Pak. J. Sci. Ind. Res. 2005 **48**(2) 113-117

Hybridization Studies in Normal and Cracked-Skull Diseased African Catfish, *Clarias gariepinus*

O. Fagbuaro*[a], J.I. Awopetu[b], M.A. Faluyi[c] and P.O. Aluko[d]

[a]Department of Zoology, University of Ado-Ekiti, PMB 5363, Ado-Ekiti, Nigeria

[b]Department of Zoology, Obafemi Awolowo University, Ile Ife, Nigeria

[c]Department of Plant Science and Forestry, University of Ado-Ekiti, PMB 5363, Ado-Ekiti, Nigeria

[d]National Institute for Freshwater Fisheries Research, PMB 6006, New Bussa, Nigeria

(received March 8, 2003; revised May 26, 2004; accepted January 25, 2005)

Abstract. Intraspecific hybridization studies were carried out in two strains of *Clarias gariepinus* with acute and mild cephalic-abnormality or cracked-skull disease and non-cephalic or normal species from Ibadan, Oyo State, Southwestern Nigeria, and the Maiduguri strain from Maiduguri, Borno State, Northern Nigeria. The highest percentage of hatchability (84.5%) was recorded in the Maiduguri strain of *C. gariepinus* and the least (67.5%) in the strain with mild cephalic-abnormality. Among the intraspecific hybrids, the highest percentage (76.7%) was recorded in the cross between the female of Maiduguri strain and the male of non-cephalic strain, while the least (72.2%) was recorded in the cross involving the female of Maiduguri strain and the male of strain with cephalic-abnormality. The maximum growth recorded was 4.321g in six weeks in the strain with mild cephalic-abnormality and minimum 3.758 g in the Maiduguri strain. Among the hybrids, maximum growth of 4.086 g was recorded in the cross between the female of Maiduguri strain and the male of non-cephalic, and minimum of 3.582 g in the cross between the female with cephalic-abnormality and the male of Maiduguri strain. There was no occurrence of cephalic-abnormality in the cross between the female of *C. gariepinus* with cephalic-abnormality and the male of Maiduguri strain.

Keywords: hybridization, cephalic-abnormality, hatchability, productivity, *Clarias gariepinus*

Introduction

Clariid catfish (*Clarias* spp., and *Heterobranchus* spp.) are highly valued edible fishes in Africa and constitute prominent commercial aquaculture species. These are widely cultivated in Africa, mainly under semi-intensive systems with an average production level of 40 metric ton/ha/yr (Hecht, 1996). *Clarias gariepinus* has been introduced for intensive culture in some European and Asian countries. Huisman and Richter (1987) reported that *Clarias gariepinus* belongs to an economically important group of fishes in the tropical and subtropical regions. According to Aluko and Popoola (2002), the rapid development of fish production, along with the introduction of super-intensive fish farming systems, have necessitated the concurrent improvement of productivity and adaptability of fish species. This has necessarily involved hybridization which simply means heterospecific insemination. The method is used to combine desirable characteristics of one species with those of another in order to produce offsprings of superior quality than the parent species. Aluko and Popoola (2002) reported that scientists and fish farmers have successfully used the methods of hybridization, selection and inbreeding for fish quality improvement. Intraspecific hybridization is the cross between strain stocks of the same species and has been applied to cause increased fish production, while interspecific hybridization is the cross between two different species. At low stocking densities, diseases are not a serious problem in polyculture or monoculture operations of African catfish. Although some fungal parasites and bacterial diseases may occur, cephalic-abnormality, also known as cracked-skull disease, is a catfish disease which has been reported from intensive pond rearing systems and hatcheries across Africa. The outbreak of the disease has been reported in the Asian catfish species *C. batrachus* and *C. macrocephalus* (Kabata, 1985), and *C. gariepinus* in Israel (Viveen *et al*., 1985), in Central Africa (Huisman and Richter, 1987) and in Nigeria (Awa and Alegbeleye, 1991).

The present study has been carried out to find a permanent solution to the problem of cephalic-abnormality in catfish culture.

Materials and Methods

Source of broodstock. Male and female brooders were obtained from different locations in Nigeria. These included

*Author for correspondence; E-mail: omofagbuaro@yahoo.com

Ibadan (Oyo State) and Maiduguri (Borno State). The affected strains, which were mainly from Ibadan and Maiduguri, were stocked separately in holding concrete tanks at the National Institute for Freshwater Fisheries Research (NIFFR), New Bussa, Niger State, Nigeria for almost two seasons for fattening and were fed on the NIFFR prepared fish feed containing 40% crude protein. Brooders were collected from the tanks by using drag-net and were separated into the two sexes.

Hormone injectcon. Synthetic ovaprim hormone was used for induced breeding of the fish at the dose of 0.5 ml/kg of fish for a latency period of 12 h.

Fertilization procedure. After the latency period of 12 h, milt was collected by sacrificing the male because of the testicular anatomy, which hampers stripping of milt in *Clarias*. Eggs were stripped from females by gently pressing the abdominal region. The collected milt was diluted by physiological saline solution (0.9% NaCl) at a ratio of 1:5 and was used in fertilizing the eggs by using a clean dry feather for mixing.

The parental and intraspecific crosses were carried out, in triplicate, as detailed below.

Parentals. The parental crosses were: female cephalic (cp) x male (cp) (acute); female cephalic (cp) (mild) x male cephalic (cp) (mild); female non-cephalic (ncp) x male non-cephalic (ncp); and female Maiduguri (mai) x male Maiduguri (mai).

Intraspecific hybrids. The intraspecific hybridization was done between: female cephalic (cp) x male non-cephalic (ncp); female cephalic (cp) x male Maiduguri(mai); female non-cephalic (ncp) x male cephalic (cp); female non-cephalic (ncp) x male Maiduguri (mai); female Maiduguri (mai) x male cephalic (cp); and female Maiduguri (mai) x male non-cephalic (ncp).

Incubation. Incubation was carried out in 30 well aerated aquarium tanks with dimensions 60 x 30 x 30 cm^3, each filled with fifteen litres of water. Egg collectors, made up of shreads of loose nylon sack called "kakabans", were placed in each aquarium for the eggs to attach. The temperature range was between 25 °C and 26 °C in the aquaria, with pH 7.1. Hatching occurred 22 h after fertilization, which lasted 6 h. Hatchlings or larvae were carefully counted. The hatching percentage was also determined for each aquarium. 100 post-hatchlings from each mating group were put in different aquaria and the length and weight measurements were done. Feeding was started on the fourth day after hatching, which was done twice a day with the zooplankton, *Moina micrura,* for a period of 10 days. The survival rate for each treatment was determined everyday by direct counting of the fry. Counting was done in small beakers with minimum stress to the fry.

After the indoor rearing, all the ten mating groups were duplicated. From each cross, 80 fry were stocked in 2 x 2 x 1 m^3 concrete tanks. The fry were fed on artificial fish diet with 40% crude protein for a period of six weeks (42 days). Weekly samplings were done for fry growth in length (cm) and weight (g).

Results and Discussion

The percentage hatchability in the ten mating groups involving intraspecific crosses of two strains of *Clarias gariepinus*, a strain with cephalic-abnormality or cracked-skull disease plus normal species of the same strain and another from a different stock was recorded (Table 1). The highest of percentage (84.5%) hatchability was recorded in the Maiduguri strain (female Maiduguri x male Maiduguri) among the parental crosses, and the least (67.5%) in the Ibadan strain with mild

Table 1. Mean percentage daily survival of four strains and six intraspecific hybrids of *Clarias gariepinus*

Mating groups (f x m)	Hatchability (%)	Mean length (mm)	Initial mean weight (g)	Final mean weight (g)	Mean weight gain (g)	Mean weight gain/day (g)
Parental strains						
cp (acute) x cp (acute)	82.5	4.6	0.02	1.25	1.230	0.123
cp (mild) x cp (mild)	67.5	4.4	0.01	0.95	0.940	0.094
ncp x ncp	69.2	3.6	0.15	0.90	0.885	0.089
mai x mai	84.5	4.4	0.01	1.05	1.040	0.104
Intraspecific hybrids						
cp x ncp	73.7	2.6	0.02	1.05	1.035	0.104
cp x mai	75.9	5.0	0.01	1.00	0.990	0.099
ncp x cp	75.5	4.6	0.02	1.05	1.030	0.103
ncp x mai	73.5	4.6	0.01	1.50	1.490	0.149
mai x cp	72.2	2.2	0.01	0.90	0.800	0.080
mai x ncp	76.7	4.5	0.01	0.95	0.940	0.094

f = female; m = male; cp = cephalic-abnormality strain; ncp = non-cephalic-abnormality strain; mai = Maiduguri strain

cephalic-abnormality [female cephalic (cp) (mild) x male cephalic (cp) (mild)].

The highest percentage of hatchability (75.9%) was recorded in the cross between the female cephalic-abnormality (cp) and male Maiduguri (mai) among the hybrids, and the least (72.2%) in the female Maiduguri (mai) and male cephalic-abnormality (cp). In this study, cases with low percentage hatchabilities may have arisen from cephalic-abnormality, poor water quality and probably over-ripening of eggs. The low hatchability of the parental strain with mild cephalic-abnormality may probably be due to other hereditary factors.

The mean length (mm) and weight (g) of hatchlings of the ten mating groups are also shown in Table 1. In the parental groups, the highest final mean weight and length of hatchlings 1.25 g and 4.6 mm, respectively, were recorded for *C. gariepinus* strain with acute cephalic-abnormality, while non-cephalic-abnormality strain had the least final mean weight and were even shorter 0.90 g and 3.6 mm, respectively. Among the intraspecific hybrids, hatchlings with shorter body (about 2.2 mm and 2.6 mm) were produced in the cross between female Maiduguri and male cephalic-abnormality, and female with cephalic-abnormality and male non-cephalic-abnormality, respectively. The hatchlings of the hybrids between female non-cephalic and male Maiduguri strain were heavier (1.50 g), while the hatchlings of the cross between the female cephalic-abnormality strain and the male Maiduguri strain were the longest. The hybrids of the cross between the female Maiduguri strain and the male cephalic-abnormality strain had the least weight (0.90 g), and were likewise shorter in length (2.2 mm).

The mean percentage of the daily survival of hatchlings of the four strains of *C. gariepinus* and their six intraspecific hybrids reared for ten days in indoor aquaria, are shown in Fig 1. Among the pure line strains, *C. gariepinus* with mild cephalic-abnormality had the highest survival rate (78.0%) and the strain with acute cephalic-abnormality had the least survival value (70.0%). Hybrids of the female *C. gariepinus* with cephalic-abnormality and the male without cephalic-abnormality had the highest survival value (89.0%), while the least (39.0%) was in the hybrids of the female *C. gariepinus* without cephalic-abnormality and the male Maiduguri strain at the end of ten days of rearing in aquaria. The reason for the possible low survival value may be due to the trait of cephalic-abnormality.

The average weight of the fry of four strains and six intraspecific hybrids of *C. gariepinus* reared in open concrete tanks for a period of six weeks are shown in Fig.2. Among the parental crosses, the *C. gariepinus* strain with cephalic-abnormality had the highest weight (7.94 g), while the least

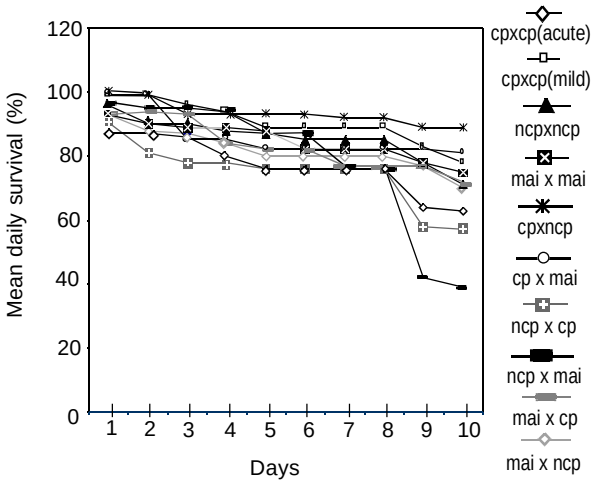

Fig. 1. Mean percentage daily survival of four strains and six intraspecific hybrids of *Clarias gariepinus* maintained in indoor aquaria.

(4.50 g) was noted in the Maiduguri strain of *C. gariepinus*. By the third week in concrete tanks, the Maiduguri strain was heavier than other mating groups (2.01 g), which however later depreciated in weight. It was observed that the disease is hereditary and that the *C. gariepinus* with cephalic-abnormality normally had high growth rate at the early stage of their life. It is possible that the abnormality hastened more digestion and assimilation of food nutrients than occurred in the other strains, suggesting that the possible cause of cephalic-abnormality of cracked-skull disease may not be due to the lack of vitamin C and other nutritional deficiencies as reported by Kabata (1985), Ashley *et al.* (1975) and de Graaf and Janssen (1996). Ashley *et al.* (1975), have further stated that the vitamin C deficiency results in poor wound healing, failure of granulation tissue to fibrose, and bizzarre development of gill, skeletal cartilages and spinal deformities. This further confirmed that cephalic-abnormality or cracked-skull disease is hereditary and could possibly be eradicated by intraspecific and interspecific hybridization of species of *Clarias*.

Awa and Alegbeleye (1991) and Huisman and Richter (1987) reported that the cause of the cracked-skull disease is phytoplankton blooms. This was impossible as there usually were traces of the cephalic-deformation few days after hatching even during the indoor rearing where there were no traces of blooms. Among the intraspecific hybrids, the female Maiduguri strain and the male non-cephalic strain hybrid showed the highest weight in the six weeks of maintenance, while the cross between the female non-cephalic strain and the male Maiduguri strain had the least weight after six weeks of maintenance outside the concrete tanks. The reason for this weight

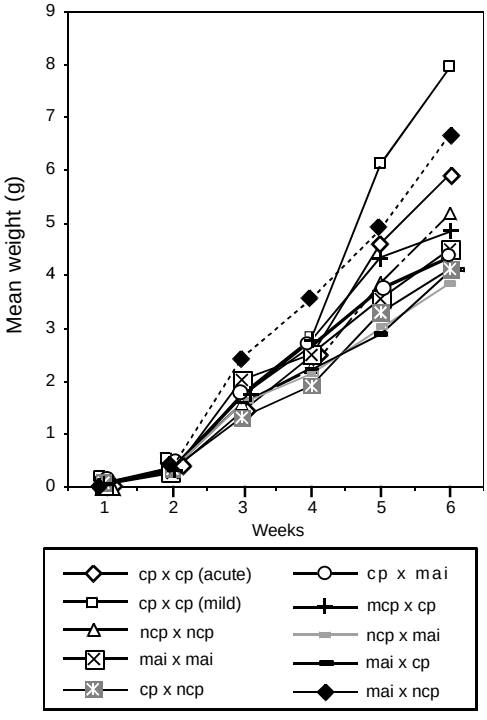

Fig. 2. Mean weight of fry of four strains and six intraspecific hybrids of *Clarias gariepinus* reared in concrete tanks.

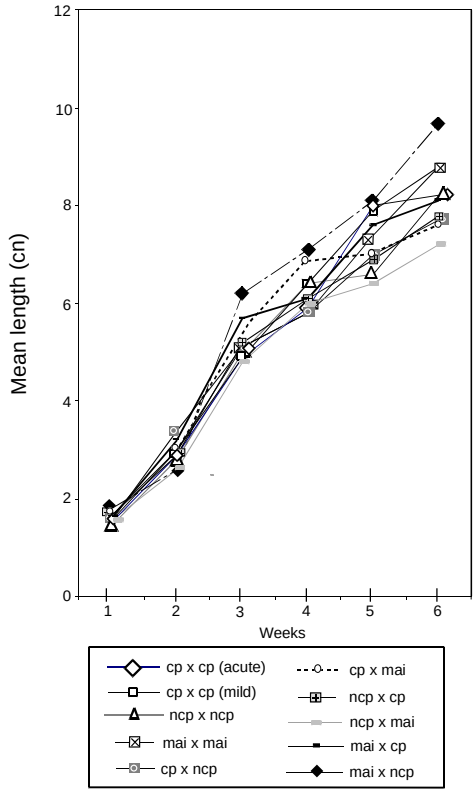

Fig. 3. Mean length of fry of four strains and six intraspecific hybrids of *Clarias gariepinus* reared in concrete tanks.

decrease may not be due to the rate of conversion of food, but may be due to other environmental factors such as stress during sampling and water quality.

The mean length of the fry of the ten genetic groups of *C. gariepinus* reared in concrete tanks for six weeks is shown in Fig.3. The highest mean length of fry (8.84 cm) was recorded for the strain with mild cephalic-abnormality of the parental group and there were no significant differences in the mean length of the parental group. Among the hybrids, the fry with short body (7.20 cm) was produced in the cross between the female non-cephalic strain and the male Maiduguri strain, while the longer ones (9.74 cm) were observed in the cross between the female Maiduguri strain and the male non-cephalic strain of *C. gariepinus.*

The percentage frequency of occurrence of the cephalic-abnormality among the fry of four strains and six intraspecific hybrids of *C. gariepinus* is shown in Fig. 4. There was a drastic reduction in the incidence of cephalic-abnormality in this study with the total elimination of cephalic problems in the cross between the strains with cephalic-abnormality and Maiduguri strain. This hybrid could probably solve the problem of cephalic-deformation, or mating of *C. gariepinus* with cephalic-abnormality with another species of *Clarias* (interspecific hybridization) that could be properly screened for

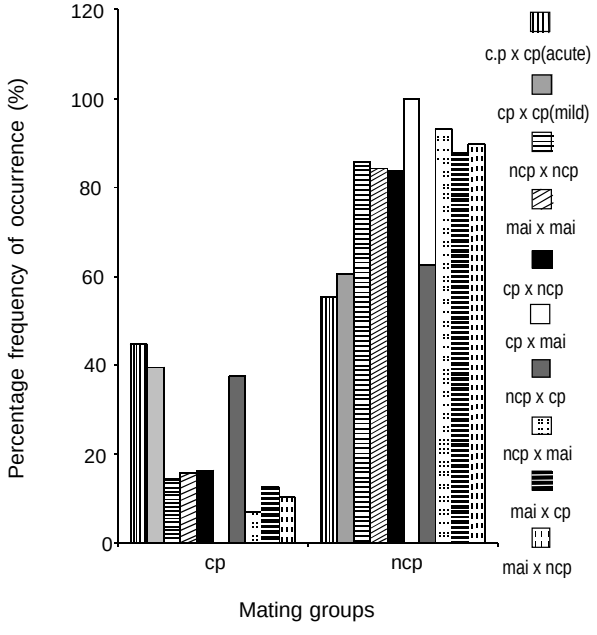

Fig. 4. Percentage frequency of occurrence of cephalic-abnormality among the fry of four strains and six intraspecific hybrids of *Clarias gariepinus* cp, cephalic; ncp, non-cephalic.

cephalic problems and could be properly paired with *C. gariepinus* in terms of chromosomes. The results obtained from this study generally showed that the traces of cephalic-abnormality are common to all strains of *C. gariepinus* regardless of the source or geographical distribution. It is, therefore, recommended that source and occurrence of cephalic abnormality (cracked-skull disease) should be the area of focus in other species of *Clarias* so as to help aquaculturists in finding total solution to this problem and possibly to overcome the problems of inbreeding in hatcheries.

Reference

Aluko, P. O., Popoola, E. O. 2002. Intraspecific hybridization studies in three wild strains of *Heterobranchus longifilis* (Valencienns, 1840). *J. Aquatic Sci.* **17:** 9-12.

Ashley, L.M., Halver, J.E., Smith, R.R. 1975. Ascorbic acid deficiency in rainbow trout and coho salmon and effects on wound healing. In: *The Pathology of Fishes*, W.E. Ribel, G.L. Migaki (eds.), pp. 769-786, The University of Wisconsin Press, Wisconsin, USA.

Awa, J.H., Alegbeleye, W.O. 1991. Occurrence and treatment of cracked-skill disease affecting *Clarias* spp. in homestead ponds in Nigeria. *J. Fish Disease* **14:** 467-473.

de Graaf, G., Janssen, H. 1996. *Artificial Reproduction and Pond rearing of the African Catfish Clarias gariepinus in Sub-Saharan Africa,* FAO Fisheries Technical Paper 362, pp. 1- 73, Food and Agriculture Organization, Rome, Italy.

Hecht, T. 1996. The culture of *Clarias gariepinus* in South Africa with comments on the subsistence aquaculture in Africa. In: *World Fisheries Congress,* pp. 121-135, Oxford and IBM Publishing, New Delhi, India.

Huisman, E.A., Richter, C.J.J. 1987. Reproduction, growth, health control and aquacultural potentials of the African catfish, *Clarias gariepinus* (Burchell, 1822). *Aquaculture* **63:** 1-14.

Kabata, Z. 1985. *Parasites and Disease of Fish Cultured in the Tropics,* pp. 1-150, Taylor and Francis, London, UK.

Viveen, W.J.R., Richter, C.J.J., Van Oordt, P.G.W.J., Janssen, J. A. L., Huisman, E. A. 1985. *Practical Manual for the Culture of African Catfish Clarias gariepinus,* pp. 1-128, Directorate General International Cooperation of Ministry of Foreign Affairs, The Hague, the Netherlands.