

INTRASPECIFIC HYBRIDIZATION AND RECIPROCAL CROSS BETWEEN CULTURED AND WILD *Clarias gariepinus*; AND EVALUATION OF THEIR PROGENIES

^{1*} ED-IDOKO, J. O., ²SOLOMON, S. G. and ²IKAPE, S. I.

¹Department of Fisheries and Aquaculture, University of Agriculture, Makurdi, Nigeria.

²Department of Biological Sciences, Benue State University Makurdi Nigeria. Centre for Food Technology and Research.

Correspondence author email: edidoko.john@gmail.com

Phone number: +2348061216970

ABSTRACT

Studies of intraspecific hybridization and reciprocal cross between cultured and wild *Clarias gariepinus* and evaluation of their progenies was carried out, with aim of evaluating the progenies of the crosses hatchability, survival, growth etc. Pure strains of cultured and wild *Clarias gariepinus* broodstock were used. Incubation, hatching, and post hatching activities were monitored in 50 litres plastic bowls. Cultured Male *Clarias gariepinus* (CCG♂) x Wild Female *Clarias gariepinus* (WCG♀) were used as Treatment 1 with Replicate and Wild Male *Clarias gariepinus* (WCG♂) x Cultured Female *Clarias gariepinus* (CCG♀) as Treatment 2 with Replicates. The result showed that in all the two genetic crosses, (CCG♂) x Wild Female *Clarias gariepinus* (WCG♀) had the highest fertilization rate (84.93%). Statistically, there was a significant difference ($p < 0.05$) in % survival at first feeding, between CCG♂ x WCG♀ (95.05%) and WCG♂ x CCG♀ (90.65%). No significant difference in Growth Rate, Specific Growth Rate (SGR) and Protein Efficiency Ratio (PER). % survival at the end of the experiment was highest with CCG♂ x WCG♀ (84.93%) compared with WCG♂ x CCG♀ (60.33%). It was concluded that fish seed from (CCG♂ x WCG♀) had better reproductive potential in terms of fertilization, hatchability, testes volume and better growth performance.

Keywords: Intraspecific, hybridization, Spawner and Maceration.

INTRODUCTION:

According to FAO (2007), aquaculture continues to grow more rapidly than the other animal food producing sectors, with an average global annual growth rate of 8.8% per year since 1970, compared to only 1.2% for capture fisheries. Culturing of fish, as opposed to capture fisheries demands a controlled practice of fish gene manipulation and crossing for improving and increasing fry, fingerlings and table size fish quality and quantity. This will help meet the ever growing global human population, and satisfy the global need for a rich source of animal protein availability. There are a number of fish species with high culture potentials in Nigeria. However, Megbowon *et al.*, (2013) stated that the African Catfish (*Clarias gariepinus*) is widely considered as the leading cultured fish in the country. *Clarias gariepinus* is the accepted and equally reared on most of the fish farms in Nigeria. It is easily crossed among the genus of *Clarias*; it has high resistance to diseases and handling stress, tolerates low oxygen, unfavourable temperature, and fast growth (Ochokwu *et al.*, 2015). In aquaculture, fish production can be improved upon through genetic improvement (Omeji *et al.*, 2013). The usual traditional method of improving fishes has been through hybridization and selective breeding

(Lakra, 2001). Fish production through hybridization is an age long practice in Africa. Hybridization in African catfishes *Clarias gariepinus*, *Clarias anguillaris*, *Heterobranchus bidorsalis* and *Heterobranchus longifilis* has been in practice in Africa (Adah *et al.*, 2014). Hybridization has been used to improve fish, increase growth rate, manipulate fish sex, production of sterile fish, and improve flesh quality, increase semen volume, increase disease resistance, and environmental tolerance (Bartley *et al.*, 2000).

Hybridization possesses two important advantages. It avoids inbreeding and it avoids contamination of the environment with sex hormones (Wohlfarth 1994). Also, Hybridization can be very attractive for aquaculture in many ways. First, it may produce sterile animals, avoiding growth loss or fragility related to sexual maturation. Hybrids sterility reduces potential interactions between domestic and wild fishes. Secondly, hybridization may lead to one sex population production which might be an advantage in case of differential growth between males and females (Chevassus 1983). Thus, there is need to improve on the existing progenies and parent stock of *Clarias gariepinus*, especially as it

is a dominant fish species in Nigerian Aquaculture. This has necessitated research into hybridization. The objectives of this study were to evaluate the hybridization and reciprocal cross between cultured and wild species of *Clarias gariepinus*, comparing the crosses progenies in terms of hatching, survival, growth, and other genetic attributes.

MATERIALS AND METHODS:

Experimental fish

The broodstock of *Clarias gariepinus* (average weight of 406.1-693.60g) males and female (438.9-577g) were obtained from River Benue and Reputable fish Farm in Makurdi. The fish obtained were transported in plastic Jericans (50 liters) to the experimental site. The brood stocks were acclimatized for 5 days in concrete ponds. They were fed 35% crude protein diet at 3% body weight twice a day before the commencement of the experiment.

Hypophysation and artificial hybridization

The fishes were sexed and separated into males and females based on their genital papillae Viveen *et al.*, (1985); the weight and length of the gravid females were measured and induced with Ovaprim synthetic hormone dosage of 0.5 ml/kg body weight for female. The male were not injected, but their reddish tip of papillae was used to determine maturity. (Omeji *et al.*, 2013). The brood fish were kept in well aerated plastic bowls of 60cm diameter × 30cm deep for 10 hours and covered with a net.

Milt collection

After 10 hours of the latency period, the milt was collected by sacrificing the male. The two testes from the male testes were removed and cleaned with a towel. The length and weight of the testes were measured using a meter rule in centimeters. The motility duration of the spermatozoa was estimated using a light microscope at 100X magnification and was expressed as percentage of motile spermatozoa. The milt from the males were collected, cut and squeezed onto a physiological solution in a Petri dish strain-wise. The eggs of the female were stripped by the gentle application of pressure on the abdomen into receptacles strain-wise.

Fertilization and Hatchability

Fertilization and hatchability rate was determined using 50 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 fertilization were considered fertilized and counted to estimate fertilization rate. The incubation was carried out through the experimental design of 2 treatments and 2 replicates each of Male Cultured *Clarias gariepinus* (CCG♂) x Female Wild *Clarias gariepinus* (WCG♀) as Treatment 1 with 2 Replicates and Male Wild *Clarias gariepinus* (WCG♂) x Female Cultured *Clarias gariepinus* (CCG♀) as Treatment 2 with 2 Replicates. After hatching, each treatment and replicate had 1000 fry each as sample. The number of hatchlings in each trough was recorded by direct counting of the hatchlings and un-hatched eggs for each cross combination. The fertilization and hatching rates were estimated as follows:

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

$$\text{Hatchability (\%)} = \frac{\text{Number of hatchling}}{\text{Total number of fertilized}} \times 100.$$

Statistical analysis

Data collected was analyzed for descriptive statistics and analysis of variance. Breeding performance was analyzed using student T-Test {GenStat 10.3DE (PC/Windows Vista)}.

RESULTS

The results of fertilization, hatchability, % survival at first feeding, of the fry to post fry progenies of the cross between CCG♂ x WCG♀ and WCG♂ x CCG♀ in outdoor plastic bowls are shown in Table 2. The highest fertilization (84.93 ± 5.07^a) was found to be in CCG♂ x WCG♀ as against WCG♂ x CCG♀ (60.33 ± 0.33^b), with a significant Difference at ($P < 0.05$). The Hatchability (90.3 ± 5.99) was found in CCG♂ x WCG♀ as against WCG♂ x CCG♀ (89.49 ± 0.61), with no significant Difference at ($P < 0.05$). The highest % Survival at 1st feeding (95.05 ± 0.15^a) was found to be in CCG♂ x WCG♀ as against WCG♂ x CCG♀ (90.65 ± 1.45^b), with a significant Difference at ($P < 0.05$).

Table 1: Water Quality Parameters in Treatment 1 and 2

	Trt 1	Trt 2
Temperature	27.00 ± 0.10	26.85 ± 0.05
TDS	116.60 ± 0.50	118.05 ± 1.50
DO	4.75 ± 0.50	4.70 ± 0.20
Ph	5.51 ± 0.03	5.83 ± 0.04
Conductivity	232.60 ± 0.50	235.50 ± 1.50

Tr1= CCG♂ x WCG♀, Tr2= WCG♂ x CCG♀

Table 2: Hatching and Survival Parameters for Treatment 1 and 2

Parameters	Trt 1	Trt 2
Wt of Males (g)	693.60 ± 68.6 ^a	406.1 ± 6.0 ^b
Wt of testis (g)	1.650 ± 0.15 ^a	0.70 ± 0.1 ^b
Wt of Females (g)	577.50 ± 27.5	438.9 ± 50.1
Wt of eggs (g)	16.85 ± 0.75	20.55 ± 0.57
Fecundity (g)	126375 ± 5625	154125 ± 5623
Fertilization	84.93 ± 5.07 ^a	60.33 ± 0.33 ^b
Hatchability	90.30 ± 5.99 ^a	89.49 ± 0.61 ^b
%Survival @1 st feeding	95.05 ± 0.15 ^a	90.65 ± 1.45 ^b

Mean in the same row with different superscripts differ significantly ($P < 0.05$) Tr1=CCG♂ x WCG♀, Tr2 = WCG♂ x CCG♀

Table 3: Growth Performance of the Crosses in Treatment 1 and 2

	Trt 1	Trt 2
Initial Wt (g)	0.34 ± 0.04	0.21 ± 0.25
Final Wt (g)	3.68 ± 0.30	3.15 ± 0.64
Wt gain (g)	3.34 ± 0.34	2.95 ± 0.62
Growth rate	0.10 ± 0.01	0.09 ± 0.01
SGR	7.45 ± 0.63	8.49 ± 0.26
PER	0.07 ± 0.00	0.07 ± 0.01
%Survival	84.93 ± 5.07 ^a	60.33 ± 0.33 ^b

Mean in the same row with different superscripts differ significantly ($P < 0.05$) Tr1=CCG♂ x WCG♀, Tr2 = WCG♂ x CCG♀

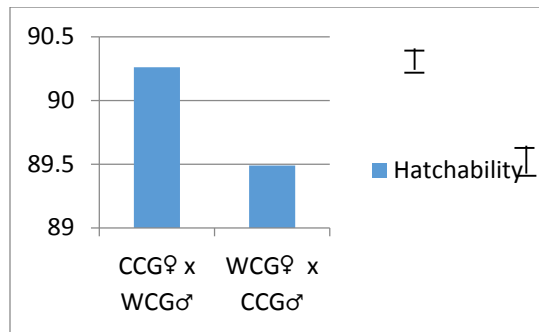


Fig. 1: Hatchability of the cross between Cultured *Clarias gariepinus* (Male) x Wild *Clarias gariepinus* (Female) and Wild *Clarias gariepinus* (Male) x Cultured *Clarias gariepinus* (Female)

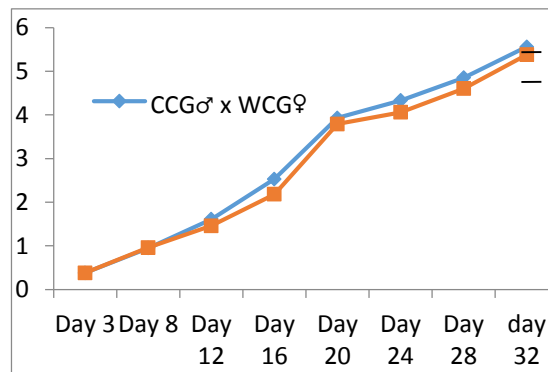


Fig. 2: Growth performance of the cross between Cultured *Clarias gariepinus* (Male) x Wild *Clarias gariepinus* (Female) and Wild *Clarias gariepinus* (Male) x Cultured *Clarias gariepinus* (Female)

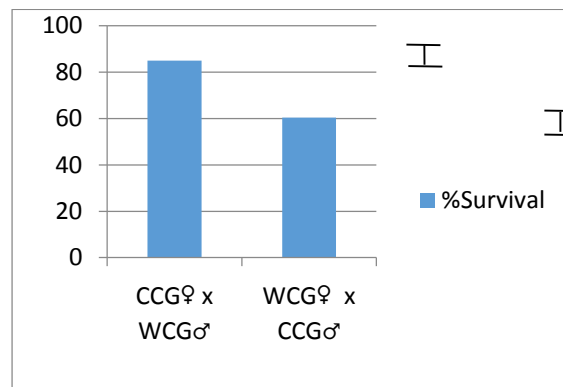


Fig. 3: %Survival of the cross between Cultured *Clarias gariepinus* (Male) x Wild *Clarias gariepinus* (Female) and Wild *Clarias gariepinus* (Male) x Cultured *Clarias gariepinus* (Female)

DISCUSSION

The higher fertilization rate (84.93%) and hatching rate (90.30%) obtained in this study is close to the findings of Omeji *et al.*, (2013) that had similar results after crossing exotic and local *Clarias gariepinus*. Shah *et al.*, (2011) also reported that the reciprocal crosses between Jamuna, Padma and

hatchery strains had comparatively similar fertilization rates (86-89%) whereas pure Jamuna strain showed the highest fertilization rate (95%). The reason for the higher fertilization rate in the crosses can be due to differences in their population. However, results from this study disagreed with Islam and Shah (2007) who obtained a lower mean fertilization rate for Jamuna strain as 75.75%, for pure Jamuna strain 75.49%

and for pure hatchery strain 65.49%. Also Tilahun *et al.* (2016) reported a lower fertilization rate (77.10%) in hybrid crosses between *Clarias gariepinus* and *C. batrachus*. Between the intra-specific crossbreeds, Cultured Male *Clarias gariepinus* (CCG♂) x Wild Female *Clarias gariepinus* (WCG) had the best hatchability rate (90.30%) while the least hatchability was observed in the cross between Wild Male *Clarias gariepinus* (WCG♂) x Cultured Female *Clarias gariepinus* (CCG♀) (89.49%). However, the trend in hatchability observed in the research favoured the parental crosses. This result agrees with the findings of Olufeagba *et al.* (2015) who obtained a low hatchability rate among the reciprocal hybrids (41.0%) when compared with the parental crosses (94.0%). Similar trends were observed by Omeji *et al.* (2013) when he crossed female exotic x male exotic *Clarias gariepinus* (52.10%) and local x exotic *Clarias gariepinus* (49.50%), and the reported works by Tilahun *et al.* (2016), Sayeed, (2015), Shah *et al.* (2011), Aluko and Ali (2001). It is however important to acknowledge that differences that arise from breeding history, may be affected by water quality, condition factor of the fish and age of the fish especially the hatching rates. Variations in seasons can also lead to differences in hatching rates, as rightly observed by Shah *et al.*, (2011) and (Ochokwu *et al.*, 2015).

The higher fecundity rate (154,125) recorded in this study was higher in the female of *Clarias gariepinus* from the Cultured Female *Clarias gariepinus* (CCG♀) agrees with the findings of Shinkafi and Ipinjolu, (2012) who reported higher fecundity in most of the larger fishes than the smaller fishes in *Achenoglanis occidentalis*. He stated that the lower the number of eggs in the species, the larger the size of eggs. However, Fecundity was also dependent on the size of fish and thus, the larger the fish, the higher its egg number and this may be due to more available visceral volume for holding the eggs. Similar results were observed by Ochokwu *et al.*, (2016).

Variation in sperm quality may be due to sex ratio, stocking density, age, size, nutrition and feeding regime; (Tahoun *et al.*, 2008). Studies have shown that qualitative parameters of the milt (sperm motility, sperm lobe length, milt volume and count) can be influenced by several factors such as feeding regime, the quality of the feed (Cerovsky *et al.*, 2009), environmental factors, variations between individual, age, weight, length of the fish Ochokwu *et al.*, (2015).

Hatchability for CCG♂ x WCG♀ (90.3%) and WCG♂ x CCG♀ (89.49%) showed no significant difference ($P < 0.05$). Though fertilization for WCG♂ x CCG♀ (60.33%) was relatively lower than CCG♂ x WCG♀ (84.93%),

the hatchability result in comparison, shows that almost all fertilized eggs from WCG♂ x CCG♀ hatched, unlike that of CCG♂ x WCG♀ that had more unhatched eggs. This suggests that hatching was strongly influenced by their maternal parent. This observation is in line with the hypothesis by Hubbs (1970) and Hester (1970), who suggest the use of hybridization to demonstrate phylogenetic relationship in fish. It is accepted that the characteristics of the embryo and the larva depend on genetic and nongenetic (phenotype-based) factors like egg size or quality (Saillant *et al.* (2001); Rideout, Trippel & Litvak (2004); Ottesen & Babiak (2007); Donelson *et al.* (2009).

Survival rate at first feeding for CCG♂ x WCG♀ (95.05%) was higher than WCG♂ x CCG♀ (90.65%). Growth rates of fish depend on a number of factors among which are the Temperature of the water, genetic factor, availability of food, feeding rate and stocking density of the fish. Even though production may increase with density, crowded populations suppress increase in weight and the individuals (Bardach *et al.*, 1972). However, the nongenetic effects (environmental effects) are often associated with maternal factors, because the female is responsible for 100% of the yolk characteristics of the egg. However, the contribution of males in the early stages of embryonic and larval development are sometimes highlighted (Rideout *et al.*, 2004; Probst *et al.*, 2006), although it may be suspected that the male effect is more related to genetic than to environmental factors (Saillant *et al.*, 2001). Thus, it is imperative know that female broodstock obtained from the wild sources developed eggs that yields more hardy fry.

Statistically, there was no significant difference ($P < 0.05$) in growth rate of CCG♂ x WCG♀ cross to WCG♂ x CCG♀. This agrees with the findings of Donelson *et al.*, (2009) who reported that maternal or paternal effect can be largely offset by factors of the rearing environment, such as the amount of food available, and in some cases, differences that may occur in early breeding period can then disappear in weeks or months after birth. Similar observations were also made by (Ottesen, Babiak & Dahle 2009). Also, Madu *et al.*, (1993) opened a similar observation that the superior growth characteristics of the hybrids will not be manifested until after 12 months culture period from fingerlings to adult.

Percentage survival at the end experiment for CCG♂ x WCG♀ (84.93%) was higher than WCG♂ x CCG♀ (60.33%) this could probably due to its hardiness and adaptation to environment. This result followed the trend of de Graaf *et al.*, (1995) and Olufeagba and Aluko (1997) who reported high survival rate of local *Clarias gariepinus*

reared under a medium stocking density for a short duration in protected tanks.

Physiochemical properties of water (pH, Total dissolved solid, temperature, conductivity and dissolve oxygen) during breeding, process agree with the findings of Onyia *et al.*, (2015) and they are all within the tolerable ranges for fish culture.

CONCLUSION:

The study revealed information on some aspects of reproduction of *Clarias gariepinus*, and methods of its reproduction, which can be used to promote and improve the management of the species in the wild and under aquaculture. The experiment also explained how the progenies of the crosses affects the fertilization rate, hatchability, survival rate and growth performance in *Clarias gariepinus* positively, thus, intraspecific hybridization of CCG♂ x WCG♀ is highly recommended. This could enhance better quality of fish production and boost aquaculture production.

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