AACL BIOFLUX
Aquaculture, Aquarium, Conservation & Legislation
International Journal of the Bioflux Society

Reproductive, survival and growth performance of intergeneric cross of Exotic Dutch *Clarias*, *Heterobranchus bidorsalis* and *Heterobranchus longifilis* in Sokoto North-West Nigeria

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Abstract. Gamete from *Heterobranchus bidorsalis* (Hb), *Heterobranchus longifilis* (Hl) and Exotic Dutch *Clarias* (EC) were used to fertilize eggs from female Exotic Dutch *Clarias* to produce pure Exotic Dutch *Clarias* and its paternal hybrids in other to assess their induced spawning, survival and growth potentials in the hatchery. The female and males were induced with Ovatide at 0.2 mL kg^{-1} and 0.1 mL kg^{-1} body weight respectively and fertilized in triplicate. Hatchlings of each cross were stocked at 500 hatchlings per plastic bowls in a completely randomized design and reared on decapsulated *Artemia* for four weeks. The percent fertilization was highest (92.67±1.76) in cross EC♂ x EC♀ and was statistically not significant from other crosses ($p > 0.05$). Highest (42.25±3.82) percent hatchability was obtained in cross Hb♂ x EC♀ but significantly not different ($p > 0.05$) from other crosses. Cross (Hl♂ x EC♀) showed significantly ($p < 0.05$) poor survival (35.93±4.23) compared to crosses EC♂ x EC♀ and Hb♂ x EC♀ on decapsulated *Artemia*. Cross EC♂ x EC♀ had the highest weight gain (0.172±0.003), percent weight gain (17171.0±318.93) and specific growth rate (7.99±0.03) with no significant ($p > 0.05$) from other crosses. Reproductive performances of the treatments were good while the offspring from cross (EC♂ x EC♀) had the best survival and growth performance.

Key Words: induced spawning, paternal hybrids, fertilization, hatchability, decapsulated *Artemia*, growth performance.

Introduction. Fish has been reported (FDF 2007) to be an important and the cheapest source of animal protein which accounts for about 37% of Nigeria's total protein requirement. Meanwhile it is undisputable that aquaculture has been deemed the only alternative for high quality protein production to meet the nutritional needs of the ever increasing world population because capture fisheries are showing precipitous decline due to over fishing, habitat destruction, and pollution (Eyo 2001; Dunham et al 2001; Olufeagba et al 2007; Adewumi & Olaleye 2011).

According to Jimoh et al (2010), Adebayo & Popoola (2008) and Potongkam & Miller (2006), fingerlings production and availability of quality fish feeds have been the bane of fish farming development in Nigeria for the past four decades and stressed the need for increased production of fingerlings to meet the ever rising fish demand.

One of the major steps in solving this problem is through hybridization which has been recognized as a tool for stock improvement and management purposes (Owodeinde et al 2012).

Moreover the importance of fish hybridization is to increase growth rate; enhance productivity through hybrid vigor and transfer desirable traits by reducing unwanted production in a production system has been emphasized (Tave 1993; Purdom 1993).

In line with these, much work have been done on intergeneric hybridization of pure *Clarias gariepinus* or *C. anguillaris* with *Heterobranchus spp*. by several researchers (Akinwande et al 2012; Nwadukwe 1995; Adeyemo et al 1994; Madu et al 1991; Hecht &

Lublinkhof 1985) but little or no work is done on hybridization of Exotic *Clarias* which is, according to Nwafili & Tianxiang (2007), a hybrid of two species of *C. gariepinus* (East Africa) and *C. anguillaris* (West Africa) with *Heterobranchus spp*. Therefore this work examined productive potentials in terms of reproductive performance (fertilization and hatchability), survival and growth potentials of the hybrid of male *Heterobranchus longifilis* and *H. bidorsalis* with female Dutch strain of *Clarias*, in the hatchery system in order to proffer solution to farmers who indiscriminately carryout crosses without following the methodological requirements of hybridization and proper identification.

Material and Method

Experimental site. The experiment was conducted from 7st of August to 20th of September 2012 at the Fish Hatchery of the Department of Forestry and Fisheries. The site is located on latitude 13^{o}07'78''N, and longitude 05^{o}12'25''E at 275 m above sea level (Google Earth 2011) in Usmanu Danfodiyo University, Sokoto.

Collection of breeders. The breeders were collected from Tee-Jay Fish Farm in Ajibesin Village, Ogidi, Ilorin, Kwara State Nigeria. The fish were transported to Sokoto in Plastic Jerry-cans after which they were acclimatized for two (2) weeks in a 1.5 m x 1.7 m x 1.7 m Breeders' Holding Tank of the Fish Hatchery. The breeders were fed a 6.0 mm Coppens commercial feed at 3% of their body weight, following the procedure of Dada et al (2010).

Species identification and selection of breeders. Four breeders consisting in males of *Heterobranchus longifilis, H. bidorsalis*, and Exotic Dutch *Clarias* and a female of Exotic Dutch *Clarias* were used for the experiment (Figures 1-3). The fish were identified using the description of Olaosebikan & Raji (2004) and the gravid female breeder was selected using procedure of Viveen et al (1985).

Figure 1. *Heterobranchus bidorsalis*.

Figure 2. Exotic Dutch *Clarias*.

Figure 3. *Heterobranchus logifilis*.

Hormonal treatment. Ovatide synthetic hormone was used for this study. The hormone is a low viscous liquid containing 20 µg Salmon GnRH and 10 mg of dopenridone, which is a dopamine antagonist. The hormone's recommended dose by the manufacturer is 0.2 mL kg^{-1} of female's body weight. Ovatide is manufactured by Hemmo Pharmaceuticals Pvt. Ltd.

Collection of milt and eggs and fertilization procedures. Collection of milt and eggs and subsequent dry fertilization were accomplished using the procedure of Viveen et al (1985).

Care of larvae. Care of hatchlings commenced immediately first hatchling was observed. Hatchlings were separated from deformed larvae, and general sanitation was done by siphoning, using 1.5 mm Rubber Hose.

Feeding experiment. *Artemia* nauplii were used in the experiment for four weeks, between 22nd of august and 20th September 2012. Three (3) days old Swim-up fry of the following crosses: Exotic *Clarias* (♂) x Exotic *Clarias* (♀) (Treatment I), *Heterobranchus bidorsalis* (♂) x Exotic *Clarias* (♀) (Treatment II), *Heterobranchus longifilis* (♂) x Exotic *Clarias* (♀) (Treatment III). Feeding was done ad-libithum in each experimental unit four times daily as follows: 9:00 am, 1:00 pm, 5:00 pm, and 9:00 pm. Uneaten feed were usually siphoned-out before each feeding, while the water volume is balanced after siphoning. About 70-80% renewal of water was made every morning, and the bowls carefully mopped with soft foam in order to remove dirt from the medium. Total renewal and washing of bowls were done weekly.

Experimental design and set-up. Each cross constituted a treatment in the study, and each treatment was replicated thrice in a completely randomized design (CRD), which makes a total of nine (9) experimental units. Nine (9) plastic bowls (30 litres water capacity) of the same colour were used for the experiment. Each experimental unit was stocked with 500 fish fry (three days old). Each bowl was filled with water up to 20 litres capacity. Stocking density (25 fry/m^2) was used for optimum growth and survival according to Sahoo et al (2005). Aeration was accomplished using a Resun Air Pump (Model ACO-008).

Monitoring of growth and mortality. Daily mortality rates were monitored. Changes in weight, and total length of fry were measured weekly. The initial total weight and total length of fish for each experimental unit were measured using JT210N Electronic Top Loading weighing balance, and a Plastic Ruler (30 cm), respectively. This was done by siphoning the fish fry in a bowl after which they were counted directly using a Plastic Strainer. The fish fry were then put in a linen cloth and quickly transferred into a pre-weighed plastic bowl containing water using feather. The length in millimeters was obtained by randomly collecting some fry from each experimental unit, and the body length measured.

Water quality parameter measurement. Two water quality parameters (pH and temperature) were monitored in this study. The pH was measured once daily while temperature was measured in the morning, afternoon, and evening. The measurement was carried-out for each experimental unit. The pH readings were taken using Jenway

3015 pH Meter. Temperature was measured using Mercury in Glass Thermometer which ranged from 0°C-100°C, calibrated at 1°C interval.

Data analytical tools. Spawning fecundity, stripping percentage, percentage fertilization, and hatchability were recorded for each treatment. The length, weight, and survival recorded weekly were used to calculate growth, survival, and condition factor as follows:

Spawning fecundity. The total number of stripped (spawned) eggs was estimated by counting number of eggs in 1 g of egg mass, and multiplied by the weight of stripped eggs according to Sahoo et al (2005).

Stripping percentage. This was calculated according to Brzuska (2003) as follows:

$$stripping\ percentage = \frac{weight\ of\ stripped\ eggs}{body\ weight} \times 100$$

Relative fecundity. This was calculated as described by Billard (1990) in Kahkesh et al (2010) below:

$$Relative\ fecundity\ = \frac{number\ of\ stripped\ eggs}{body\ weight} \times 100$$

Percent fertilization. To determine this, 50 eggs were taken from each experimental unit about 20 minutes after fertilization. The eggs were observed under Kyowa Electronic Microscope (Model: XSZ-21) at 40 magnification, translucent eggs containing embryonic eyes were counted as fertilized; while opaque eggs were considered unfertilized. This was then calculated according to Adebayo & Popoola (2008) as follows:

$$percent\ fertilization\ = \frac{number\ of\ fertilized\ eggs}{total\ number\ of\ eggs\ counted} \times 100$$

Percent hatchability. Hatchability was determined by direct counting of 100 fry in each experimental unit to obtain a known weight. The total numbers of hatchlings were then estimated using gravimetric method. It was calculated as in Akinwande et al (2012) as follows:

$$percent\ hatchability\ = \frac{number\ of\ hatchlings\ (two\ day\ old)}{total\ number\ of\ eggs\ fertilized} \times 100$$

Survival rate. It was calculated after Akinwande et al (2012):

$$survival\ rate\ (\%)\ =\ \frac{Ni - Nf}{Ni} \times 100$$

Where N_i = initial number of fish at the beginning of experiment; N_f = final number of fish at the end of experiment.

Weight gain (WG). The weight gain recorded was computed according to Sveier et al (2000):

$$(WG) = Final\ weight\ (g) - Initial\ weight\ (g)$$

Percent weight gain (PWG). This was calculated as follows (Olvera-Novoa et al 1990):

$$PWG = \frac{final\ mean\ weight\ (g)\ - initial\ mean\ weight\ (g)}{initial\ body\ wieght}$$

Specific Growth Rate (SGR). This was calculated according to Castell & Tiews (1980):

$$SGR\ (\%) = \frac{\log_e W_f - \log_e W_i}{times\ (days)} \times 100$$

Where; Log_e = Natural logarithm; W_i = initial weight (g) of fish at the beginning of the experiment; W_f = final weight (g) of fish at the end of the experiment.

Condition factor (K). Condition factor (K) of fish fry in each experimental unit was calculated at the beginning, and at the end of the experiment adopting the procedure of Bagenal & Tesch (1987):

$$K = \frac{100W}{L^3}$$

Where: W = weight of fish (g); L = total length of fish (cm).

Statistical analysis. Data collected on fertilization, hatchability, survival and growth were subjected to analysis of variance (ANOVA), and means were separated using New Duncan's Multiple Range Test (DMRT) (Gomez & Gomez 1984). The analysis was carried-out using the SPSS V: 16.0 (2007) package for Windows.

Results and Discussion. The results on hormone administration, brood stock body weight, eggs weight, and quantity of hormone administered were presented in Table 1. The female weighed 3.1 kg and was administered 0.62 mL of ovatide (0.2 mL kg^{-1}). The size of the male and female breeders ranged from 0.9-3.1 kg and were above the recommended minimum size of 0.5 kg gravid *Clarias gariepinus* (Viveen et al 1985).

The eggs were mature, shiny light-green and adhesive. Latency period of 9 hours was observed at a mean temperature of 29±0.40^{o}C. The latency period of 9 hours and incubation period of 21 hours at 29^{o}C and 28.5^{o}C respectively agrees with that of Nwadukwe (1995) where latency period of 7-10 hours was observed at 26-29^{o}C with carp pituitary extract. The 21 hours incubation period of this study agrees with the report of Ajana & Anyanwu (1995) who observed 16-22 hours incubation period at a mean temperature of 30^{o}C and Aluko et al (1994) who reported incubation at 22 hours at temperature of 24^{o}C which indicates the dependence of embryonic development on temperature. The difference in incubation period might be due to difference in temperature and efficacy of the hormone.

Spawning fecundity was 27,100, while stripping percent was 11.77%. This agrees with Khan et al (2006) and Sahoo et al (2005) who recorded high fecundity on *Clarias gariepinus, C. batrachus* and *Labeo rohita,* respectively when Ovatide was induced at 0.2 mL kg^{-1}.

The result on percent fertilization and hatchability (Table 2), showed no significant difference ($p > 0.05$) among all the treatments with the highest 92.67±1.76 in treatment I (EC ♂ x EC ♀). This showed that fertilization was successful among the treatments. The high rate of fertilization obtained in this study was appreciable than 87.5% highest percent fertilization for parental crosses of *C. gariepinus* using Ovaprim synthetic hormone recorded in Akinwande et al (2012).

The percent hatchability was generally low among the treatments and there was statistically no significant difference ($p > 0.05$) among treatments, it was however highest in treatment II (Hb ♂ x EC ♀) 42.25±3.82, while treatment III (Hl ♂ x EC ♀) 27.16±4.93 recorded the lowest percent hatchability. The low trend of hatchabilty in this experiment considerably differed from other studies, where hatchability of 79% using Ovaprim was recorded for *C. gariepinus* (Abubakar et al 2013) in the same study area. Lower hatchability was however observed by Nwadukwe (1995) for Hl ♂ x EC ♀ where percent hatching ranged between 40-71%. The higher fertilization rate and the consequent low hatchability could be as result of the overriped eggs observed which might have resulted in egg mortality after fertilization. This was similar to the finding of Sahoo et al (2005). It is however important to acknowledge that differences that arise

from breeding history, age and water quality according to Ataguba et al (2009) can affect hatchability in this experiment.

The result of survival rate showed that treatment I and II differed from treatment III where the lowest survival rate (35.9±4.23) was obtained (Table 3). This observation differed from the study of Olurin & Aderibigbe (2006), and Akinwande et al (2012) who recorded higher values (80% and 86% respectively) for *C. gariepinus,* and 70% for hybrid crosses of male *H. longifilis* and female *C. anguillaris* (Akinwande et al 2012). The lower survival rate in treatment III might be due to high cannibalism that was recorded. The general lower survival rate in this study can be attributed to mortalities resulting from the weekly sampling stress since the fry were very fragile at this stage.

The weight gain, percent weight gain, body length increase and the specific growth rate in this study (Table 3) showed declining trend along crosses of EC ♂ x EC ♀, Hl ♂ x EC ♀ to Hb ♂ x EC ♀, indicating better growth performance in treatment I (EC ♂ x EC ♀) but the cross Hl ♂ x EC ♀ compete favourably with cross (EC ♂ x EC ♀) in weight gain and percent weight gain with no statistical significance ($p > 0.05$). The highest values recorded for weight gain, percent weight gain, and specific growth rate for treatment I (EC ♂ x EC ♀) agreed with the findings of Ataguba et al (2009) who observed 10.57±0.835g weight gain, and 13.70±0.167 specific growth rate (SGR) for offspring of *C. gariepinus* in 15 days. Ataguba et al (2009) recorded 7.05±0.530 mg weight gain, and 11.43±0.502 specific growth rate for *H. longifilis* (male) cross with *C. gariepinus* (female). But in variant with the finding of Madu & Aluko (1999) who observed better (13.6) mean specific growth rate for offspring of male *H. longifilis* when crossed with female *C. anguillaris* in 3 weeks when compared with (12.7) SGR of pure line *C. anguillaris*. The growth variation observed in this study however could be attributed to environmental factors and genetic incompatibility of the Exotic Dutch *Clarias* with the two *Heterobranchus* species used for crossing. The lowest survival rate recorded for treatment III (Hl ♂ x EC ♀) might have given them space advantage and surface area which resulted in better growth performance than in treatment II (Hb ♂ x EC ♀).

The initial condition factor (0.51±0.02) was better in (EC ♂ x EC ♀), The mean initial total length (0.58±0.01) was found to be significantly higher in treatment I (EC ♂ x EC ♀) when compared to other treatments which might have resulted from the phenotipic impact of the male Exotic *Clarias* which was longer than the male *H. bidorsalis* and *H. longifilis* respectively (Table 4). The final condition factor however took a different dimension where treatment III (Hl ♂ x EC ♀) showed a better (0.59±0.06) well being which could be attributed to the low survival rate recorded at the end of the experiment.

The mean water temperature and pH values recorded (Table 5) during the experimental period were within the temperature (25^oC-32^oC) and pH (6.5-9) range recommended by Boyd & Lichtkoppler (1979). The relative stability of minimum and maximum morning, afternoon and evening temperatures (Table 5) could also have influenced the better growth performance recorded in the whole treatment.

Table 1

Summary of induced ovulation and spawning operation

Parameter	*Female Exotic Clarias*	*Male Exotic Clarias*	*Male H. bidorsalis*	*Male H. longifilis*
Body weight (kg)	3.1	1.6	1.5	0.9
Weight after stripping (kg)	2.65	-	-	-
Quantity of hormone administered (ml)	0.62	0.16	0.15	0.08
Latency period (hours)	9	9	9	9
Weight of stripped eggs (g)	365			
Nature of eggs ovulated	Matured and golden light-green			
Spawning fecundity	27,100			
Stripping percent	11.7			
Mean temperature during ovulation (^{o}C)	29±0.05			
Mean temperature at incubation (^{o}C)	29.9±0.4			

Table 2

Percent fertilization and hatchability of the different crosses

Parameter	*Treatment I (EC ♂ x EC ♀)*	*Treatment II (Hb ♂ x EC ♀)*	*Treatment III (Hl ♂ x EC ♀)*
Estimated numberof eggs	3700	3700	3700
Duration of incubation (hours)	22	22	22
Estimated number of hatchlings	3904	4690	3015
% Fertilization	92.67±1.76	88.67±5.2	89.33±1.76
% Hatchability	35.17±4.10	42.25±3.82	27.16±4.93

Means in row are not significantly different.

Table 3

Summary of survival and growth performance of fry at the end of feeding with decapsulated *Artemia*

Parameter	*Treatment I (EC ♂ x EC ♀)*	*Treatment II (Hb ♂ x EC ♀)*	*Treatment III (Hl ♂ x EC ♀)*
Duration of experiment (days)	28	28	28
Total initial fish number	1500	1500	1500
Total final fish number	764	760	539
Initial body weight (g)	0.5±00	0.65±0.03	0.69±0.11
Initial body length (cm)	0.58 ± 0.01^{a}	0.39 ± 0.01^{b}	0.39 ± 0.00^{b}
Mean length increase (cm)	2.22±0.16	2.13±0.12	2.19±0.06
Final body length (cm)	2.77±0.06	2.53±0.12	2.61±0.16
Mean weight gain (g)	0.172 ± 0.003^{a}	0.109 ± 0.003^{b}	0.148 ± 0.047^{ab}
Mean percent weight gain	17171.0 ± 318.93^{a}	8350.0 ± 3316.4^{b}	11749.0 ± 577.7^{ab}
Mean specific growth rate	7.99 ± 0.03^{a}	6.87 ± 0.11^{b}	7.26 ± 0.47^{ab}
Initial condition factor	0.51 ± 0.02^{b}	2.15 ± 0.14^{a}	2.29 ± 0.41^{a}
Final condition factor	0.81 ± 0.03^{a}	0.68 ± 0.04^{ab}	0.59 ± 0.06^{b}

Means in row with superscript are significantly different ($p < 0.05$).

Table 4

Morphometric measurement of male breeders

Feature	*Abbreviation*	*Length (cm) H. bidorsalis*	*Length (cm) H.longifilis*	*Length (cm) Exotic Clarias*
Total length	TL	62	49	68
Standard length	SL	54	42.5	60
Predorsal distance	PDD	2.3	3.5	2.5
Dorsal fin length	DFL	22	14.3	40
Anal fin length	AFL	26	17.3	28
Pectoral fin length	PFL	5.5	6.3	8
Pectoral spine length	PSL	5	5	5
Distance between dorsal and caudal fin	DDCF	0.6	4	3
Distance between occipital process and dorsal fin	DODF	5	5	5
Caudal peduncle depth	CPD	3.5	3.7	4
Body depth at anus	BDA	6.5	5.7	7.5
Head length	HL	15.5	13	16
Head width	HW	11	8	9
Snout length	SNL	8.5	6	8.5
Inter orbital distance	ID	8	5.2	6.3
Eye diameter	ED	0.9	0.6	0.6
Length of occipital fontanelle	OFL	4.5	0.3	3
Width of occipital fontanelle	OFW	0.8	0.4	0.4
Adipose fin length	ADFL	12	9	-
Space between dorsal and adipose fin	SBDAF	1.5	4	-
Dorsal ray count	DRC	44	27	57

Table 5

Average water quality values during the experiment

Temperature (^oC)	*Morning*	*Afternoon*	*Evening*	*pH*
Minimum	27.00	28.00	27.00	7.10
Maximum	30.50	31.50	31.00	7.80
Mean (±SE)	28.70±0.73	29.80±0.64	29.20±0.72	7.44±0.14

Conclusions. The findings show that the intergeneric crossing of female Dutch Exotic *Clarias* with male *H. bidorsalis and H. longifilis* will produce offspring poor survival and growth performance when compared with the pure line Exotic Dutch *Clarias* which has superior performance.

Acknowledgements. Thanks goes to the head of Unit Fisheries Programme for giving us all the support needed to use the available facilities in the hatchery.

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Received: 23 July 2013. Accepted: 17 October 2013. Published online: 12 November 2013.
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How to cite this article:
Ipinjolu J. K., Abubakar M. Y., Magawata I., Buko M. I., 2013 Reproductive, survival and growth performance of intergeneric cross of Exotic Dutch *Clarias*, *Heterobranchus bidorsalis* and *Heterobranchus longifilis* in Sokoto North-West Nigeria. AACL Bioflux 6(6):571-581.