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Full Length Research Paper

Hybridization between Indian catfish, ♀*Heteropneustes fossilis* (Bloch) and Asian catfish, *Clarias batrachus* ♂ (Linn.)

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Success has been achieved in intergeneric hybridization between two air breathing catfishes by crossing Indian catfish, *Heteropneustes fossilis* (Bloch) and Asian catfish, *Clarias batrachus* (Linn.) by *in vitro* fertilization technique. Four experimental trials were attempted and the highest fertilization (55.44%) and hatching (50.49%) rates were observed in the experiment IV. After 90 days of rearing, the growth rate of hybrids (65.3 ± 0.78 mm) was found to be higher than *H. fossilis* (55.2 ± 3.76 mm) and less than the *C. batrachus* (75.7 ± 0.9 mm). The survival rate of hybrids (24.75%) was observed to be less than *H. fossilis* (75.70%) and *C. batrachus* (73.13%). The karyological examination revealed that the hybrids possessed intermediate chromosomes (2n=53) between *H. fossilis* (2n=56) and *C. batrachus* (2n=50). Morphologically, the hybrids resembled one of the parent's *C. batrachus* and the dorsal fin was found almost in confluent with the tail fin.

Key words: *Heteropneustes fossilis, Clarias batrachus,* intergeneric hybridization, chromosomes, *in vitro* fertilization.

INTRODUCTION

Indian catfish, *Heteropneustes fossilis* (Bloch) commonly known as 'singhi' is found in several Asian countries such as India, Bangladesh, Pakistan, Nepal, Srilanka, Myanmar and Indonesia (Smith, 1945). The Asian catfish, *Clarias batrachus* locally known as 'Magur' is a favourite food fish in India and other Asian countries including, Bangladesh, Thailand, Vietnam, Malaysia and Indonesia (Mollah and Karim,1990). Interspecific hybridization was successfully obtained in many fish and shellfish genera and families (carps, catfishes, cichlids, moronids, salmonids, sparids, sunfishes, oysters, crayfish), and others (Bakos et al., 1978; Chevassus, 1983; Refstie, 1983; Hulata, 1995; Harrell, 1997; Lawrence et al., 2000) as a means of improving production traits (growth rate, survival, disease resistance) as well as to manipulate sex ratios. Sometimes, an interspecific hybrid does not exhibit heterosis for any trait, but is still quite important for aquaculture application as it expresses a good combination of beneficial traits from both parent species (Hulata, 2001). The literature on the hybridization studies in fishes were very scanty and only very few reports are available.

Catfish hybrids were reported in *Clarias macrocephalus* × *C. batrachus* (Boonbrahm et al., 1977), *C. batrachus* or *C. macrocephalus* × *Pangasius sutchi* (Tarnchalanukit, 1986) and *Clarias gariepinus* × *Heterobranchus longifilis* (Hecht and Lublinkhof, 1985). Hybrids between *C. gariepinus* and *Clarias batrachus* were produced by Mollah and Karim (1990). Sahoo et al. (2003) successfully produced interspecific hybrids between *C. batrachus* and *C. gariepinus.* Legendre et al. (1992) reported that the reciprocal intergeneric hybrid catfish between *C. gariepinus* and *H. longifilis* can be produced. Hybridization between *H. fossilis* and *C. batrachus* were reported by Padhi et al. (1995) and Mukhopadhyay

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(1999). Khan et al. (2000) reported on the mass production of hybrid magur and evaluated its culture potential in Bangladesh.

Extensive culture of *H. fossilis* is prevalent in India, but with less growth, when compared to *C. batrachus.* The culture of *C. batrachus* was rare in India due to short supply of quality seeds. The main objective of this study was to standardize induced spawning and artificial intergeneric hybridization between female *H. fossilis* and *C. batrachus* male to find out the performance of the hybrids to overcome the problems faced in the culture of *H. fossilis* and *C. batrachus*.

MATERIALS AND METHODS

Matured brooders of *H. fossilis* were collected from the Thamirabarani river basin of Tirunelveli district in southern part of India and *C. batrachus* were collected from the Bhubaneswar district of Orissa in North India. The fishes were maintained in round cement tanks of 2 m diameter at a stocking density of 20/tank sex-wise and species-wise separately and fed twice a day at 2% of their body weight with laboratory made pellet feed containing 38.78% crude protein. Before starting, hybridization induced breeding of *H. fossilis* and *C. batrachus* were standardized. The following specimens were selected for induced breeding of *H. fossilis*: 12 females, 230 to 280 mm total length (weight 100 to 170 g); six males, 310 to 315 mm T.L. (weight 105 to 125 g) and *C. batrachus*: 12 females 250 to 300 mm T.L. (weight 100 to 150 g); six males, 270 to 288 mm T.L. (weight 120 to 160 g). The following specimens were used for the hybridization experiment; *H. fossilis*: four females, 230 to 270 mm T.L. (weight 90 to 110 g); and *C. batrachus*: four males, 280 to 300 mm T.L. (weight 230 to 300 g).

The females of both species were injected with hormone (Ovatide at 1.5 ml/kg) intramuscularly just below the dorsal fin and above the lateral line during late evening. The males and females of both species were kept in separate round plastic troughs. The top of the troughs were covered with net to prevent the jumping of the fishes. After 10 to 14 h of injection, the females were checked for the release of eggs. The eggs of female *H. fossilis* were hand stripped by gently pressing the abdomen on an enamel tray containing Hanks balanced salt solution (HBSS). Each batch of eggs consisted of 500 ± 50 numbers. The milt was collected from the male *C. batrachus* by surgically removing the testis. Sperm suspension was prepared with the help of a mortar and pestle containing HBSS. Motility was checked by adding a drop of water to the milt and observed under microscope. *In vitro* fertilization was done by gently mixing the sperm suspension with stripped eggs for 2 to 3 min after adding pond water using a bird feather. The fertilized eggs were observed under the Nikon-Zoom stereo microscope. These eggs formed the hybrid groups (*H. fossilis* × *C. batrachus*). Similarly, *in vitro* fertilization was done for *H. fossilis* and *C. batrachus* to serve as control groups. Immediately after fertilization, the eggs from different batches were maintained in separate plastic troughs of size (1.5′ × 1.0′). The sub samples (150) were used for the determination of fertilization and hatchability. The number of live eggs was estimated within 3 to 4 h after fertilization.

After 72 h of fertilization, the embryos hatched out from the fertilized eggs. Water exchange was done five times a day in the hatching trays; no aeration was provided. The embryos were nourished by the yolk sac up to 72 h after hatching. From the 3rd day, the larvae were fed *ad libitum* with *Artemia* nauplii for a period of one week and partly replaced by zooplanktons collected from the fertilized carp culture ponds for two weeks. Water was exchanged daily twice in the larval rearing tanks and the waste and uneaten food were siphoned out. After 15 days of rearing, the larvae were stocked in round cement tank and fed with frozen *Artemia* and powdered pellet feed. Water was exchanged daily in the fry rearing tanks. At the end of every month, 25 randomly sampled fish were taken for assessment of growth rate of both control and hybrids. Analysis of the data was carried out using two ways, analysis of variance (ANOVA) and Student's t-test was used to compare the mean values of control and hybrid groups.

The technique of Kligerman and Bloom (1977) was followed with simple modifications for the preparation of chromosomes from the larvae. The hatchlings were allowed to swim in a 0.005 to 0.1% colchicines solution for 4 h and cell suspension was made by cutting into small fine pieces. The cell suspension was given hypotonic treatment in 0.4% KCl solution for 30 to 45 min. After hypotonic treatment, the tissues were fixed in Carnoy's fixative containing 3:1 ratio of methanol: acetic acid for 30 min. Then, the suspension was centrifuged for 10 min at room temperature. The supernatant was decanted and again overlaid with the fixative. Then, the suspension was kept in refrigerator overnight for thorough fixation. The contents were mixed and again centrifuged for 10 min. The supernatant was decanted and the centrifugation was repeated two times. A drop of the cell suspension with fixative was dropped from a Pasteur pipette held at a height of 1 to 1.5 feet to a clean slide and gently warmed over a spirit lamp. The slides were air dried at room temperature and stained in 5% Giemsa solution for 30 min. The slides were washed with tap water, air dried and observed under Trinocular Nikon microscope (Labophot) under oil immersion objective (100x) and photographed. Well spread chromosome spreads were obtained from the larvae of *H. fossilis*, *C. batrachus* and hybrids.

RESULTS

Induced breeding of *H. fossilis* and *C. batrachus* was standardized before attempting hybridization of the two species. The mean fertilization and hatching percentage of control groups of *H. fossilis* and *C. batrachus* is presented in Table 1. *In vitro* fertilization of *H. fossilis* eggs was done using the sperm of *C. batrachus.* The fertilization and hatching percentage observed in the hybridization between *H. fossilis* x *C. batrachus* is presented in Table 2. In the experiment I, the fertilization rate achieved was only 3.3% due to poor condition of the female; maybe the eggs stripped were immature. Almost all the embryos died just before hatching. In the experiment II, the fertilization rate achieved was 36.66%. The fertilized eggs hatched out after 19 to 20 h of fertilization at a water temperature of 26±1°C. After 24 h, the mortality noticed among the hatchlings may be due to sudden fall of temperature (22 ± 2°C) of water in the hatching troughs in the early morning because heavy rain due to cyclone weather. In the experiment-III, the fertilization rate achieved was 43.33%. The fertilized eggs hatched out 19 to 21 h after fertilization at a water temperature of 26±1°C. The hatching rate was reduced to 26.66%. In experiment-IV, the fertilization rate achieved was 55.44%. The fertilized eggs hatched out after 20 to 22 h after fertilization at a water temperature of 26±1°C. The hatching rate observed was 50.49%.

The survival rates of *H. fossilis*, *C. batrachus* and hybrids are presented in Table 3. After 30 days of rearing, the survival rate of *H. fossilis*, *C. batrachus* and hybrid were 85.98, 84.91 and 45.54% and after 90 days,

Table 1. Mean percentage of fertilization and hatching of the control groups of *H. fossilis* and *C. batrachus.*

Species	Fertilization (%)	Hatching (%)
H. fossilis	78.20 ± 5.8	77.52 ± 7.0
C. batrachus	76.33 ± 5.8	79.69 ± 9.4

Table 2. Fertilization and hatching percentage of hybrids (*H. fossilis* x *C. batrachus*) at different experimental trials.

Trial	Female *H. fossilis*		Male *C. batrachus*		Fertilization %	Hatching %
	Length (mm)	Weight (g)	Length (mm)	Weight (g)		
Expt-I	240	95	290	280	3.3	-
Expt-II	250	100	280	230	36.66	-
Expt-III	230	90	295	270	43.33	26.66
Expt-IV	270	110	300	300	55.44	50.49

Table 3. Survival rate of *H. fossilis*, *C. batrachus* and hybrids (*H. fossilis* x *C. batrachus*) after 90 days of rearing period.

Day	Survival (%)		
	H. fossilis	*C. batrachus*	Hybrid
30	85.98	84.91	45.54
60	80.84	78.79	35.64
90	75.70	73.13	24.75

Table 4. Mean growth of larvae (mm) of *H. fossilis, C. batrachus* and hybrids (*H. fossilis* x *C. batrachus*) after 90 days of rearing period.

Group	Number of days		
	30 mm	60 mm	90 mm
H. fossilis	11.9±0.94	30.7±0.90	55.2±3.76
C. batrachus	14.1±1.14	43.6±1.11	75.7±0.90
Hybrids	12.6±1.28	39.2±1.08	65.3±0.78

***No significant difference (P<0.05) in the growth of *H. fossilis*, *C. batrachus* and hybrids during the early stage of growth.

the survival rate reduced to 75.70, 75.13 and 24.75%, respectively.

The growth rate of *H. fossilis*, *C. batrachus* and hybrids is presented in Table 4 and Figure 1. The growth rates of hybrids were found to be intermediate (65.3±0.78 mm) between *H. fossilis* and *C. batrachus*. The growth rate of C. *batrachus* was found to be greater (75.7 ± 0.9 mm) than that of the *H. fossilis* (55.2 ± 3.76 mm) after rearing period of 90 days but there was no significant difference (P<0.05) in the growth of the *H. fossilis*, *C. batrachus* and hybrids during the early stage of growth.

The karyological investigation revealed that, the diploid chromosome number of *H. fossilis* was found to be 2n=56 and *C. batrachus* was found to be 2n=50. The chromosomal number of the hybrid (*H. fossilis* × *C. batrachus*) was found to be intermediate between this two species 2n=53 (Figures 5 to 7).

DISCUSSION

In agreement to the present study, Padhi et al. (1995) reported that the reciprocal hybrids between *H. fossilis* and *C. batrachus* can be produced and the fertilization

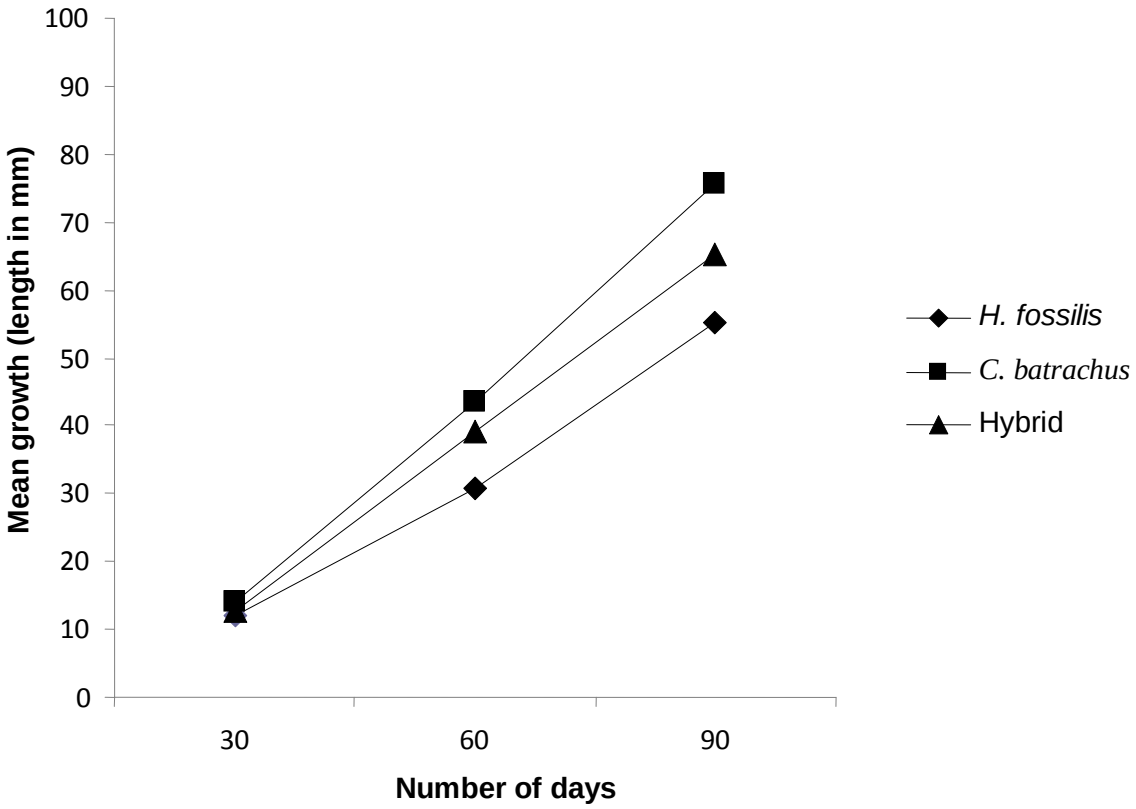

Figure 1. Mean growth of *H. fossilis*, *C. batrachus* and hybrids (*H. fossilis* x *C. batrachus*) for a period of 90 days.

rate varied between 60 and 75 % but their rate of survival reduced (0.8 and 0.9%) due to high rate of mortality of the hatchlings when the transition from endogeneous to exogeneous feeding take place. Mukhopadhyay (1999) made a similar study and reported the survival of hybrids of female *C. batrachus* and male *H. fossilis* was very poor and most of the embryos died during embryogenesis or after hatching. Only two viable fry were produced out of which one survived for one month. The reciprocal crosses between female *H. fossilis* and *C. batrachus* male met success only up to hatching stage and concluded that the large scale mortality of hybrids occurs either before or after hatching which indicates incompatibility of their respective genome. On the contrary, in the present study encouraging results were obtained and more than 50% of fertilization and hatching rates were observed among the hybrids. Discouraging results were reported in other catfish species by Nam et al. (2001) who stated that the intergeneric hybrids (female catfish, *Silurus asotus* x male mud loach *Misgurnus mizolepis*) did not hatch although the embryonic development of hybrid proceeded until late gastrula stage.

Legendre et al. (1992) concluded that the survival of hybrids *H. longifilis* x *C. gariepinus* (6±2.8%) was very low and was strongly influenced by the maternal parent. The growth result of hybrids showed faster growth than *H*. *longifilis* and was considerably similar to that of *C. gariepinus.* These results are similar to the present study that, hybrids show faster growth rate than the *H. fossilis* and hybrids are more profitable than *H. fossilis* for fish culture.

According to Hubbs (1955), hybrids are intermediate for those characteristics which distinguish the parents but a bias towards one parents or the other is very common. In the present study, the hybrids of female *H. fossilis* and male *C. batrachus* showed a characteristic external morphology of *C. batrachus* and the dorsal fin almost confluent with the tailfin (Figures 2 to 4). Similar observation was reported by Padhi et al. (1995) that in the reciprocal hybrids between *C. batrachus* and *H. fossilis*, all the progeny were found to have a long dorsal fin and closely resembled morphologically to one of the parent's *C. batrachus*. Mollah and Karim (1990) reported that hybrids between female *C. batrachus* and male *C. gariepinus* are phenotypically similar to *C. batrachus*, but the growth rate is similar to that of *C. gariepinus*.

The chromosomal complement studied among different groups of catfishes was found to be in agreement with the report of Padhi et al. (1995). Similar chromosome count in the hybrids (2n=53), and the average of the chromosomal number of the two parental species was

Figure 2. Morphology of *H. fossilis* (90 days old).

Figure 3. Morphology of *C. batrachus* with long dorsal fin confluent to tail (90 days old).

Figure 4. Morphology of hybrid (*H. fossilis* x *C. batrachus*) with long dorsal fin confluent to tail similar to that of *C. batrachus* (90 days old).

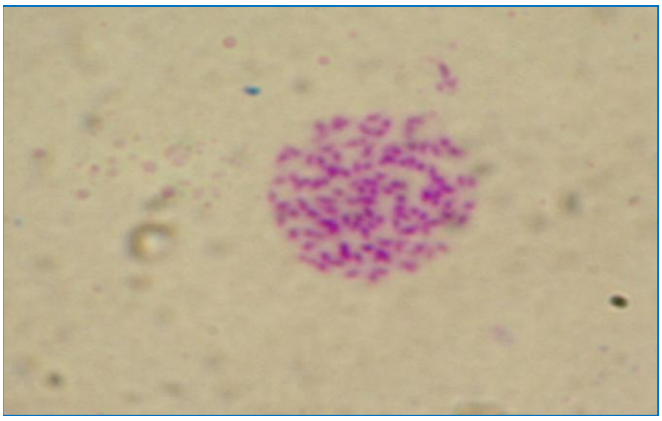

Figure 5. Metaphase chromosomes (100X) of *H. fossilis* (2n=56).

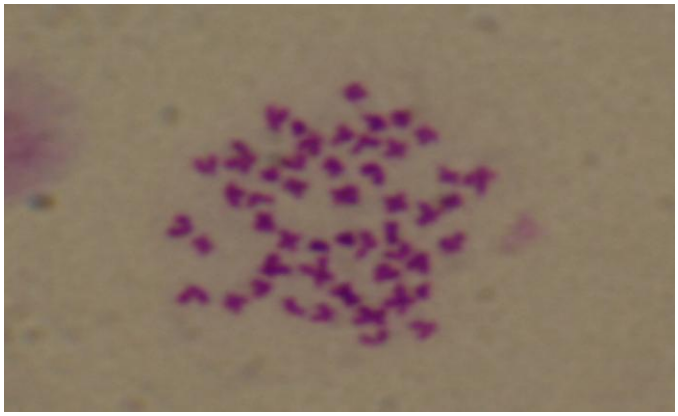

Figure 6. Metaphase chromosomes (100X) of *C. batrachus* (2n=50).

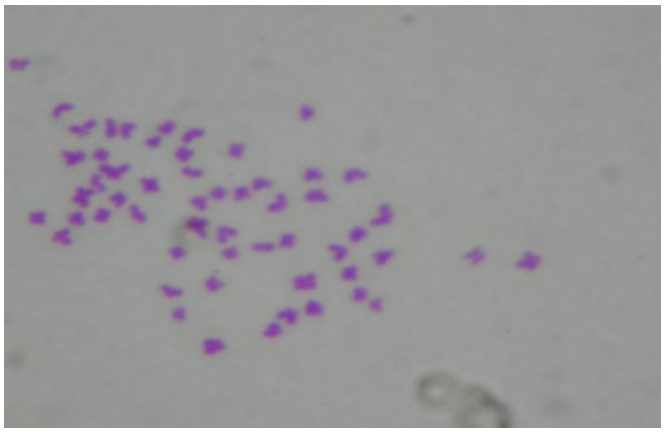

Figure 7. Metaphase chromosomes of hybrid catfish (*H. fossilis* x *C. batrachus*) (2n=53).

found. Thus, the study indicates that successful intergeneric hybrids can be produced among catfishes

using artificial *in vitro* fertilization techniques. Further study is required for large scale production of catfish hybrids that can be exploited for commercial catfish culture.

REFERENCES

Bakos J, Krasznai Z Marian T (1978). Cross-breeding experiments with carp, tench and Asian phytophagous cyprinids. Aquacult. Hungarica 1:51-57.

Boonbrahm M, Tarnchalanukit W, Suraniranat P (1977). Experiments of hybridization of freshwater catfish, *Clarias macrocephalus* Gunther and *Clarias batrachus* L. Research report. Kasetsari University, Bangkhem, Bangkok.

Chevassus B (1983). Hybridization in fish. Aquaculture 33:245-262.

Harrell RM (1997). Hybridization and genetics, pp. 217-234, in Striped Bass and other Morone Culture, edited by R.M. Harrell. Elsevier Science Publishers, Amsterdam,The Netherlands.

Hecht T, Lublinkhof W (1985). *Clarias gariepinus* x *Heterobranchus longifilis* (Clariidae) Pisces): a new hybrid for aquaculture. South African J. Sci. 81:620-621.

Hubbs CL (1955). Hybridization between fish species in nature. Systematic Zool. 4:1-20.

Hulata G (1995). A review of genetic improvement of the common carp Cyprinus carpio L.) and other hybrids by crossbreeding, hybridization and selection. Aquaculture 129:143-155.

Hulata G (2001). Genetic manipulations in aquaculture: a review of stock improvement by classical and modern technologies. Genetica 111:155-173.

Khan MMR, Mollah MFA, Ahmed GU (2000). Mass production of hybrid magur and its culture potential in Bangladesh. Aquacult. Res. 31:467-472.

Kligerman AD, Bloom SE (1977). Rapid chromosome preparations from solid tissues of fishes. J. Fish. Res. Board Can. 34:266-269.

Lawrence CS, Morrissy NM, Vercoe PE, Williams IH (2000). Hybridization in Australian freshwater crayfish production of all-male progeny. J. World Aquacult. Soc. 31:651-658.

Legendre M, Teugels GG, Cauty C, Jalabert B (1992). A comparative study on morphology, growth rate and reproduction of *Clarias gariepinus* (Burchell, 1822), *Heteropneustes longifilis* Valenciennes, 1840, and their reciprocal hybrids (Pisces, Claridae). J. Fish. Biol. 40:59-79.

Mollah MFA, Karim M (1990). First record of induced breeding of African Magur Clarias gariepinus (Burchell) in Bangladesh. J. Inland. Soc, Ind. 22:52-54.

Mukhopadhyay SK (1999). Survival of hybrids between two Indian air-breathing catfishes *Clarias batrachus* (female) x *Heteropneustes fossilis* (male) and their reciprocal crosses. J. Inland. Soc. Ind. 31(1):60-63.

Nam YK, Cho HJ, Im JH, Park IS, Choi GC, Kim DS (2001). Production of all-female diploid and triploid far eastern catfish, *Silurus asotus* (Linnaeus): survival and growth performance. Aquacult. Res. 32:991-997.

Padhi BK, Datta P, Mandal RK (1995). Reciprocal hybridization between two freshwater catfishes: *Heteropneustes fossilis* (Bloch) and *Clarias batrachus* (Linn.). Ind. J. Exp. Biol. 33:433-436.

Refstie T (1983). Hybrids between salmonids species. Growth and survival in seawater. Aquaculture 33:281-285.

Sahoo SK, Giri SS, Sahu AK, Ayyappan S (2003). Experimental hybridization between *Clarias batrachus* (Linn.) x *Clarias gariepinus* (Bur.) and performance of the offspring in rearing operations. Asian Fish. Sci. 16:157-166.

Smith HM (1945). The freshwater fishes of Sian or Thailand. United States National Museum Bulletin, 188, Smithsonian Institute, Washington, D.C., 622 pp.

Tarnchalanukit W (1986). Experimental hybridization between catfishes of the families Clariidae and Pangasiidae in Thailand. Environ. Biol. Fish. 16:317-320.