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Hybridization between threatened freshwater catfish *Mystus gulio* (Hamilton & Buchanan) and *Mystus montanus* (Jerdon) by artificial fertilization

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Inter-specific hybrids were produced between the threatened catfish species *Mystus gulio* × *Mystus montanus.* The differences in percentage of fertilization and hatching between control and interspecies were significant. The survival of hybrid was significantly lower (24.80 ± 4.3%) when compared to control (95.1 ± 3.5%). Time difference in yolk absorption by hybrid (73.30 h) was higher than that of control (72 h). When compared to interspecific fertilized egg the hatching time (24-25 h) and viability of larvae of the control were significantly better. In hybrids more deformed hatchlings (52.7 ± 4.2 %) were noticed than the control (24.80 ± 4.3%).

Keywords: Artificial fertilization, Hybridization, *Mystus gulio, M. montanus*, Threatened catfish

In aquaculture practices, hybrids play a pivotal role for their faster growth, survival, colour, fin shape and structure and improvement of genetic strains[1-3]. Interspecific hybridization of different genetic types is an alternative to conventional selective breeding of fishes to produce qualitative or quantitative changes in commercial traits[4]. Interspecific cross-breeding may produce hybrids with precious characteristics like sterility, monosex, disease resistance and enhanced growth for their culture. Interbreeding with parental species of hybrid will come from competition, predation, genetic introgression of gene from one species to another species[5,6]. Hence, artificial hybridization between catfishes viz, *Clarias gariepinus* × *Heterobranchus longifilis*[7], *C. batrachus* × *Heteropneustes fossilis*[8], *H. fossilis* × *H. microps*[9] and *Mystus cavasius* × *M. seenghala*[10] and *Clarias macrocephalus* × *C. gariepinus*[11] has been attempted. African catfish *Clarias gariepinus* × *C. macro cephalus* hybrid created by artificial cross males of an alien species of Thai walking catfish (*C. gariepinus*) with females of native species (*C. macrocephalus*) are attractive to farmers in Thailand[12] The present investigation on interspecific hybridization deals with two threatened freshwater catfishes[13] viz, *Mystus montanus* and *M. gulio*, commonly known as "Narikeluliru" and "Manchakeliru" which are important food fish in Tamilnadu and Kerala[14] and to evaluate their growth performance with an ultimate objective of commercial culture and conservation.

Materials and Methods

Mystus montanus (10-12 cm; 100-120 g) and *Mystus gulio* (20-25 cm; 150-200 g) were collected from river Tamirabarani, Tirunelveli Dist, Tamilnadu (8.44°N, 77.44°E) during May 2006. The collected individuals were transported to CARE Aquafarm and acclimatized in earthen ponds (7.5m × 5m × 1.5m). During the stocking period, the brooders were fed with finely chopped chicken intestine *ad libitum*[15]. Matured *M. montanus* (6 males and 3 females) and *M. gulio* (6 males and 3 females) were selected by sexual dimorphism for artificial fertilization (Figs 1 and 2). Profused spawning was induced by a single intramuscular injection of ovaprim (Syndel Laboratory, Canada) of 0.5 ml/kg body weight to both males and females (105–138g) and were kept separately in cement tanks (500 litres).

The eggs were collected from *M. montanus* by stripping method. The testes were dissected out from *M. gulio* and ground in a glass homogenizer using 0.7% sodium chloride as extender [8] and the milt was applied to a bowl containing *M. montanus* eggs. Water was added after 2 min and the eggs and the milt were mixed well. The ova and spermatozoa were incubated together for 10-15 min at room temperature (28ºC ±1ºC) in petridishes to allow fertilization. The

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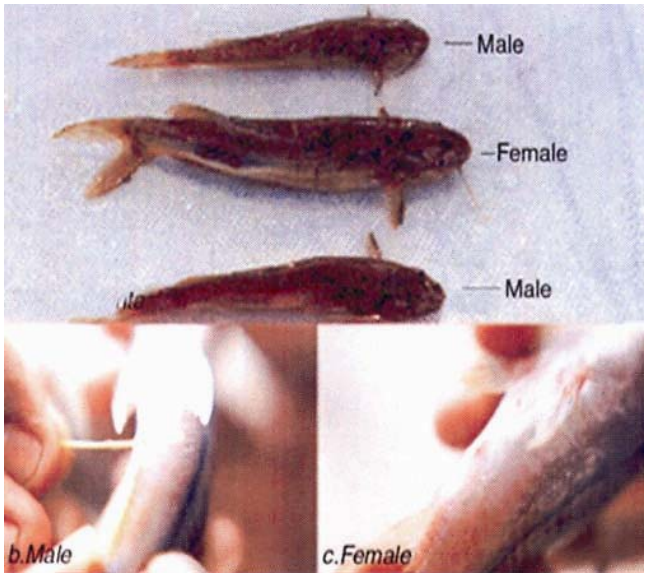


Fig.1—Sexual dimorphism of *Mystus montanus*

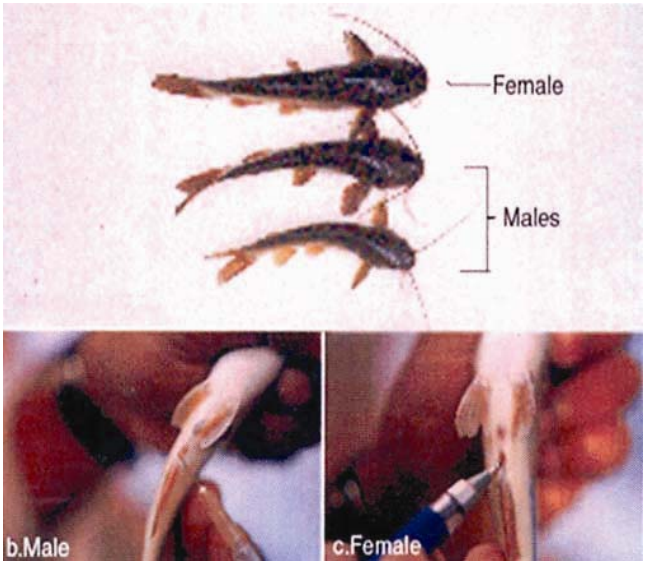


Fig.2—Sexual dimorphism of *M. gulio*

Table 1—Details of artificial fertilization of hybrid and control [Values are mean ± SD from 5 observations]

Weight of spawner (g)		Ovaprim	Fertilization	Incubation	Hatching	Survival
Female	Male	(ml/kg)	(%)	period	(%)	(%)
Control 105 *M.montanus*	98 *M.montanus*	0.5	95.1± 3.5[a]	22 ± 0.51	80.2 ± 5.2	57.06 ± 4.3[a]
Control 138 *M.gulio*	120 *M.gulio*	0.5	90 ± 0.8[a]	23 ± 0.47	78 ± 6.53	82 ± 1.41[a]
Interbreed120 *M.gulio*	96 *M.montanus*	0.5	78.3 ± 5.2[b]	24 ± 0.33	52.7 ± 4.2	24.80 ± 4.3[b]

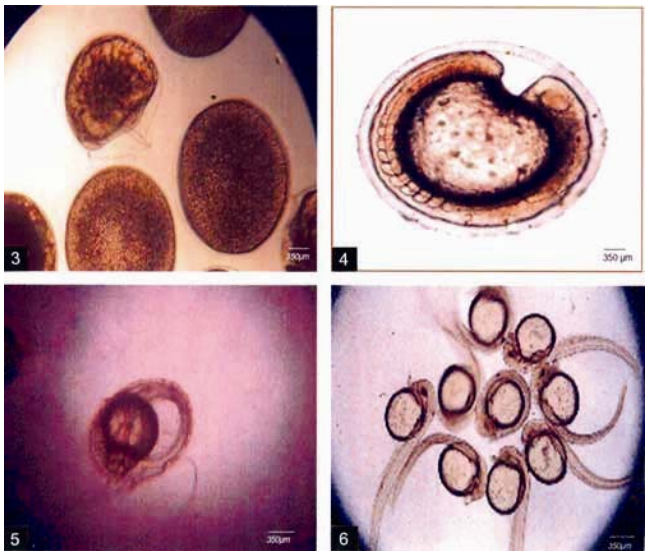
Values shown under similar alphabets did not significantly vary at $P<0.001$ and <0.05 level by one way ANOVA and Turkey's multiple range test

excess sperm suspension was washed out two times using distilled water. The fertilized eggs were collected from the bowl and grouped into six batches of 100 each in separate petridishes[15]. The percentage of fertilization was calculated from the fertilized eggs[16]. The percentage of hatching was estimated from the number of hatchlings following Lagler[17]. The developmental stages were observed under Nikon microscope (U III E-400 Eclipse). The data were subjected to one-way ANOVA and Turkey's multiple range tests[18].

Results and Discussion

In the present study, the incubation period showed a slight difference between the hybrid (24 h) and the control (22 h). But according to Sridhar and Haniffa[9] the incubation period was the same (24 h) in *H. fossilis* × *H. microps* hybrids and control *H. fossilis*. Glamuzine *et al.*[19] reported that the embryonic development was slightly faster throughout the embryogenesis of the hybrid *Epinephelus costae* × *E. marginatus*.

The control as well as the cross fertilized eggs (Fig. 3) were transparent and adhesive in nature. The number of eggs fertilized by the control (95.1 ± 3.5%) was higher than the interspecific cross (78.3 ± 5.2%) (Table 1). After the fertilization, the first cleavage took a longer duration of 0.35 h in hybrids whereas only 0.25 h in the control. Eight somatic stage was reached after 9 h (Fig 4). The appearance of heart rudiments was observed after



Figs 3–6: 3—Fertilized eggs; 4—9 hr old embryo; 5— Just hatched hybrid hatchling; 6— One day old hatchlings

Figs 7–10: 7—Two day old hatchling; 8— Three day old hatchlings; 9— Abnormal head of hybrid hatchling; 10— Deformed hatchling showing blunt tail and lack of barbells

17 h in the control as well as the hybrids. After 24 h, hatching occurred both in the control and hybrid (Fig 5).

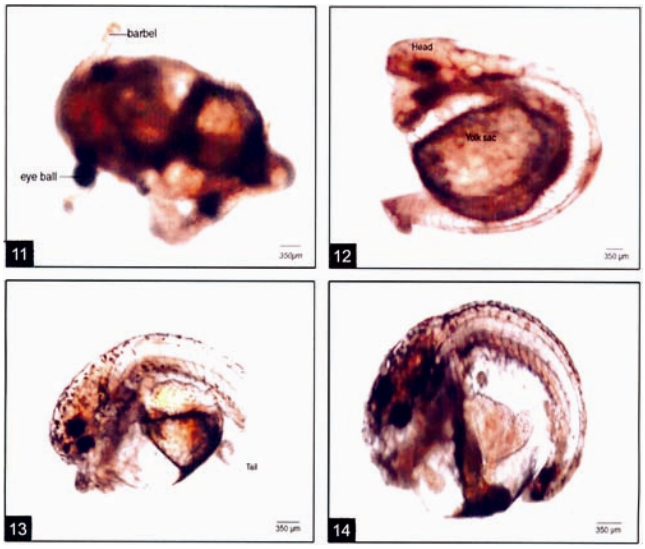
The development of the hybrids was slightly slower than that of the control in the present study (Table 2). When compared to the control, the hybrid mortality was severe (63%) during the early developmental stages especially during the hatchling stage and the difference in survival was statistically significant (P<0.05) between the control (69.53 ± 2.85 %) and the hybrids (24.80 ± 4.3 %)

Table 2—Developmental stage of interspecific fertilized egg and control (*M. montanus* and *M. gulio*).

Developmental stage	Time after fertilization (h)		
	Hybrid	*M. montanus*	*M.gulio*
Blastodisc formation	00.25	00.20	00.20
1st cleavage	00.35	00.25	00.25
2nd cleavage	00.50	00.30	00.30
4 cell stage	01.05	00.40	00.40
8 cell stage	1.20	01.00	01.05
16 cell stage	1.50	1.25	1.25
32 cell stage	2.10	1.45	1.55
Morula stage	3.25	2.50	2.55
Yolk plug stage	5.10	4.30	4.20
Elongation of yolk mass	6.40	6.00	6.00
Appearance of embryonic rudiment	9.20	8.50	8.20
8 somite stage	10.50	10.20	10.25
Appearance of optic rudiment (10 somite stage)	11.15	10.30	10.40
12 somite stage	12.30	11.05	11.20
Elongation of tail	14.00	13.25	13.35
Appearance of heart rudiment	18.20	17.15	17.45
Appearance of pectoral fin buds	19.10	18.20	18.40
Twisting movement of the embryo	22.00	21.30	21.50
Just hatched larva	24-25	23-24	24
Yolk absorption	73.30	72.00	72

(Figs 6 and 7). Hybrid deformities were noticed both in embryos and hatchlings. The deformities included abnormal head (Fig. 9), bent tail, bent trunk and lack of barbels (Fig. 10), protrusion of eye ball and shrinkage (Fig. 11), curved spinal cord (Fig. 12), rudimentary tail (Fig. 13), blunt trunk and lack of caudal fin (Fig. 14). Similar deformities were noticed among hybrids of *C. batrachus* × *C. gariepinus*[20] and *M. cavasius* × *M. seenghala*[10]. After 74 h when the yolk sac was absorbed the hybrid post larvae attained 2.8 mm length whereas the control reached only 2.4 mm length (Fig. 8). The fertilization frequency of the interspecific fertilized eggs in the present study (78%) was slightly higher when compared to earlier reports on *H. fossilis* (50% and above) using D-Lys65 GnRH-A[21] and *M. cavasius* × *M. seenghala* 75%[10].

In the present, study the survival of interspecific fertilized hatchlings was comparatively less (24%) when compared to the control (57%) and the difference was statistically significant (P <0.01). The survival of the viable hybrids was strongly influenced



Figs 11–14: 11—Protrusion of eye ball and shrinkage of hybrid hatchling; 12— Curved spinalcord of hybrid hatchling; 13—Rudimentary tail of hybrid hatchling; 14— Bent trunk and lack of caudal fins of hybrid hatchling

by their maternal parent[7]. Laywonyawu *et al*[22] reported that post larval performance of the hybrid produced from the crosses between *C. macrocephalus* (female)) × *C. gariepinus* (male) was better than the reciprocal *C. batrachus* × *C. gariepinus* hybrids which have not reached fry stage and took more than 6 months[20]. The other cross was performed between air breathing catfishes *C. batrachus* (female) × *H. fossilis* (male) and the produced hybrids survived upto 8 months[23]. But in the present study, the hybrid hatchlings survived till the absorption of yolk sac (73 h) and after that they died within five days. Death could be attributed to incomplete mouth formation and lack of feeding. Further studies on hybridization between *M. montanus* (male) and *M. gulio* (female) are in progress to arrive a conclusion.

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