



## Intra-specific Hybridization between Two Strains of *Clarias gariepinus* from South-West and North Western Nigeria

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### ABSTRACT

Studies of intra-specific hybridization between *C. gariepinus* from Ibadan and Katsina in the South-west and North western Nigeria was carried out, under hatchery conditions, with the aim of estimating their aquaculture potential. The broodstock of *C. gariepinus* were procured from University of Ibadan research farm and Zobe Dam in Katsina state. The result showed that in all the four genetic crosses, fertilization rate (98.0%), hatchability (88.0%), milt volume (9.0ml), sperm motility (62.0s) and testes length (10.74cm) of those from the South West (Ibadan) were significantly ( $P < 0.05$ ) higher. It was followed by the hybrids between Ibadan and Katsina strains which had 94.0% fertilization rate. The crosses between the Katsina strain had significantly ( $P < 0.05$ ) higher 84.0% hatchability compare to their hybrids (78.0%). The crosses within Ibadan strains had the highest (198,205) number of eggs and ovary weight (192.80g). Progeny from Ibadan gave the best result. The breeding potentials of *Clarias gariepinus* strain from Ibadan and Katsina were better when crossed breed. If several selective breeding and back crossing of the strains and one of the parents are carried out between Ibadan and Katsina there is the possibility of obtaining fish seed of better reproductive potential in terms of fertilization, hatchability, testes volume and better growth performance. It can reduce the occurrence of undesirable traits.

**Key words:** Intraspecific, hybridization, *Clarias gariepinus* South-West, Northwest, Nigeria

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### 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. There are a number of fish species with high culture potentials in Nigeria. However, Megbowon *et al.* (2013) stated that the African Catfish (*C. 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). In selective breeding, the target could be for qualitative or quantitative traits. Induced breeding with hormone either the pituitary gland or synthetic hormone is the most method employed in aquaculture (Rottmann *et al.*, 1992). Crossing of different strains have resulted in the production of quality hybrids with desirable characters. (Marjorie *et al.*, 2005) stated that the offspring of distinctly different parental types produces a new, uniform phenotype with a combination of characteristics from the parents.

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

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fish, increase growth rate, manipulate fish sex, production of sterile fish, improve flesh quality, increase semen volume, increase disease resistance, and environmental tolerance (Bartley *et al.*, (2000). Hybridization is used to produce offspring that performs better than both parental species (positive heterosis). Hybridization is the generation of a new form of plant or animal either naturally or by human intervention through combining the genes of two different species or subspecies. Fish hybridization is when two different species, genera or families are crossed, crossing of the first filial generation, backcrossed or out crossed to give the hybrid of desired qualities. Fish hybridization is an essential genetic technique that removes undesirable characteristics while retaining the desirable ones.

Research has been carried out on intra-specific hybridization. Legendre *et al.* (1992) reported the cross between *Clarias gariepinus* and *Heterobranchus longifilis* to produce viable reciprocal hybrids with their survival rates being similar to those found in the maternal species, (Diyaware and Onyia, 2014) reported high growth rate in cross between *Clarias anguillaris* and *Heterobranchus bidorsalis*. While (Omeji *et al.*, 2013) reported Intra-specific hybridization of local and exotic *Clarias gariepinus*, where survival and fecundity was improved. (Tilahun *et al.*, 2016) had a better fertilization and hatchability rate in the hybrids when he assessed the reproductive performance, growth and survival of hybrids of African catfish (*C. gariepinus*) and Indian catfish (*Clarias batrachus*) compared to their parental lines cross. The growth performance of intraspecific hybridization of wild strains of *Clarias gariepinus* from Nigeria water was investigated by Megbowon *et al.* (2014). Olney and McBride, (2003) also investigated the intraspecific Variation in Batch Fecundity of American Shad: Revisiting the Paradigm of Reciprocal Latitudinal trends in Reproductive Traits, while Preliminary report on genetic improvement of *H. longifilis* through intraspecific hybridization of different strains from Nigeria was evaluated by Olufegba and Okomoda, (2015) and Solomon *et al.* (2015) evaluated the Intraspecific morphological variation between cultured and wild *Clarias gariepinus* (Burchell) (Clariidae, Siluriformes). Intraspecific hybridization involves combining different strains of specie. It involves crossing of fish that belong to the same species. While Onyia *et al.* (2010) reported that cross-breeding of *Clarias anguillaris* strains could be advantageous because of the better performancethe crossbreedingy. They also reported high hatching success and survival rates of *C. anguillaris*. The need for high quality fish seed has necessitated research into hybridization. The objectives of this study were to evaluate the milt volume, sperm length, fertility rate, hatchability, and weight of ovary of *Clarias gariepinus* strains from Ibadan (South West) and Katsina (North West) Nigeria.

## MATERIALS AND METHODS

### Study area

The experiment was conducted in fish hatchery of the Teaching and Research fish farm of the Department of Fisheries and Aquacultural Technology, Federal University Dutsinma, Katsina State. Dutsinma is a Local Government Area in Katsina state, it lies on latitude 12°27'18N and longitude 07°29'29E. Dutsinma is bounded by Kurfi and Charanchi Local Government Areas to the north, Kankia Local Government area to the east, Safana and Dan-Musa Local Government Areas to the west, and the Matazu LGA to the southeast. The Dutsinma Local Government Area has land size of about 552.323 km<sup>2</sup> with a population of 169,829 recorded during the 2006 national census. The people are predominantly farmers, cattle rearers and traders. Rainfall is between May and September with a peak in August. The average annual rainfall is about 700 mm. The pattern of rainfall in the area is highly variable. (Iguisi *et al.*, 2012). The mean annual temperature ranges from 29 °C –38 °C.

### Experimental fish

The broodstock of *C. gariepinus* (average weight of 550-1500g) males and female (650-1300g) were collected from two geopolitical zones of Nigeria; South West from teaching and research farm University of Ibadan and North West from Zobe Dam in Dutsinma Local Government Area. The fish obtained from Ibadan were transported in plastic troughs (60cm diameter × 30cm deep) Dutsinma, Katsina State. The broodstock were acclimatized for 5 days in 10 m<sup>2</sup> earthen 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 Ovulin at a dosage of 0.5 ml/kg body weight for female and 0.25 ml/kg body weight for male (Omeji *et al.*, 2013). The brood fish were kept in well aerated plastic bowls of 60cm diameter × 30cm deep for 9 hours and covered with a net.

### Milt collection

After 9 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 centimetres. The motility duration of the spermatozoa was estimated using a light microscope at 100 X 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. The milt was then added onto the stripped eggs in the following cross combinations replicated in triplicate: Female Ibadan x male Ibadan (IB x IB), Female Ibadan x male Katsina (IB x KT), Female Katsina x male Katsina (KT x KT), Female Katsina x male Ibadan (KT x IB). Females are mentioned first in this study. The crosses were repeated three times in complete randomized block design (CRBD) manner.

### 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 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: Fertilization rate (%) = Number of fertilized eggs ÷ Total number of eggs x 100, Hatchability (%) = Number of hatchling ÷ Total number of fertilized x 100, Gonadosomatic index = ovary ÷ body weight (g) x 100, Pseudogonadosomatic index = weight of egg mass x 100 ÷ body weight before inducement – weight of striped eggs (Richter *et al.*, 1987; Viveen *et al.*, 1985).

### Statistical analysis

Data obtained from the experiment were subject to a One-Way-Analysis of Variance (ANOVA). Duncan Multiple Range Test DMRT, Duncan (1985) was used to determine the differences between the means (P=0.05) using SPSS version 20.0.

## RESULTS

Table 1 shows the gonadosomatic index of *C. gariepinus* for Ibadan and Katsina. The result revealed that the female had high fecundity rate (198,205), compared to the broodstock obtained from Katsina (163,054). There was significant (P<0.05) difference in the weight of the ovary between Ibadan and Katsina.

The mean sperm volume and motility of *C. gariepinus* from Ibadan and Katsina are shown in Table 2. The males from Ibadan had the highest milt volume (9.0 and 7.0ml), testes weight (8.40 and 6.20g), motility (62.0 and 54.0seconds) and testes length both right and left (4.24 and 3.30, 6.50 and 4.50cm). The result shows that there was significant (P<0.05) difference in males from Ibadan and Katsina.

Table 1: Mean fecundity and gonadosomatic index of female *C. gariepinus* from South-west and North-west Nigeria

Strains	Weight (g)	Total length (cm)	Weight of ovary (g)	Number of eggs	GSI (%)	PGSI
Ibadan	1300.0 <sup>a</sup>	50.0 <sup>a</sup>	192.8 <sup>a</sup>	198,205.0 <sup>a</sup>	12.92 <sup>b</sup>	17.41 <sup>b</sup>
Katsina	650.0 <sup>b</sup>	44.0 <sup>a</sup>	132.1 <sup>b</sup>	163,054.0 <sup>b</sup>	16.89 <sup>a</sup>	25.51 <sup>a</sup>
SEM	0.57 <sup>*</sup>	0.57 <sup>ns</sup>	0.58 <sup>*</sup>	0.57 <sup>*</sup>	0.06 <sup>*</sup>	0.06 <sup>*</sup>

Means in each column with the same superscript are not significantly different (P>0.05)

Table 2: Mean sperm volume, weight, and semen motility of *C. gariepinus* from Ibadan and Katsina

Strains	Weight (g)	Length (cm)	Testes Weight (g)	Length of Right testes (cm)	Length of Left testes (cm)	Milt Volume (ml)	Motility Duration (S)
Ibadan	1500.0 <sup>a</sup>	56.40 <sup>a</sup>	8.40 <sup>a</sup>	4.24 <sup>a</sup>	6.50 <sup>a</sup>	9.00 <sup>a</sup>	62.00 <sup>a</sup>
Katsina	550.0 <sup>b</sup>	49.75 <sup>b</sup>	6.20 <sup>b</sup>	3.30 <sup>b</sup>	4.50 <sup>b</sup>	7.00 <sup>a</sup>	54.00 <sup>b</sup>
SEM	28.86 <sup>*</sup>	0.05 <sup>*</sup>	0.58 <sup>*</sup>	0.01 <sup>*</sup>	0.58 <sup>*</sup>	0.57 <sup>ns</sup>	0.57 <sup>*</sup>

Means in each column with the same superscript are not significantly different (P>0.05).

Table 3 shows the fertilization and hatching rate of intra-specific hybridization between Ibadan and Katsina strains of *C. gariepinus*. The highest fertilization (98%) and hatchability (88%) were recorded in parent broodstock from Ibadan, followed by the hybrids between Ibadan and Katsina which had (94%) fertilization. There was significant (P<0.05) differences between the fertilization rate and hatchability among the pure lines and the hybrids.

Table 3: Mean fertility and hatching rates of intra-specific hybridization between Ibadan and Katsina strains of *C. gariepinus*

Genetic Groups	No. of fertilized Eggs	Fertilized %	No. of hatched eggs	Hatchability %
Parental crosses				
IB x IB	49.0 <sup>a</sup>	98.0 <sup>a</sup>	44.0 <sup>a</sup>	88.0 <sup>a</sup>
KT x KT	43.0 <sup>b</sup>	86.0 <sup>b</sup>	42.0 <sup>ab</sup>	84.0 <sup>ab</sup>
SEM	0.57 <sup>*</sup>	1.15 <sup>*</sup>	1.15 <sup>ns</sup>	2.30 <sup>ns</sup>
Ib x Kt				
IB x KT	47.0 <sup>a</sup>	94.0 <sup>a</sup>	39.0 <sup>b</sup>	78.0 <sup>b</sup>
KT x IB	38.0 <sup>c</sup>	77.0 <sup>c</sup>	38.0 <sup>b</sup>	77.0 <sup>b</sup>
SEM	0.57 <sup>*</sup>	1.15 <sup>*</sup>	0.57 <sup>ns</sup>	1.15 <sup>ns</sup>

Means in each column with the same superscript are not significantly different (P>0.05)

Table 4 shows the mean water quality parameters during the 48 hours incubation and hatching. Temperatures ranged from 29.20 – 29.79, pH, dissolved oxygen and conductivity ranged from 7.10 – 8.44, 5.16 – 5.86, and 30.55 – 30.86 respectively.

Table 4: Mean Water quality parameters within 48 hours of incubation in the hatchery

Cross combinations	Temperature (°C)	pH	DO mg/l	Conductivity
Parental crosses				
Ibadan x Ibadan	29.20	7.40	5.86	30.55
Katsina x Katsina	29.67	7.10	5.60	32.60
Hybrids				
Ibadan x Katsina	29.64	8.44	5.16	31.86
Katsina x Ibadan	29.79	7.10	5.24	33.24

## DISCUSSION

The higher fertilization and hatching rates obtained in this study is incongruent with the findings of Omeji *et al.* (2013) that had similar results after crossing exotic and local *C. gariepinus*. Shah *et al.* (2011) also reported that the reciprocal crosses between Jamuna, Padma and hatchery strains had comparatively lower fertilization rates (86-89%) whereas pure Jamuna strain showed the highest fertilization rate (95%). The reason for the lower fertilization rate in the crosses can be due to differences in their population. While it disagreed with Islam and Shah (2007) who obtained a higher 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 low fertilization rate (77.10%) in hybrid crosses between *C. gariepinus* and *C. batrachus*. Between the four intra-specific crossbreeds, female Ibadan x male Ibadan had the best hatchability rate (88.0%) while the least hatchability was observed in the cross between female Katsina x male Ibadan (77.0%). However, the trend in hatchability observed in the research favoured the parental crosses. This result of hatchability between Ibadan and Katsina *C. gariepinus* and their reciprocal hybrids agrees with Olufeagba *et al.* (2015) who obtained a lower 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 between female exotic x male exotic *C. gariepinus* (52.10%) and local x exotic *C. 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 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 (198,205) recorded in this study was higher in the female of *C. gariepinus* from Ibadan which agrees with Shinkafi and Ipinjolu, (2012) who reported higher fecundity in most of the larger fishes than the smaller fishes in *A. 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 was observed in Hirpo, (2013). The milt volume (9ml) and sperm length (right 4.24cm and left 6.50cm) was significantly higher ( $P < 0.05$ ) in broodstock from Ibadan compared to Katsina, which had milt volume (7ml) and sperm length (right 3.30cm and left 4.50cm) respectively. The differences could be attributed to the weight of the fish Ibadan had (1500g) compared to Katsina (550). 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), season of the year (Hajirazae *et al.* (2010), stress, uptake of nutritive and genetic materials, physiochemical properties of water (pH, salinity and temperature and dissolve oxygen) (Brooks *et al.*, 1997). Meanwhile temperature, Dissolve Oxygen and pH during breeding, process agree with the findings of Onyia *et al.* (2015).

## Conclusion

The study revealed information on some aspects of reproduction of *C. gariepinus* in Zobe dam in Katsina State. It is important parameters for stock assessment, understanding of the population dynamics and methods of reproduction, which can be used to promote and improve the management of the species in the wild and under aquaculture. The most plausible causes of poor performance of broodstock are poor management practice, stress, poor health condition before breeding, small size of brood fish and lack of maintenance of genetic diversity. If a good attention is paid in the above mentioned factors, it will result to quality eggs, sperm; thereby increase fertility, hatchability and

fecundity in fish. Thus, the intra-specific hybridization and strain, crossing method used in this research can be used to boost aquaculture production.

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