 to stripping, failed to come into contact with sperm, or died during the early stages of embryogenesis. Physical damage to the eggs during mixing with milt cannot be excluded as a cause of egg infertility. Eggs that do not come into contact with sperm after being exposed to water die faster than those that do not develop into embryos. Ataguba and Angela (2024) found no change in fertilization rates between *C. gariepinus* and *H. bidorsalis* crosses.

Hatchability rate observed at T_1 and T_2 in this experiment was higher than what Ataguba and Angela (2024) reported with ♀*C. gariepinus* × ♂*H. bidorsalis* (52.57%) and ♀ *H. bidorsalis* × ♂ *C. gariepinus* (70.57%). This may be due to different factors, including physico-chemical parameters of the culture medium, as was stated by Aliu and Obasogie (2006). Hatchability rate was recorded as highest in T_3 (77.00%) and lowest in T_4 (72.87%), suggesting variability in reproductive success even among crosses with similar growth outcomes. Meanwhile, the hatching rates recorded in this experiment were not compared to those obtained in the crosses between *C. gariepinus* and *H. longifilis* (84.16% to 87.43%) by Ataguba et al. (2009). Agnèse et al. (1995) reported higher values (90.37 to 92.96%) for *H. longifilis* breeding, whereas Tiogué et al. (2018) found values ranging from 68.07 to 72.82% in a closed loop for *C. gariepinus* triggered by fertilization. However, the present hatching rate is higher than the 51.64 to 65.58% reported by Tiogué et al. (2018) in *C. gariepinus* caused by hypophysation in closed or static conditions. Furthermore, Ataguba and Angela (2024) found low values in all genetic categories tested (41.84% to 62.12%). T1 may be preferable in hatchery settings due to its high fertilization rate, although its growth rate is the slowest.

Conclusion

The study found that reciprocal hybridization had a considerable impact on growth and reproductive features in farmed catfish. In terms of growth performance, the hybrid T_2 outperforms the other four reciprocal crosses tested throughout a 120-day culture period. However, hatchery success may benefit more from T_1 or T_3, underlining the necessities of selecting the appropriate cross according on production objectives (for example, seed production vs. grow-out performance). The findings emphasize the importance of selecting optimal parent combinations and cross directions based on specific production objectives, such as seed production or table fish farming. Furthermore, the observed differences in hybrid performance highlight the significance of reciprocal crossing as a strategic breeding technique for harnessing maternal and cytoplasmic factors to produce desirable aquaculture traits.This research provides important insights into the genetic improvement of African catfish species trough hybridization. It lays the groundwork for future selective breeding initiatives aimed at increasing production, promoting genetic variety, and ensuring the sustainability of catfish aquaculture in Nigeria and other developing countries.

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Authors' Contribution: Ezeafulukwe CF, Njoku DC, Osuigwe DI and Adaka GS conceptualized and supervised the work; and read the manuscript. Ogueri C, Ajima MNO, Utah C, Iwuji, NG, Bunu AJ, Uzoma JI Okonkwo JC and Ahaotu EO conducted the experiments, collected and analyzed the data; and prepared the manuscript. All authors read and approved that last manuscript version.

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