GROWTH PERFORMANCE AND SEX RATIO IN INTERSPECIFIC AND INTERGENERIC HYBRIDS OF THREE *Clariid* CATFISH SPECIES

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ABSTRACT

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The growth performance and sex ratio the intergeneric and interspecific hybrids of three Clariid catfishes namely; Heterobranchus longifilis, Clarias gariepinus and Clarias anguillaris were studied (October 2009 - October 2010) in experimental earthen ponds of the Federal College of Freshwater Fisheries Technology, New-Bussa, Niger State. Six weeks old fingerlings (3.6±1.12 g) of the various nine mating combinations produced by hypophysation and artificial fertilization were stocked in triplicates in earthen ponds of 4m2 for one year (365 days). The intergeneric hybrids of Heterobranchus longifilis male (♂) x Clarias gariepinus female (♀) had the highest specific growth rate (0.4%) and mean weight gain (240.42 g) which was significantly different ($p<0.05$) from the other mating combinations. The intergeneric hybrids with male H. longifilis had higher weight increase. This suggested that, there was much paternal influence of H. longifilis when crossed with female Clarias species. Offspring's of Clarias gariepinus (♂) x H. longifilis (♀) also had the highest survival (93%) which was however not significantly different ($p>0.05$) from 88% recorded in H. longifilis male (♂) x Clarias gariepinus female (♀). The interspecific hybrids of C. gariepinus and C. anguillaris also showed improved growth performance over the parental C. anguillaris. The intergenric hybrids with male Clarias have sex ratios of 1: 2.53 and 1: 2.27 which significantly deviated from the expected 1:1 common to the other mating combinations. Artificial propagation of the interspecific hybrid of C. gariepinus (♂) X C. anguillaris (♀) and the intergeneric hybrids with male H. longifilis may further improve the aquaculture productivity of these Clariid fishes in Nigeria.

Keywords: *Clariid catfish, Hybridization, Sex ratio,*

INTRODUCTION

The development of experimental tools for studying physiology, clarification of taxonomic relationship, the production of genetic variance for selection programme, the control of sex ratio and production of superior fish for culture can be achieved through logical approaches to hybridization (Purdom, 1993). The success of interspecific hybridization may vary and this depends on the genetic structure, mating combination, gamete compatibility and gene flow pattern of the parental species. A non-genetic factor which includes selection, handling, and breeding also influences hybridization success (Rahman *et al*., 2013). The development of hybrids between superior stocks of *O. niloticus* and *O. mossambicus* has the potential to combine the greater growth potential of the former with the saline tolerance of the latter and thus does appear to represent a potential commercial application for hybridization in brackish water aquaculture. Hybrids of Florida red- strain hybrid (O. *mossambicus* x *O*. *Urolepis hornorum*) (Ernest *et al*, 1991) and O. *niloticus* x *O. aureus hybrids* (Lahav and Lahav, 1990; Wohlfarth, 1994) also show enhanced salinity tolerances. Cross breeds of different strains of European catfish (*Silurus glanis*) also exhibited higher adaptability under warm water conditions and mixed feeding regimes (Bartley *et al*., 2001). In crossbreed of the strains of the Chum salmon, there was however no heterosis effect for growth compared to the parental species (Dunham, 1996). Hybridization between some species, such as *O. niloticus* and *O. aureus*, results in predominately male offspring (Rosenten and Hulata, 1994). Other tilapia crosses that produce mainly male offspring include, *O. niloticus x O. urolepis hornorum* or *O. macrochir* and *O. mossambicus x O. urolepis hornorum* (Wolhfarth, 1994). Conversely, the cross between striped bass and yellow bass produced 100% females (Wolters and Demay, 1996). This can be desirable for culture purposes where there are; growth differences between the sexes; sex- specific products such as caviar are wanted; or reproduction needs to be controlled like in overpopulation and stunting in tilapia production ponds. Hybridization between species can also result in offspring that are sterile or have diminished reproductive capacity (Dunham, 2001). As with monosex production, the production of sterile hybrids can reduce unwanted reproduction or improve growth by energy diversion from gametogenesis Sterile hybrids or those with reduced fertility have been reported between in the Black crappie x White crappie (Hooe *et al*, 1994) and the Red sea bream x Gilt head sea bream (Hulata,1995). The hybrid between African (*Clarias gariepinus*) and Thai (*C. macrocephalus*) catfish combines the fast growth of the African catfish and the desirable flesh characteristics of the Thai catfish (Na-Nakorn, 2004). The hybrid cross between *Heterobranchus* and *Clarias* is receiving considerable attention in Africa particularly, Nigeria. These hybrids have been reported to show heterosis (Aluko, 1998; Nlewadim, 2002; Diyaware and Onyila, 2014). Eight fast growing and viable intergeneric hybrids of these catfish species which showed superior growth over the parent up to the fingerlings stage (Aluko, 1999) but their suitability for aquaculture has not been thoroughly evaluated. Detailed and extend study on the growth and

survival of the interspecific hybrids of *C. gariepinus* and *C.anguillaris* at the grow-out stage has not been carried out. However, the viability of its production (Akinwande *et al*, 2011) and the growth from the fry to fingerlings stage (Akinwande *et al*, 2012) has been reported. This present study aimed at assessing the growth performance and sex ratio of the interspecific and intergeneric hybrids of *H. longifilis*, *C. gariepinus* and *C. anguillaris* at the grow- out stage for one year in order to know the aquaculture potentials of these various catfish hybrids.

MATERIALS AND METHODS

Study area

The studies were conducted between October 2009 - October 2010 at the Federal College of Freshwater Fisheries Technology New-Bussa in Borgu Local Government Area of Niger State (9^o 53'N 4^o 31' E).

Collection of broodstocks and artificial Induced breeding

The parental broodstocks used in this study; *C. gariepinus, C. anguillaris* and *H. longifilis* were obtained from the concrete tanks of the Fish Genetic Improvement Unit of the National Institute for Freshwater Fisheries Research, New Bussa and taken to the Hatchery Complex of Federal College of Freshwater Fisheries Technology. Sexually matured males (with turgid reddish genital papillae at its tip and gravid females(740g-820g), which oozes out greenish-yellow eggs on gentle application of pressure on the abdomen were selected. Hypohysation and artificial fertilization were carried out as described by Akinwande *et al*, (2011) to produce the following mating combinations ; male (♂) x female (♀)

Clarias gariepinus (♂) x *Clarias gariepinus* (♀)
Clarias anguillaris (♂) x *Clarias anguillaris* (♀)
Heterobranchus longifilis (♂) x *Heterobranchus longifilis* (♀)

The mating combinations were separated into interspecific and intergeneric hybrids as follows:

Interspecific hybrids
Clarias gariepinus (♂) x *Clarias anguillaris* (♀)
Clarias anguillaris (♂) x *Clarias gariepinus* (♀)

Intergeneric hybrids
Clarias gariepinus (♂) x *Heterobranchus longifilis* (♀)
Heterobranchus longifilis (♂) x *Clarias gariepinus* (♀)
Clarias anguillaris (♂) x *Heterobranchus longifilis* (♀)
Heterobranchus longifilis (♂) x *Clarias anguillaris* (♀)

Hybrids and parents were produced on the same day in each experiment and were raised indoor in aerated tanks for six weeks.

Experimental set up

Initial pooled weight of six weeks old fingerlings (3.6±1.12 g) from each mating combination was taken using an Acculab sensitive weigh balance calibrated to nearest 0.1 g. Sixty (60) fingerlings from each mating combinations were stocked outdoor in earthen ponds (2m x 2m x 1.m) at a stocking density of 5 fish/m^2 (20 fingerlings/tank) in triplicate batches from each mating group. The ponds were initially fertilized with chicken manure, filled to 70cm of its height two week earlier before stocking and fenced with mosquito net to prevent predators from entering the ponds. One third of the water volume in each pond is pumped out weekly and refreshed with clean bore-hole water. Fingerlings were fed with 40% crude protein commercial diet (Table 1) twice daily at 5% of their biomass, while monthly weight were also recorded for twelve (12) months of grow-out studies in the earthen pond.

The monthly survival of the offspring of the various mating combinations was also recorded. Growth parameters were estimated using the following formulae according to Fagbenro, (1996);

$$Weight\ gain(WG) = (Wf - Wi)g$$

$$Specific\ growth\ rate(SGR)\% = \frac{LgWf - LogWi}{t} x100$$

$$Survival(\%) = \frac{Ni - Nf}{Ni}\ x100$$

Where Wf = Mean final body weight of fish, Wi = Mean initial body weight of fish, t = Number of days of experiment, Nf = final number of fish at end of experiment and Ni= initial number of fish at beginning of experiment.

Sex ratio of offspring of the various mating combinations

The total number of fish from the experimental ponds containing the product of each mating combination was collected at end of the twelve month of growth studies. The sex was determined by visual examination of the genital papilla and the number of males and females in each mating combination were recorded.

Statistical analysis

Variations in the data generated from parameters in the various mating combination were evaluated using One Way Analysis of variance (ANOVA). Significant different treatment means were separated at 5% probability level using Ducan multiple Range Test. Chi square analysis was also used to test if there is any association between the mating combinations and sex of the offspring produced as well as to test whether the male and female ratio deviated from the expected (1:1).

RESULTS AND DISCUSSION

The percentage composition of the feed ingredients fed the fishes during the experimental period is shown in Table 1. At the end of twelve (12) months of grow-out studies, *C. gariepinus* attained the highest mean weight gain of 214.42 g among the parental group (Table 2.) which was significantly different ($p<0.05$) from that of *C. anguillaris(*166.4g) and *H. longifilis(*155.4 g). The offspring of *C. gariepinus* also had the highest weight gain/day of 0.59g compared to 0.46 g in *C. anguillaris* and 0.43g for *H. longifilis*. The mean weight gain and weight gains per day in the reciprocal interspecific hybrids were not significantly different ($p>0.05$). Considering all the offspring of the various mating combinations, the intergeneric hybrids of *H. longifilis* (♂) x *C. gariepinus* (♀) had the highest mean final weight of 240.2g followed *C. gariepinus* (♂) x *H. longifilis* (♀) while *H. longifilis* had the least final mean weight among the offspring of the nine mating combinations (155.4g). *C. gariepinus* also had the highest specific growth rate (SGR) of 0.5% compared to *C. anguillaris* (0.46%) and *H. longifilis* (0.45%) and is not significantly different ($p>0.05$) among offspring of the parentals. The interspecific hybrid of *C. gariepinus* (♂) X *C. anguillaris* (♀) had a SGR value of 0.47% which was non-significant from 0.46% obtained in its reciprocal C. *anguillaris* (♂) X C. *gariepinus* (♀) hybrid (Table 2.0). Amongst the intergeneric hybrids, *H. longifilis* (♂) X *C. gariepinus* (♀) had the highest SGR value of 0.49% and the least value was recorded in *C. anguillaris* (♂) X *H. longifilis* (♀). In all the nine mating combinations, the offspring of the parental *Clarias gariepinus* had the highest SGR value (0.50%) and least value of 0.45% in *H. longifilis*.

Table 1: Composition of experimental diet (40% crude protein) fed the fishes during the experimental period

Ingredients	Composition (%)
Fish meal	15.43
Soya bean meal	31.10
Groundnut cake	28.26
yellow maize	23.21
Vegetable oil	1.00
Vitamin premix	1.00

Table 2: Growth performance of offspring of the various mating combinations at the end of twelve months of growth studies

Mating combinations	Initial mean weight (g)	Mean weight gain (g)	Final mean weight (g)	Specific growth rate (%)	Weight gain day^{-1}
Parentals					
C. g (♂) x *C.g* (♀)	3.40a	214.42b	217.82b	0.50 a	0.59ab
C. a (♂) x *C.a* (♀)	3.12a	166.4a	169.53a	0.46 a	0.46a
H. l (♂) x H. l (♀)	3.20 a	155.4a	158.64a	0.45a	0.43a
Interspecific hybrids					
C. g (♂) x *C. a* (♀)	3.36a	172.4a	175.7ab	0.47 a	0.47a
C. a (♂) x C. g (♀)	3.18 a	168.3a	171.48ab	0.46 a	0.46a
Intergeneric hybrids					
H. l (♂) x *C. g* (♀)	4.62b	240.42c	245.04c	0.49a	0.66b
C. g (♂) x *H. l* (♀)	4.30ab	226.4bc	230.70c	0.48a	0.62b
H. l (♂) x *C. a* (♀)	4.12ab	218.74b	222.86bc	0.48a	0.60ab
C. a (♂) x *H. l* (♀)	3.21a	177a	180.21ab	0.47a	0.49a

$^{a\,b\,c}$ –values with different superscripts in a column are significantly different from each other ($p<0.05$). *C.g- Clarias gareipinus*, *C.a- Clarias anguillaris*, *H.l- Heterobranchus longifilis*

The result of this growth studies at the end of 365 days rearing period reveals that offspring of the intergeneric hybrids of *H. longifilis* (♂) X *C. gariepinus* (♀) had the best mean weight gain. The offspring of the interspecific hybrids had higher weight gain and specific growth rate over one of the parentals; which is *C. anguillaris*. In an interspecific hybridization study carried out by Sahoo *et al*, (2003), hybrids of C. *batrachus* (♂) x *C. gariepinus* (♀) and *C. gariepinus* (♂) x *C. batrachus*(♀) hybrids reached average weight of 550.71 g and 341 g respectively at six month of culture, which were higher compared to the final body weight of the parental *C. batrachus*

(69.1g). Similar result also was obtained in the growth performance of the interspecific hybrid of *C. gariepinus*(♂) *a*nd *C.anguillris*(♀) at the fry to fingerlings stage (Akinwande *et al*., 2012) The result of this present study further suggests that the aquaculture potntials of *C. anguillaris* can be improved upon by producing its interspecific hybrids for grow-out purpose. The advantage of the increase in growth rate of the interspecific hybrids over the parental *C. anguillaris* in pond culture indicates a lucrative and promising catfish culture. Results obtained in the study also revealed that two other intergeneric hybrids; *C. gariepinus* (♂) X *H. longifilis* (♀) and *H. longifilis* (♂) X *C. anguillaris* (♀) were second best in mean weight gain when all the mating combinations were compared together. This shows that there was so much paternal influence of *H. longifilis* when crossed with female *Clarias spp*. Several intergeneric hybrid crosses of *H. bidorsalis* and *C. gariepinus,* hybrids with paternal *Heterobranchus* had similarly been reported to perform better than those with paternal *Clarias* (Aluko and Ali, 2001; Nlewadim, 2002; Diyaware and Onyia, 2014). Intergeneric hybrids between the red sea bream and common dentex also exhibit faster growth in cage culture (Colombia *et al*, 1998). Heterosis for growth in hybrid fishes depend on several conditions, such as the type of species crossed, culturing environment, type of fish feed and other management strategies. Low mean weight was attained in the offspring's of various mating combinations (158.64 g - 245.04 g) in this study and this could be due to the stocking density (5 fish per m^2) of fishes in this study which is relatively high for catfish culture in earthen ponds and also properly due to the culture method adopted in this study, which was a partial flow-through system in which the pond water was partially refreshed once in a week.

The survival of the offspring of all the nine mating combinations at the end of 12 month of grow-out studies are shown in Figures 1, 2 and 3. Among the parental mating combinations; *C. gariepinus* and *C. anguillaris* recorded the same highest survival (85%) which was significantly different ($p<0.05$) from the value obtained in *H. longifilis* (76.3%). Offspring of *C anguillaris* however had more relatively stable survival rate from the second month of stocking till the end of twelve month.

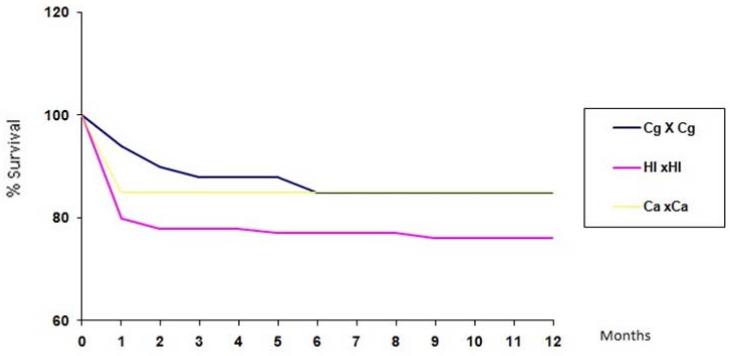

Fig 1: Monthly survival of the offspring of the parentals in grow-out earthen ponds

The interspecific offspring of *C. gariepinus* (♂) X *C. anguillaris* (♀) had higher survival of 84% when compared to 78% obtained from its reciprocal *C. anguillaris* (♂) X *C. gariepinus* (♀) hybrid cross, which was however not significant ($p>0.05$).

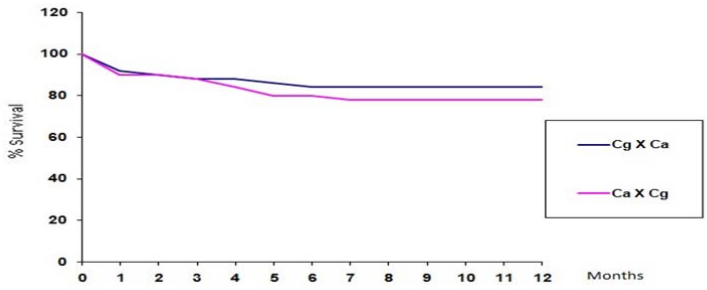

Fig 2: Monthly survival of the offspring of the interspecific hybrids in grow-out earthen ponds

A comparison of the intergeneric hybrids shows that they exhibited varying degrees of survival rate as shown in Fig 3.0. Offspring of *C. gariepinus* (♂) X *H. longifilis* (♀) had the highest value of 93%, followed by *C. anguillaris* (♂) X *H. longifilis* (♀) with 90%. The survival rates of these two mating combinations were not

significantly different (P<0.05) from 88% obtained in *H. longifilis* (♂) X *C. gariepinus* (♀). The least survival was obtained in *H. longifilis* (♂) X *C. anguillaris* (♀) with value of 83%.

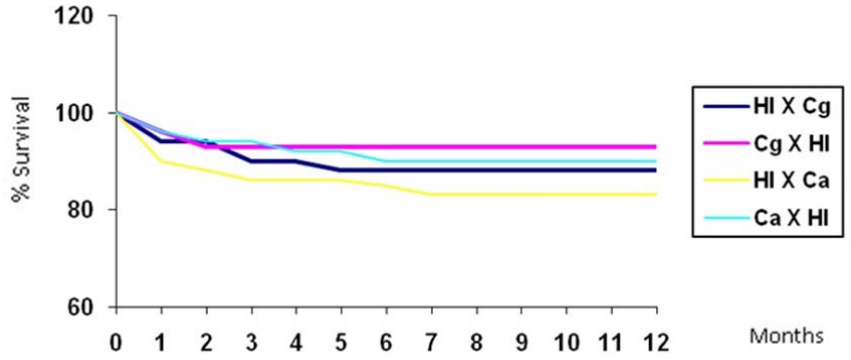

Fig 3: Monthly survival of the offspring of the intergeneric hybrids in grow-out earthen ponds

Considering the survival of all the products of the various mating combinations, the offspring of the intergeneric combination; *C. gariepinus* (♂) X *H. longifilis* (♀) was best in terms of survival at the end of the grow-out period. The offspring were also more stable as they maintained 93% survival from the second month up to the end of grow-out period. The offspring of the parental *H. longifilis* had the least survival (76.3%). The survival of *H. longifilis* fell as low as 80% even in the first month indicating the fact that they could not quickly adapt very well in the outdoor earthen ponds immediately after stocking (Fig 1.). The survival rate of the interspecific hybrids of *C. gariepinus* (♂) X *C. anguillaris* (♀) in this study was very high and not significantly different from the parental which shows the aquaculture potentials of these hybrids.

Chi square (x^2) analysis of the males and females offspring (at 12months) indicated that there was a significant level of association between mating combination and the sexes of offspring produced (P<0.05). An analysis to evaluate if there was any departure from the expected 1:1 ratio of male and female (Table 3 .0) revealed that two intergeneric mating combinations produced offspring with sex ratio that significantly deviated from the 1:1 ratio. These were the products of *C .gariepinus* (♂) x *H. longifilis* (♀) and *C .anguillaris* (♂) x *H. longifilis* (♀) with sex ratio of 1:2.53 and 1:2.27 male to female ratio respectively. All the other mating combinations had off springs that did not depart from the 1:1 male to female ratio.

Table 3: Sex ratio of offsprings of the various mating combinations

Mating combinations	No of males (M)	No of females (F)	Sex Ratio (M:F)
C.g (♂) x *C.g* (♀)		36	1:1.13^{NS}
C.a (♂) x *C.a* (♀)	32	29	1:0.91 ^{NS}
H.l (♂) x *H.l* (♀)	31	37	1:1.19 ^{NS}
C.g (♂) x *C.a* (♀)	30	35	1:1.16 ^{NS}
C.a (♂) x *C.g* (♀)	28	34	1:1.21 ^{NS}
H.l (♂) x *C.g* (♀)	31	39	1:1.26 ^{NS}
C.g (♂) x *H.l* (♀)	21	53	1:2.53*
H.l (♂) x *C.a* (♀)	29	37	1.1:27 ^{NS}
C.a (♂) x *H.l* (♀)	22	50	1 :2.27*

*-significant different, ^{NS}- no significant difference. *C.g- Clarias gareipinus*, *C.a- Clarias anguillaris*, *H.l- Heterobranchus longifilis*

A nominal view of the number of male and female offspring produced, showed there was a general trend towards the production of more females by most of the mating combinations. A significant number of females were recorded from the intergeneric mating combinations with maternal *H. longifilis* in this study. (Nlewadim, 2002) also reported similar result in hybridization in two strains of female C. *gariepinus* which were crossed and male *H. bidorsalis* in which the offspring's had sex ratios of 1:3.71 and 1:18.5. It could therefore be that there is paternal influence of *Heterobranchus* species in the production of more female offspring when crossed with females of other *Clariid* catfishes. Hybridization can lead to production of offspring's with varying sex ratio (Budd *et al*, 2015) and has been reported in several species of Tilapia and in the Bass (Wolter and De–May, 1996). Markers assisted selection has also been used to manipulate sex ratio in Tilapia (Bensten *et al.,* 2012) and Turbot (Martinez *et al*., 2009). According to Baroiller, *et al*, (2001), the molecular mechanism of sexual development in teleost fish is evolutionarily plastic and requires complex regulatory pathways that are governed by genetic factor, environmental cues or on an interaction between the two (genotype x environment interactions).

The applications of genomic approach in recent times haves greatly contributed to the rapid discerning of gene and gene function associated with the sex differentiation in commercial important fish species(Heule *et al*, 2014). In *C. gariepinus,* sex specific marker (*CgaY1* and *CgaY2*) has been identified using randomly amplified polymorphic DNA (Pandian, 1999; Kovac *et al*, 2000). The sex determining mechanism in the *Clariid* catfishes has not been extensively studied and so the genetic basis for having more female in the intergenric hybrids using male *Heterobranchus* species has obtained in this study cannot be deduced.

CONCLUSIONS AND RECOMMENDATIONS

This study show that the intergeneric hybrids of the *Clariid* catfishes has higher growth and survival in the grow-out stage in earthen ponds compared to the parental *Heterobranchus longifilis* , *Clarias gariepinus* and *Clarias anguillaris*. The interspecific hybrid of *C. gariepinus* (♂) X *C. anguillaris* (♀) also had higher growth and survival than the parental *C. anguillaris*. The aquaculture potential of our indigenous *Clariid* catfishes can be improved by artificial propagation of the interspecific hybrid using *C.gariepinus* (♂) X *C. anguillaris* (♀) and intergeneric hybrids having paternal *Heterobranchus longifilis* by fish breeders. Such fingerlings are encouraged to be sold out to fish farmers which will improve their production output and increase profit.

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