

Experimental Hybridization between Catfish *Clarias batrachus* (Linn.) x *Clarias gariepinus* (Bur.) and Performance of the Offspring in Rearing Operations

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Abstract

Four combinations of crossbreeding were conducted to study the effect of hybridization between *Clarias batrachus* × *Clarias gariepinus* on the performance of F₁ progeny. For the purpose, fishes were induced bred to get pure as well as crossbred offspring of catfish, *C. batrachus* and *C. gariepinus*. No significant difference was observed in the rate of fertilization among the breeding combinations. However, a significant reduction (P<0.05) in per cent hatching was noticed due to crossbreeding between *C. batrachus* (+)* × *C. gariepinus* (-)*. A substantial number (30%) of hybrid offspring produced from *C. gariepinus* eggs were deformed and all the deformed larvae died during indoor rearing. The crossbred larvae were of greater body length in comparison to the pure bred offspring. It was also observed that the larvae of *C. batrachus* and hybrids produced from its eggs were significantly higher in initial average body weight than either the *C. gariepinus* or the hybrids produced by using *C. gariepinus* eggs.

In indoor rearing systems, *C. gariepinus* or the hybrids produced from its eggs attained significantly greater body length and weight than that of *C. batrachus* parents. However, during the outdoor rearing period the length as well as the weight of the fingerlings were significantly different among the combinations with the highest in hybrids produced from *C. gariepinus* females, followed by pure *C. gariepinus*, hybrids produced from *C. batrachus* eggs and the pure *C. batrachus*, in significantly (P<0.05) descending orders. In the growout culture system the growth of both hybrids was significantly greater than that of pure *C. batrachus*. The per cent survival of hybrids produced from *C. gariepinus* eggs was significantly lower in all their life stages in comparison to the other combinations. The similar phenotypic resemblance and faster growth of *Clariid* hybrids in comparison to *C. batrachus* indicated the scope of a promising catfish culture in Asian countries.

Introduction

The Asian catfish, *C. batrachus* is considered as a highly prized fish in India because it has fewer intermuscular bones, good taste, flavor, and more

*(+) *male; (-) female*

recuperative and medicinal qualities compared to carps. However, low fecundity and slow growth rate of the species do not favor remunerative returns from the culture system. On the contrary, the African catfish, *C. gariepinus* is well recognized as the fastest growing *Clariid*, but the massive body size and appearance of this species decrease its desirability among consumers in India. The very high cannibalistic nature in rearing and culture system is another persistent drawback associated with this species. To overcome such obstacles, researchers in several countries have been trying to crossbreed this species with their native species. Various interspecies and intergeneric hybridizations with *C. gariepinus* have been carried out. The hybrids of *C. gariepinus* × *Heterobranchus longifilis* (Legendre et al. 1992; Nwadukwe 1995) were produced successfully, with very good culture potential. Hybridization between *C. batrachus* × *C. gariepinus* was reported feasible but with limited success (Rahman et al. 1995). However, their reciprocal hybrids suffered from high mortality. The study reported here with *C. batrachus* and *C. gariepinus* was undertaken to study the potential of the hybrid progenies with respect to their rate of survival and growth potential for rearing in a pond culture system to get a table sized marketable fish.

Materials and Methods

Induced breeding

C. batrachus fingerlings, produced at the Central Institute of Freshwater Aquaculture, Bhubaneswar, were reared in earthen ponds of 0.01 ha area, maintained with stagnant water. Each pond was stocked with 500 fingerlings of 5.22 ± 0.24 g (ave. body weight), raised to 150 g size, in a culture period of nine months (September to May). *C. gariepinus* of both sexes with an average weight of 150 ± 3.33 g, were collected from the local fish farmers during May 1998, and reared in 4 m³ cement cisterns (4 x 1 x 1 m) for two months with a stocking density of 20 fish per cistern (average biomass 3 kg per cistern). The cisterns were provided with intermittent flow-through system and water height was maintained at 0.5 m. Water temperature varied from 27 to 30°C during the entire rearing period. Maximum precautions were taken to prevent the escape of *C. gariepinus* into the natural water body. Both species were fed on balanced pelleted diet containing 30% crude protein (CP) and 3.5 kcal gross energy (GE) per 100 g of feed. Body weight increment of 10 randomly chosen fish was recorded at 15 day intervals to adjust the daily ration requirement with the change in body weight. Feed was provided daily at 4 P.M. at 3% of the wet body weight of fish.

During the breeding season (July to September), both species were collected from the rearing systems for breeding and hybridization experiments. Males and females were selected on the basis of sexual identification as described by Rao et al. (1994) for *C. batrachus* and Viveen et al. (1986) for *C. gariepinus*. The brood fish were distributed species wise into four replicates, comprising 2 males and 3 females, in each replicate and kept in 4 x 1 m ce-

ment cisterns with a water depth of 0.5 m. The cement cisterns were placed outdoor under natural photoperiodic condition. All the cisterns were provided with intermittent flow-through water system. The water temperature and dissolved oxygen level of cistern water varied, respectively from 27 to 29°C and 5.8 to 6.3 ppm. The replicates were bred one-by-one at weekly intervals. Before breeding, the females of each replicate were injected intramuscularly with Ovaprim (sGnRHA + Domperidon), an inducing agent for ovulation.

The female *C. gariepinus* was stripped at 7 h post injection as suggested by Viveen et al. (1986) while *C. batrachus* was stripped at 14 h of Ovaprim injection as practiced by Rao et al. (1994). The inducing agent was so injected that both species were stripped at the same time in the morning. The eggs were collected in a sterilized enamel tray from each individual and tentatively separated into two equal parts. One part of egg was utilized to produce pure breeds and the other part for the production of hybrids. The testes were removed from the males after dissecting the fishes and squeezed to prepare sperm suspension. The sperm suspension were kept separately and used within two minutes. The obtained suspension was mixed with the eggs of the homologous and heterologous species in separate trays to produce pure as well as hybrid progenies. In each replicate, fishes were bred in four combinations, listing the females of the cross first, *C. batrachus* (+) × *C. batrachus* (-) (Cb x Cb), *C. batrachus* (+) × *C. gariepinus* (-) (Cb x Cg), *C. gariepinus* (+) × *C. batrachus* (-) (Cg x Cb) and *C. gariepinus* (+) × *C. gariepinus* (-) (Cg x Cg). From each combination 800 to 1000 eggs were used for fertilization. For hatching, 250 eggs were kept in each triplicate 30 cm diameter incubation tray, maintained a water height of 6 cm, and were provided with continuous water flow (1 l·min). The water temperature and dissolved oxygen level of the water in hatching trays varied from 27 to 30°C and 5.7 to 6.1 ppm, respectively during incubation period.

The translucent eggs containing embryonic eyes were considered fertilized and counted to calculate per cent fertilization. Opaque eggs were considered unfertilized. The total number of spawn hatched, hatching per cent and total number of deformed larvae were counted from each incubation tray before releasing them into the indoor rearing tanks. After hatching more than 50% of the eggs, the length of the larvae, along with the size of the yolk sac were measured to below the nearest half millimeter using an ocular micrometer under a compound microscope at a magnification of 10x. Wet weight of the larvae was recorded to an accuracy of 0.01 mg using a digital electronic balance (Sartorius make).

Indoor rearing of larvae

All the normal larvae of *C. batrachus*, *C. gariepinus* and their hybrids were transferred into indoor hatchery tanks (1 m × 0.5 m) to raise them into fry. Water depth in the tanks was maintained at 25 cm throughout the rearing period of 14 days. One hundred pieces of pure and hybrid larvae from each combination were stocked in triplicate tanks. The larvae were fed on mixed live plankton at hourly intervals. At the end of the rearing period the change in

body length, weight, total biomass and per cent survival were recorded from each individual cistern.

Nursery rearing of fry

The triplicate numbers of 12 rectangular cement tanks (4 m × 0.5 m) with 30 cm water depth were used for the outdoor rearing of fry. The rearing tanks were prepared as described by Rao et al. (1994) for *C. batrachus* and kept in an open area under natural light condition. One-third of each tank surface was covered by palm leaves to provide shade to fish during sunny days. Each tank was stocked with 200 pieces of fry and reared for a period of 30 days to get the fingerlings. The change in body weight and length of fry were recorded at 15 day intervals. The fish were fed on laboratory prepared pelleted feed (2 mm dia), containing 30% crude protein. The daily ration was fed at 10% of wet body weight in two equally divided meals, at 10 A.M. and 4 P.M. At the end of the experiment, changes in body length, weight, total biomass and per cent survival were recorded.

Culture

After 30 days nursery rearing study, *C. batrachus* (pure and their hybrids) were reared for another 80 days to get 5 to 6 g size fishes to stock the grow out culture ponds. Fingerlings of Cb x Cb and Cb x Cg were stocked at a density of 5000/ha in triplicate 0.01 ha ponds maintained with a water depth of 1m. Since fewer fingerlings were available, duplicate ponds were stocked for the Cg x Cb grow out culture. Throughout the culture period, fish were fed on a formulated feed (30% CP and 3.5 Mcal GE/kg feed) at 5% of the wet body weight, daily and feeding adjustment was made as per the body weight changes recorded during sampling at 15 day intervals. Total daily ration was offered in two divided meals at 8:30 A.M. and 3:30 P.M. At the end of the growout culture period of 180 days, the ponds were completely dewatered to recover all fish. Body weights were measured and the per cent survival was calculated. Average daily body weight gain was calculated by subtracting initial body weight from final body weight, divided by the number of days of growout culture.

Statistical analysis

Data were analyzed using variance component analysis (Snedecor and Cochran 1967) while difference between the means was examined using Duncan's multiple range tests.

Results

Table 1 shows that the rate of fertilization of eggs is similar in all the treatments. Incubation time of crossbreeds followed the maternal parent. Em-

bryonic mortality started after 2 to 3 h and highest mortality was recorded at 8 to 9 hours of incubation in crossbreeds. The per cent hatching of Cb x Cg hybrid larvae was significantly reduced ($P < 0.5$). It was also observed that crossbreeding of Cg x Cb produced the highest number of deformed larvae in all the four replicates. The deformities included bent tail, bent trunk, acephali and rudimentary tail and the larvae were unable to move. Following hatching, the deformed larvae died within 8 to 10 days. Recovery of normal larvae was more than 68% in all combinations except in hybrids (60%) produced using *C. gariiepinus* eggs.

Larval biometry was measured at the time of more than 50% hatching. The length and height of the yolk sacs (Table 2) did not indicate any difference between Cb x Cb and Cb x Cg. Their yolk sac length were significantly greater ($P < 0.05$) in comparison to that of Cg x Cb, and all the three were significantly greater compared to Cg x Cg. The average length of the crossbred larvae was significantly greater ($P < 0.05$) 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. gariiepinus* and the hybrids produced from using *C. gariiepinus* eggs but was significantly less in comparison to pure *C. batrachus* larvae or its hybrids.

After 14 days of indoor rearing (Table 3), the pure *C. gariiepinus* fry (Cg x Cg) attained significantly greater body length in comparison to fry produced from the other three breeding combinations. There was no significant difference in length between Cb x Cg and Cg x Cb fry. The Cb x Cb fry attained the

Table 1. Effect of crossbreeding of *C. batrachus* x *C. gariiepinus* on (Mean \pm SE) fertilization %, hatching % and larval quality. The data are based on the means (n=3) of 4 replicates. Data are arc sin transferred.

Combination	Fertilization (%) (NS)	Hatching (%)	Deformed larvae (%)	Normal larvae (%)
Cb x Cb	53 \pm 3.6	55 \pm 3.3 ^a	20 \pm 3.5 ^{ab}	71 \pm 3.5 ^{ab}
Cb x Cg	49 \pm 4.8	38 \pm 3.7 ^b	22 \pm 1.9 ^{ab}	68 \pm 2.1 ^{ab}
Cg x Cb	51 \pm 4.7	40 \pm 5.1 ^{ab}	30 \pm 4.6 ^a	60 \pm 4.6 ^b
Cg x Cg	56 \pm 3.0	57 \pm 2.1 ^a	17 \pm 1.2 ^b	73 \pm 1.3 ^a

Mean values bearing different superscripts in a column differ significantly ($P < 0.05$)
NS: Nonsignificant

Table 2. Mean \pm SE of yolk sac and larval biometry of *Clariids* (hybrids and pure) for four replicates at more than 50% of hatching. The data (N) are based on the pooled means (n=3) in each replicate.

Combination	Yolk sac length (mm)	Yolk sac height (mm)	Larval length (mm)	Larval weight (mg)
Cb x Cb	22 \pm 0.04 ^a	1.5 \pm 0.02 ^a	4.6 \pm 0.15 ^b	3.2 \pm 0.07 ^b
Cb x Cg	2.1 \pm 0.03 ^a	1.4 \pm 0.01 ^a	5.1 \pm 0.09 ^a	4.0 \pm 0.09 ^a
Cg x Cb	1.3 \pm 0.02 ^c	1.0 \pm 0.02 ^c	5.2 \pm 0.06 ^a	2.4 \pm 0.13 ^c
Cg x Cg	1.2 \pm 0.01 ^b	1.0 \pm 0.04 ^b	4.7 \pm 0.05 ^b	2.4 \pm 0.11 ^c

Mean values bearing different superscripts in a column differ significantly ($P < 0.05$)
N = Biometry of 20 larvae were recorded.

least length ($P < 0.05$) in comparison to that of others at the end of indoor rearing. The body weight at the end of the 14th day was similar in all the offsprings except that of pure *C. batrachus*, which was significantly lower than the other crosses. The final biomass followed the similar trend as of final average body weight. The rate of survival was highest in Cb x Cg and differ significantly from Cb x Cb fry which was also significantly higher than either Cg x Cb or Cg x Cg.

During nursery rearing, the hybrid fry produced from Cg x Cb attained significantly greater length and weight compared to that of other breeding combinations, in the first fortnight. In the second half of the rearing period, length of fry was significantly different among all breeding combinations with greatest in Cg x Cb followed in order of decreasing length by Cg x Cg, Cb x Cg and Cb x Cb (Table 4). The weight of the fry showed the same trend as that of

Table 3. Change in body weight, biomass and length (Mean \pm SE) of the *Clariid* offsprings in four consecutive indoor rearing each of 14 days. The data (N) are based on the pooled means ($n=3$) for each replicate during rearing study.

Combination	Initial biomass of larvae (mg)	Final total fry length (mm)	Final fry wet weight (mg)	Final biomass of fry (g)	Survival (%)
Cb x Cb	336.7 \pm 8.8 ^b	17.0 \pm 0.2 ^c	56.8 \pm 2.9 ^b	4.7 \pm 0.1 ^b	83.0 \pm 2.5 ^b
Cb x Cg	410.0 \pm 10.0 ^a	20.1 \pm 0.4 ^b	67.0 \pm 5.6 ^a	5.8 \pm 0.1 ^a	86.0 \pm 2.0 ^a
Cg x Cb	250.0 \pm 5.8 ^c	20.3 \pm 0.3 ^b	71.0 \pm 9.2 ^a	5.6 \pm 0.3 ^a	78.0 \pm 4.0 ^c
Cg x Cg	246.7 \pm 6.7 ^c	21.6 \pm 0.3 ^a	72.0 \pm 2.0 ^a	5.7 \pm 0.2 ^a	80.0 \pm 2.1 ^c

Mean values bearing different superscripts in a column differ significantly ($P < 0.05$)

N = Biometry of 20 fry were recorded

Table 4. Mean \pm SE of survival and growth performance of different pure as well as cross-breeds after 30 days of nursery rearing. Data are based on the means ($n=20$) of triplicate tanks.

Parameters	Combination			
	Cb x Cb	Cb x Cg	Cg x Cb	Cg x Cg
Initial total fry length (mm)	17.0 \pm 0.2 ^c	20.1 \pm 0.4 ^b	20.3 \pm 0.9 ^b	21.6 \pm 0.8 ^a
Total fry length after 15 days (mm)	41.3 \pm 2.3 ^c	45.5 \pm 2.1 ^b	59.8 \pm 2.6 ^a	46.6 \pm 3.4 ^b
Total fingerling length (mm)	51.4 \pm 2.8 ^d	55.4 \pm 1.2 ^c	68.1 \pm 2.4 ^a	59.1 \pm 2.1 ^b
Initial wet weight of fry (mg)	56.8 \pm 3.0 ^b	67.0 \pm 5.6 ^a	71.0 \pm 9.2 ^a	72.0 \pm 2.0 ^a
Initial biomass of fry (g)	11.4 \pm 0.1 ^c	13.4 \pm 0.1 ^b	14.2 \pm 0.1 ^a	14.4 \pm 0.2 ^a
Wet weight of fry after 15 days (mg)	555 \pm 76.0 ^c	625 \pm 45.4 ^c	1602 \pm 119.4 ^a	1124 \pm 114.7 ^b
Wet weight of fingerling (mg)	985 \pm 163.1 ^d	1130 \pm 73.8 ^c	2110 \pm 177.6 ^a	1650 \pm 22.5 ^b
Final biomass of fingerlings (g)	148 \pm 1.3 ^a	115 \pm 13.1 ^b	106 \pm 14.6 ^{bc}	76 \pm 1.9 ^c
Survival (%)	76 \pm 2.3 ^a	51 \pm 3.1 ^b	25 \pm 2.0 ^c	23 \pm 2.1 ^c

Mean values bearing different superscripts in a column differ significantly ($P < 0.05$)

length for all the combinations. The highest final biomass was achieved by pure *C. batrachus* and the biomass of pure *C. gariepinus* was significantly ($P < 0.05$) lowest. At the end of the rearing period, per cent survival of Cb x Cb fingerlings was greater in comparison to Cb x Cg, which was significantly higher than Cg x Cb and Cg x Cg. No significant morphological differences could be observed at fry stage among different breeding combinations.

Less variation in growth of fingerling was observed in hybrids produced from Cb x Cg but a much more variable growth was obtained from Cg x Cg. It was interesting to note some anophthalmic or mesophthalmic Cg x Cb hybrid fingerlings. All the blind fingerlings had deep black body colour. The body coloration, head shape and the snout of the hybrids resembled to those of *C. batrachus* parents at their fingerling stage.

During the grow out culture study, the average daily body weight gain and per cent survival of hybrid produced from Cb x Cg were significantly greater compared to that of Cb x Cb and Cg x Cb (Table 5). The hybrids produced from Cg x Cb grew faster than *C. batrachus* pure (Cb x Cb), but attained less body weight in comparison to that of Cb x Cg. The per cent survival of Cg x Cb hybrids was significantly lower and Cb x Cg was significantly higher among the treatments. However, neither in the rearing nor in the culture systems cannibalism could be observed in the offspring of Cb x Cb, Cb x Cg and Cg x Cb combinations.

Discussion

The feasibility of crossbreeding between *C. batrachus* and *C. gariepinus* and the culture of hybrid offspring in the grow out system was demonstrated in the present study. The latency and incubation period of *C. batrachus* were 14 h and 24 to 26 h, respectively which agrees to the earlier observation of Rao et al. (1994) in the same species. In agreement to Viveen et al. (1986) in the present study, the latency period of 7 h and 18 to 20 h incubation time were observed at the water temperature of 27 to 30°C in *C. gariepinus*. There was no difference between the hatching time of pure to that of their hybrids. The

Table 5. Mean \pm SE of survival, growth (g) and body weight gain of catfishes in grow out culture ponds

Combination	Wet body weight at the time of stocking (g)	Wet body weight at the end of 3 months (g)	Wet body weight at the end of 6 months (g)	Survival (%)	Average daily wet body weight gain (g)
Cb x Cb	6.0 \pm 0.58 ^c	26.4 \pm 2.17 ^b	69.1 \pm 3.81 ^c	62.7 \pm 2.60 ^b	0.4 \pm 0.02 ^c
Cb x Cg	34.6 \pm 9.35 ^a	130.0 \pm 11.55 ^a	550.7 \pm 6.61 ^a	93.0 \pm 2.30 ^a	3.0 \pm 0.16 ^a
Cg x Cb	13.0 \pm 2.00 ^b	34.1 \pm 10.00 ^b	341.0 \pm 58.00 ^b	28.0 \pm 4.61 ^c	1.8 \pm 0.32 ^b

Mean values bearing different superscripts differ significantly ($P < 0.05$)

per cent hatching was always higher in pure compared to hybrids in all replicates. The hybrid progeny attained higher body weight compared to parents, which was also observed earlier in another catfish hybrid, *C. gariepinus* x *H. longifilis* (Legendre et al. 1992; Nwadukwe 1995). In the present study a good number (30%) of larvae produced from *C. gariepinus* (+) x *C. batrachus* (-) cross were deformed and abnormal when compared to the other crosses, which were also observed by Rahman et al. (1995) in the same hybrid larvae. The deformity of larvae might be due to the hybridization effect. The hybrids of *C. gariepinus* (-) x *C. batrachus* (+) exhibited less mortality, less deformity and higher survival in fry and fingerling stages compared to the reciprocal cross.

The yolk sac length and height of pure *C. batrachus* larvae and its hybrid were similar to each other and were greater than that of pure *C. gariepinus* or its hybrids. This could be due to the bigger sized *C. batrachus* eggs. The higher mean wet weight of hybrid *C. batrachus* larvae than that of pure larvae could be due to the greater length of hybrid larvae.

The growth response of hybrids produced either from *C. batrachus* or *C. gariepinus* eggs was neck to neck and was similar to that of their *C. gariepinus* mother but was superior to the *C. batrachus* parental controls during fry stage. The higher final biomass of hybrids and *C. gariepinus* pure in fry stage might be due to better survival and growth during fry stage. The per cent survival of Cb x Cg and Cg x Cb hybrid fry were 86 ± 2 and 78 ± 4 , respectively. In contrast to the present results, Richter et al. (1995) observed that all the embryos produced by *C. gariepinus* (+) x *C. batrachus* (-) and their reciprocals died within 20 h of fertilization. Laywornyawut et al. (1992) also could not get any viable fry from hybridization between *C. macrocephalus* (-) x *C. gariepinus* (+). The significant growth difference was observed among the hybrids during fingerling stage. The hybrids produced by using *C. gariepinus* eggs grew faster than the other combinations at the fingerling stage. The per cent survival of *C. gariepinus* hybrids was similar to that of pure *C. gariepinus* but significantly less when compared to pure *C. batrachus* and its hybrids. The better growth performance of pure *C. gariepinus* and hybrids produced using its eggs in comparison to either pure *C. batrachus* or its hybrid at fry and fingerling stages might be due to their low per cent survival. The lower final biomass of hybrids and pure *C. gariepinus* in fingerling stage was due to their significantly lower survival, although growth was higher. During the study, size dependent mortality could be observed in pure *C. gariepinus* and the low survival of fingerlings could be attributed to the predatory nature of the species, agreeing with the earlier findings (Rahman et al. 1992; De-Graaf et al. 1995). The occurrence of anophthalmic or mesophthalmic conditions in Cg x Cb hybrids might be due to the effect of hybridization.

During six months of culture period Cb x Cg and Cg x Cb hybrid reached average body weights of 550.7 ± 6.61 g and 341 ± 58.00 g, respectively, which were higher compared to the final body weight of *C. batrachus* parent (69.1 ± 3.81 g avg.) during that period. Rahman et al. (1995) reported an average of 150 to 250 g body weight achievement by *C. batrachus* (+) x *C. gariepinus* (-) hybrid during a 4-months culture period. The growth of hybrids could not be compared to that of *C. gariepinus* parent because the culture of later species is

not permitted at the Institute. All the marketable hybrids produced from *C. batrachus* mother and about 75% hybrids produced from *C. gariepinus* mother looked like pure *C. batrachus* in their body phenotype, while average 25% hybrids produced from *C. gariepinus* eggs had spots on their body resembling that of *C. gariepinus*.

The study clearly indicated that hybridization between *C. batrachus* × *C. gariepinus* was successful and 78 to 86% hybrid fry survived in indoor rearing system. The rate of survival in nursery rearing and grow out pond was also 25 to 51% and 28 to 93%, respectively. Resemblance of hybrids produced from *C. batrachus* eggs and a high survival rate indicated that the offspring could be well advocated for culture practices as replacement for *C. batrachus*. Advantages of the significant increase in growth rate of hybrids over the native Clariid *C. batrachus*, and the complete absence of cannibalism in the hybrids at different stages of rearing and in pond culture indicate a lucrative and promising catfish culture.

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