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Induced breeding and early growth of progeny from crosses between two African clariid fishes, *Clarias gariepinus* (Burchell) and *Heterobranchus longifilis* under hatchery conditions

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ABSTRACT

Objective: Hybridization of *Clarias gariepinus* (Cl) and *Heterobranchus longifilis* (Ht) and determination of their early growth parameters in the hatchery.

Methodology and results: Four genetic crosses were made. These were (♀ × ♂): Cl X Cl, Ht X Cl, Cl X Ht and Ht X Ht. Triplicate batches of eggs from each cross were fertilized and incubated in 60L plastic aquaria with flow through water system using rubber type mosquito mesh netting as substrate. Variations in hatching rate and survival rates were not significantly different (P>0.05) for the crosses but fertilization rate in the cross Ht♀ X Cl♂ was statistically different from the other crosses (P<0.05, F-LSD). Specific growth rate (SGR) ranged from 11.43±0.502 (Cl♀ X Ht♂) to 13.70±0.167 in the Cl X Cl cross and these differed significantly from each other (P<0.05). In terms of growth, the pure breed Cl X Cl performed better than the other crosses, followed closely by the Ht♀ X Cl♂ cross which performed better than the other hybrid (Cl♀ X Ht♂).

Conclusion and application of findings: Survival rates of larvae up to the first feeding stage are similar in all the genetic groups investigated. The pure *Heterobranchus longifilis* cross (Ht X Ht) gave the highest fertilization and hatching rate in comparison with the other genetic groups while pure breed Cl X Cl performed better than the other crosses in terms of growth. This implies that breeding exercises which involve pure catfishes should be employed more often to maximize fry production.

Key words: African Catfish, hybridization, fertilization, growth rate, survival rate.

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INTRODUCTION

The future of aquaculture lies in production of species that are able to grow fast, adapt to changing climatic conditions and also remain capable of withstanding the rigours of an enhanced production system. Aquaculture is the world's fastest-growing food-producing sector. The sub-sector's expansion began in the 1970s, stimulated by advances in hatchery technology and pond husbandry. According to the FAO (2007), aquaculture continues to grow more rapidly than all 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. The contribution of aquaculture to global supplies of fish, crustaceans, mollusks and other aquatic animals, increased from 3.9% of total production by weight in 1970 to 27.1% in 2000 and 32.4% in 2004. This growth is however impeded by a lack of adequate attention to genetics and selective breeding, leading to stagnating yields (CGIAR, 2006).

Genetic technologies can be utilized in aquaculture for a variety of reasons, not just to improve production but also marketability, culturability and the conservation of natural resources (Bartley, 2002). Biotechnology has opened a new window for development of genetic resources in aquaculture. However, the application of biotechnology and genetics to aquaculture in Africa has been constrained by various factors (Mugabe, 2002; Changadeya *et al*., 2003). Various concerns about its safety have limited its application hence the need to rely on selective breeding and hybridization for the improvement of genetic resources in aquaculture.

Hybridization between some species of tilapias such as *Oreochromis niloticus* x *O. aureus* results in the production of predominantly male offspring and reduces unwanted natural reproduction in grow-out ponds (Rosenstein & Hulata, 1993). Hybridization of the African catfishes *Clarias gariepinus*, *Heterobranchus bidorsalis* and/or *Heterobranchus longifilis* has been reported by Nwadukwe (1995), Nlewadim *et al.* (2004) and Salami *et al.* (1993). The African catfish x Thai catfish hybrid (*Clarias gariepinus* x *C. macrocephalus*) is preferred to the Thai catfish because it has the desired flesh quality of the Thai catfish and the fast growth of the African type (Bartley *et al.,* 1997).

Legendre *et al.* (1992) investigated hybridization of the two African catfishes: *C. gariepinus* and *H. longifilis* and reported viability in reciprocal hybrids with their survival rates being similar to those found in the maternal species. Hybrid morphology was intermediate to that of the parents and no difference in external morphology was observed between the reciprocal hybrids. Both reciprocal hybrids and parental species displayed an equilibrated sex ratio.

This study investigated the fertilization, hatching and survival rates of larvae of cross breeds and pure breeds of two African catfishes. The growth rates of the progeny from the crosses were also studied.

MATERIALS AND METHODS

Broodstock of known breeding records were obtained from the University of Agriculture Makurdi Teaching and Research Farm. Species used include *Clarias gariepinus* and *Heterobranchus longifilis*. Mating combination is in the order (♀× ♂):

1. *C. gariepinus* × *C. gariepinus* (Cl X Cl)
2. *H. longifilis* × *H. longifilisI* (Ht X Ht)
3. *H. longifilis* × *C. gariepinus* (Ht X Cl)
4. *C. gariepinus* × *H. longifilis* (Cl X Ht)

Selection of broodfish and induced breeding: Females were selected based on their oocyte diameter being 1.3 – 1.5 mm. Oocytes were obtained by applying a slight pressure on the abdomen. Thirty to forty oocytes were selected randomly and measured using a micrometer screw guage. Selected females and males were transferred into separate indoor tanks in the hatchery a day prior to hormone treatment.

Oocyte maturation and ovulation was induced by a single intramuscular injection of Ovaprim at a dose of 0.5 ml kg^{-1}. After ovulation, eggs were collected by manually pressing the abdomen in the direction of the caudal fin into a dry clean plastic bowl. Milt was obtained via surgical removal of the testes. Sperm was extended into 0.9% NaCl solution. Ova were fertilized by adding the extended milt mixture.

Fertilization, incubation, hatching and survival of larvae: Triplicate batches of eggs from each cross were fertilized and incubated in 60L plastic aquaria with flow through water system using rubber type mosquito mesh netting as substrate. Three females and two males were used for each cross. A control sample of eggs that were not inseminated was used to determine fertilization. The time taken for these control eggs to become opaque (dead eggs) was noted, after which the brownish/greenish eggs in the incubation tanks were

termed as fertilized. The hatching rate of each genetic group was evaluated 24 – 48 hours depending on temperature after fertilization. The number of eggs spawned was calculated by weighing 1g of eggs from each species (triplicate determinations were made). The number of eggs in one gram was counted and the average of the three counts was taken as the number of eggs in 1g of eggs for the two species. The total weight of eggs spawned for each female was noted and this was multiplied by the average number of eggs already determined to be present in each gram of eggs. Fertilization rate and the number of hatched larvae were determined as described by Ella (1987).

Percentage survival from hatching to first feeding was estimated using 50 hatchlings from each cross counted into triplicate 5L plastic bowls. Water in these bowls was changed once daily. Time of first feeding was viewed as the moment when the larvae showed signs of exogenous feeding which was monitored closely especially at 72 hours after hatching. Survival up to first feeding stage was determined by counting the remaining larvae in each bowl.

Growth rate of larvae: To compare the growth of the two African catfishes and their crossbreeds from the first feeding stage up to day 15 after hatching, 150 larvae from each genetic group were stocked separately in triplicate 60L plastic aquaria with constant aeration. Water was changed every four days. Larvae were fed *ad libitum* twice daily with dried decapsulated cysts of *Artemia* sp. After one week of feeding, the fish were introduced gradually to an artificial dry diet of coppens (200 – 300 µm) catfish feed. *Artemia* and the artificial dry diet were offered alternately to the larvae during the weaning period which lasted four days. To follow the growth rate, 40 larvae were collected from each aquarium every four days using a fine mosquito mesh net. These were weighed on a sensitive balance while they remained in a plastic bowl containing water whose weight had been pre-determined. At the end of the trial, the number of surviving fry in each tank was determined and their bulk weights recorded.

Parameters determined include:

- Final mean weight (mg);
- Weight gain,: $W_1 - W_0$ (mg);
- Growth rate,: $\frac{W_1 - W_0}{t}$ and
- Specific growth rate (SGR): $100 \times \frac{LnW_1 - LnW_0}{t}$

Where: W_0 = initial mean body weight,

W_1 = final mean body weight and t = time in days.

Data analysis: Fertilization, hatching, growth and survival rates were compared using one-way analysis of variance (ANOVA) and Fisher's LSD to determine significant differences among means.

RESULTS

Fertilization rates for the crosses ranged from 85.66±1.159 (Ht♀ X Cl♂) to 90.14±0.745 (Ht X Ht) while hatching rate ranged from 84.16±1.793 (Ht♀ X Cl♂) to 87.43±3.833 (Ht X Ht) (table 1). The highest survival rate (90.00±1.155) was observed in the cross Ht X Ht whereas, the lowest survival rate (86.00±1.155) was observed in the cross Cl♀ X Ht♂. However, variations in hatching and survival rates were not statistically different (P>0.05) for the crosses but fertilization rate in the cross Ht♀ X Cl♂ was statistically different from the other crosses (P<0.05, F-LSD). Mean water temperature in the incubating tanks was 23.2°C while air temperature was 24.0°C. Hatching was observed after 24 hours of fertilization in all crosses.

Table 1: Fertilization, hatching and survival rates (72 hours post hatching) of pure lines and cross breeds of two African catfishes, *Clarias gariepinus* (Cl) and *Heterobranchus longifilis* (Ht).

Cross	Fertilization Rate (%± S.E.)	Hatching Rate (% ± S.E.)	Survival Rate (% ± S.E.)
Cl X Cl	89.84 ± 0.773[a]	84.92 ± 1.195[a]	88.67 ± 2.404[a]
Ht X Cl	85.66 ± 1.159[b]	84.16 ± 1.793[a]	89.33 ± 1.333[a]
Cl X Ht	88.57 ± 0.577[a]	84.26 ± 3.478[a]	86.00 ± 1.155[a]
Ht X Ht	90.14 ± 0.745[a]	87.43 ± 3.833[a]	90.00 ± 1.155[a]

Values in the same column with different superscripts differ significantly from each other (P<0.05)

The final mean weight gain for the crosses Cl X Cl (12.12 ± 0.933) and Ht♀ X Cl♂ (11.54 ± 0.660) were statistically the same (P>0.05) but larger than those of the crosses: Cl♀ X Ht♂ (8.60 ± 0.562) and Ht X Ht (8.01 ± 0.540) which were also not statistically different (P>0.05) from each other. The same pattern for weight gain was observed in growth rates for the four crosses. The growth rate for Cl X Cl (0.71mg.day^{-1}) and that for Ht♀ X Cl♂ (0.67mg.day^{-1}) were not statistically different (P>0.05). These were however significantly different from 0.47mg.day^{-1} and 0.44mg.day^{-1} for Cl♀ X Ht♂ and Ht X Ht, respectively. Specific growth rate (SGR) ranged from 11.43±0.502 (Cl♀ X Ht♂) to 13.70±0.167 in the Cl X Cl cross. Significant differences (P<0.05) in SGR were observed between all the crosses following the same pattern as the growth rates. There was however no significant differences in the survival rates of the various crosses reared for the period of 12 days. Growth pattern differed with similarity being between the crosses Cl X Cl and Ht♀ X Cl♂ while Cl♀ X Ht♂ and Ht X Ht had a similar pattern (Figure 1).

Table 2: Growth and survival rates of pure lines and cross breeds of *Clarias gariepinus* (Cl) and *Heterobranchus longifilis* (Ht) between the 1st and the 15th day after hatching.

Cross	Final Mean weight (mg ± S.E.)	Weight Gain (mg ± S.E.)	Growth Rate (mg ± S.E.)	SGR (% day $^{-1}$± S.E.)	Survival Rate (% ± S.E.)
Cl X Cl	12.12 ± 0.933^{a}	10.57 ± 0.835^{a}	0.71 ± 0.056^{a}	13.70 ± 0.167^{a}	91.11 ± 2.352^{a}
Ht X Cl	11.54 ± 0.660^{a}	9.98 ± 0.592^{a}	0.67 ± 0.040^{a}	13.35 ± 0.230^{a}	92.22 ± 0.588^{a}
Cl X Ht	8.60 ± 0.562^{b}	7.05 ± 0.530^{b}	0.47 ± 0.040^{b}	11.43 ± 0.502^{b}	91.33 ± 1.018^{a}
Ht X Ht	8.01 ± 0.540^{b}	6.59 ± 0.558^{b}	0.44 ± 0.040^{b}	11.50 ± 0.599^{b}	92.89 ± 1.176^{a}

Values in the same column with different superscripts differ significantly from each other (P<0.05)

DISCUSSION

The unregulated mating of species by hatchery managers remains a major problem in the Nigerian aquaculture industry since this trend produces crossbreeds which can be intentional or un-intentional. However, when these are sold out, control of further breeding exercises is taken off the hands of the breeder who produced the parental stock. Groblar *et al* (1992) and Teugels *et al.* (1992) reported that selection of broodstock in the African catfish has largely been through a disjointed, isolated and occasional effort unlike in the case of the American catfish *Ictalurus punctatus*. Fertilization, hatching and early survival of larvae are vital for successful aquaculture of the African catfishes especially as it relates to their crossbreeds.

The mean fertilization and hatching rates for the four genetic groups investigated in this study were quite high. This not withstanding, the pure *Heterobranchus longifilis* cross (Ht X Ht) gave the highest fertilization and hatching rate in comparison with the other genetic groups. Survival rates of larvae up to the first feeding stage were found to be similar in all the genetic groups investigated. Nguenga *et al* (2000) reported high fertilization rates for crosses of various strains of *H.longifilis* ranging from 87.1 to 95.2%. The hatching rates reported in this study were comparably high with a range of 82.2 to 89.4%, although the present study deals with inter-generic hybridization.

Lower hatching rates have been reported for *Clarias gariepinus* by various authors. de Graaf *et al* (1995) reported an average rate of 59.1% in the rainy season for *C. gariepinus* in the Republic of Congo, while Macharia *et al* (2005) reported a rate as low as 4% for *C. gariepinus* eggs incubated on a nylon substrate, which is very low compared to our results even though nylon net was used as hatching substrate. It is however important to acknowledge that differences that arise from breeding history, age and water quality can affect hatching rates. Variations in seasons can also lead to differences in hatching rates, as rightly observed by de Graaf *et al* (1995). So long as fecundity does not drop, hatching rates and survival rates of larvae remain the key to viable and economically beneficial production of catfish fry and fingerlings.

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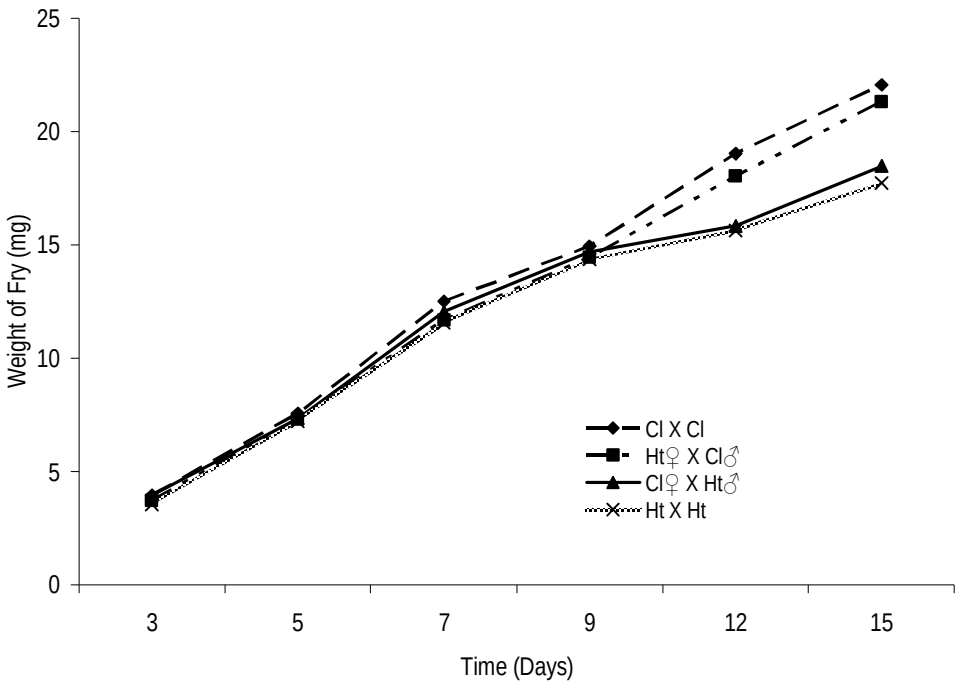

Figure 1: Mean weight of fry from crosses of two African catfishes *Clarias gariepinus* (Cl) and *Heterobranchus longifilis* (Ht) reared for 12 days.

The survival rates of progeny of the inter-generic crosses were similar to those of the pure breeds after rearing for 12 days. On the survival rates aspect, our results are higher than the range of 81.4 to 88.7% reported for strains of *H. longifilis* crossbreeds by Nguenga *et al.* (2000) after rearing for a period of 12 days. The growth rate of the crosses Cl♀ X Ht♂ and Ht X Ht were significantly different from the other crosses ($P<0.05$). The final mean weights of the various genetic groups which ranged from 8.01 - 12.12mg were lower than the 140.8 - 178.5mg reported for various strains of *H.longifilis* by Nguenga *et al.* (2000). This can possibly be attributed to differences in the husbandry system. While this study employed a static system with water aeration, Nguenga *et al.* (2000) used a flow through system. Build up of metabolic by-products can also significantly affect growth.

Specific growth rates were higher for Cl X Cl and Ht♀ X Cl♂ being 13.70%.day^{-1} and 13.35 %.day^{-1}, respectively. These were significantly different from values for the other two crosses ($P<0.05$) but similar to those reported by de Graaf *et al* (1995) for *C. gariepinus* reared for a short period after hatching. After investigating hybridization between two catfishes *Clarias batrachus* (Linn.) x *Clarias gariepinus* (Bur.) and the performance of the offspring in rearing operations, Sahoo *et al* (2003) discovered that the average length of the crossbred larvae was significantly greater than that of the pure breeds. A significantly greater average larval weight was observed in the hybrids produced from the *C. batrachus* eggs in comparison to parent *C. batrachus*. The larval weight did not vary between pure *C. gariepinus* and the hybrids produced from using *C. gariepinus* eggs but were significantly less in comparison to pure *C. batrachus* larvae or its hybrids.

Our findings showed that the survival rates of larvae up to the first feeding stage are similar in all the genetic groups investigated. The pure *Heterobranchus longifilis* cross (Ht X Ht) gave the highest fertilization and hatching rate in comparison with the other genetic groups. In terms of growth, the pure breed Cl X Cl performed better than the other crosses and was followed closely by the Ht♀ X Cl♂ cross which performed better than the other hybrid (Cl♀ X Ht♂).

The aquaculture implication of this research lies in the fact that breeding exercises should focus on the pure breed catfishes with the view to achieving greater survival and better growth rates of fry up to stocking stage.

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