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Hybridization of *Clarias* spp. and *Heteropneustes fossilis* in Tripura, India

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Authors' contributions

This work was carried out in collaboration between both authors. Author RKS conceptualized the study, designed the study, performed the literature searches, wrote the protocol and wrote the first draft of the manuscript. Author HS managed the analyses of the study and revised the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

The study was conducted to evaluate the viability of hybridization of different species of catfishes found in India. During different interspecific and intergeneric hybridization, maximum fertilization was found in cross II i.e. *Clarias gariepinus* ♂ x *Clarias batrachus* ♀ with 80% success and minimum success (40%) was encountered in cross IV (*Clarias gariepinus* ♂ x *Heteropneustes fossilis* ♀). Incubation period varied from 21 to 34 hours in different crosses with 35-75% hatching success. Incubation of eggs were done in modified 'Flow-through System'. The absorption of yolk sacs in all the crosses was noticed within 4-5 days time of development. All the larvae were fed with mixed zooplankton from 2nd day onwards (before absorption of yolk sac) which accelerated the survival rates of the larvae. A formulated feed was also used to feed the larvae. Maximum survival rate was found in cross II from 0 to 15 days of larval rearing. Growth performance was also encouraging in the cross II after 15 days of rearing as compared to other crosses.

Keywords: Catfish; propagation; hybridization; ovaprim.

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1. INTRODUCTION

The Asian catfishes, *Clarias batrachus* , *C. macrocephalus* and *Heteropnuestis fossilis* are the most important species cultured in Asia, while the sharp tooth or the Nile catfish *Clarias gariepinus* (= lazera) is the cultured species in Africa [1,2]. [3] observed that *C. gariepinus* will grow to an average 200 g in 60 days of rearing whereas the local magur (*C. batrachus*) will take one year to reach that weight and *H. fossilis* is expected to attain an average weight of about 60 gm within 6 months of rearing period [2].

In Tripura, *Clarias batrachus and H. fossilis* are the most valued table fish among the different air-breathing catfishes. However, of late, a significant decline in both the population are noticed in open waters systems like derelict and swampy areas, due to severe habitat stress like over exploitation, pollution, rapid siltation, etc.

Further, an exotic species of magur i.e., African magur (*Clarias gariepinus*) has taken illegal entry into Tripura from neighbouring country Bangladesh through private pisciculturists [4]. The species has created a great sensation among local aqua culturists for its fast growth rate and many aqua farmers have taken to its culture as well. Owing to its diversified feeding habit, the fish has got itself established in local waters and posing a threat to the native fish species of the State. At present, most of the *Clarias* catfish farmers in Southeast Asia produce exclusively hybrids [5].

On the other hand, very little information is available regarding hybridization of these air-breathing catfishes. However, hybridization between *Clarias batrachus* and *Heteropneustes fossilis* has been reported by [6,7]. [8] has reported the successful hybridization of *Clarias gariepinus* with local *Clarias batrachus* in Bangladesh. [9] have succeeded in crossing the native catfish (*C. macrocephalus)* with African catfish (*C. gariepinus)* using HCG in Philippines. [10] has indicated that the possibilities of interspecific and intergeneric hybridization of these species for producing a valid species in Tripura. The study was conducted to evaluate the hybridization success of different catfishes in Tripura condition.

2. MATERIALS AND METHODS

The present investigation performed in the Laboratory of Tripura Fisheries Training Institute (TFTI), Udaipur, South Tripura during the period of 26.5.1997 to 18.5.1997.

2.1 Brood Stock and Hormone Treatment

Matured healthy males and gravid females were collected from the TFTI farm and wild (Harijala wetland, Udaipur, Tripura) during breeding season (May-June). They were acclimatized in the laboratory condition for a week before breeding in a plastic pool (6 ft. dia. x 3 ft. height) with the water level of 50 cm and running water facility. Feeding was done with formulated feed consisting of decomposed mustard oilcake + fish meal + rice bran + yeast + multiplex. Occasionally *C. gariepinus* was fed with freshly chopped flesh of freshwater mussel (*Lamellidens marginalis)*.

The hormone treatment was conducted in two phases, first on 26.5.97 (6 pm to 7 pm) and second on 30.5.97 (6.30 pm to 7 pm) in the TFTI laboratory. Ovaprim were injected at a dose of 1.0, 1.5 and 2.0 ml/kg of female fish and 0.5 and 1 ml/kg of male fish between the base of the genital papilla and anal fin (intramuscular). Special type of tuberculin sterile syringe and precision glide needle (1 cc 26 G1/2) were used for the injection. After administration of hormones, the fish were kept species-wise in the big size aluminium hundy for induction of the brooders. One male fish of all the species were kept in each hundy.

2.2 Preparation of Milt Suspension

Milt suspension was prepared from the testis with seminal vesicles of the excited males. Male fish were anaesthetised with clove oil (@50µl/l water) before dissection. Testis with seminal vesicles was taken out species-wise by dissecting the abdomen. Immediately the dissected testis was macerated and stored the milt in 0.9% sodium chloride solution for fertilization of stripped eggs for interspecific and intergeneric hybridization.

2.3 Stripping and Hybridization

After 12-14 hours of second injection, the fully responded females were stripped species-wise and eggs were collected in a series of disinfected (with $KMnO_4$) dried enamel tray (47 x 33 x 8 cm^3 and 36 x 31 x 6 cm^3).The preserved milts were immediately mixed with the stripped ova for the

fertilization by adding little amount of water in the following combinations:

Pure cross

Cross I : *C. batrachus* ♂ x *C. batrachus* ♀

Interspecific cross

Cross II: *C. gariepinus* ♂ x *C. batrachus* ♀
Cross III: *C. batrachus* ♂ x *C. gariepinus* ♀

Intergeneric cross

Cross IV: *C. gariepinus* ♂ x *H. fossilis* ♀
Cross V: *H. fossilis* ♂ x *C. gariepinus* ♀

After fertilizing the ova, sperm dilution were carried out 2-3 times by adding seasoned tap water for proper washing to accelerate the development of eggs. Stripping was started at 7 am and completed the mixing of milts in different combinations within 9 am on 31.5.97. After stripping, all the female and undissected male brood fishes were kept in a dilute $KMnO_4$ solution and released in the plastic pool. All the trays with fertilized eggs were left undisturbed by putting clean water for 30 minutes.

2.4 Incubation of Eggs

All the labelled fertilized eggs were transferred carefully to the modified 'Flow-through System' within 1 hour and marked accordingly. Before putting the eggs, the hatchery was cleaned thoroughly with $KMnO_4$ solution. Continuous feeble water flow @ 6-7 ml/second was maintained for over all cleanliness of each bowl. Disinfected (with $KMnO_4$ or formalin dilute) *Hydrilla* plants were used as the substrate for the hatching. Aeration was provided in all the bowls for maintaining desired O_2 level (>5 mg/l) for proper development and hatching. Fertilized eggs of the crosses were examined under microscope to note all the developmental stages.

2.5 Estimation of Fecundity, Fertilization Rate and Hatching Rate

2.5.1 Fecundity

For estimation of fecundity, 5 ripe female fish was randomly selected. In the present study, fecundity of various sizes of fish was estimated by gravimetric method. The fecundity of 5 specimens was computed by counts of ova in 5 samples of 1.0 g from each ovary following standard method.

2.5.2 Fertilization rate

The transparent eggs were considered as fertilized ones whereas the opaque eggs were considered as dead eggs. The fertilization rate was calculated with the following formula by randomly sampled 100 - 150 eggs from each bowl:

Fertilization rate (%) = (Number of fertilized eggs/ Number of total eggs) × 100

2.5.3 Hatching rate

The rate of hatching was calculated by the following formula:

Hatching rate (%) = (Number of hatchling/ Number of total eggs) ×100

2.6 Formulation and Preparation of Feed

Seven ingredients were used to prepare the feed for larval rearing. First of all, poultry eggs were boiled and crushed properly. Then vitamin-B complex forte with vitamin-C (5 mg), yeast (5 mg), glucose-D (10 mg), starch (100 mg), amoxicillin-dispersible (10 mg) and multiplex (mineral mixture) (10 mg) were dissolved in 100 ml of distilled water and then mixed with 100 g of crushed poultry eggs. After mixing all the ingredients, the feed mixture was cooked for 10-15 minutes. After cooling, the cooked product was passed through a household mincer having 2 mm diameter aperture. The feed strands were dried in an oven at 50ºC to contain about 15% moisture, packed in dried polythene bag and stored in a dry place.

2.7 Larval Rearing

After 2 days of hatching, the hatchlings were shifted to the different size aquarium (56 x 25 x 28 cm^3, 57 x 30 x 29 cm^3, 76 x 31 x 31 cm^3, 83 x 30 x 47 cm^3 and 92 x 46 x 46 cm^3) with 6 inch water depth. Provided with cleaned *Pistia* plants and stones as hiding place for the fish. Aeration was provided to maintain dissolved oxygen.

Every day during 4-5 pm, fish larvae were fed with natural foods, i.e. minute zooplankton. Occasionally, prepared artificial feed was also given up to 10 days of rearing. After 10 days of rearing, the larvae were transferred into specially

prepared outdoor cement cistern (382 x 99 x 90 cm^3), big size aquarium (185 x 48 x 48 cm^3) and plastic pool (6 ft. dia. x 3 ft. height) for further development of the larvae and fed with zooplankton and formulated feed @ 5% body weight or as demand feed during evening hours.

2.8 Water Quality Management

Different water quality parameters viz., temperature, pH and dissolved oxygen (DO) content were monitored during incubation of injected brooder / hatching / larval rearing by using mercury - in - glass Celsius thermometer (0-50ºC), GripH meter (Systronic Company make) and Aqua Merck Kit respectively.

For maintaining water quality in different steps, the 0.01 mg/l $KMnO_4$, 2 mg/l dispersible Amoxicillin, 100 mg multiplex per litre of water were used (Table 1).

Every day morning, leftover feed as well as faecal matters were collected through siphoning from the larval rearing aquarium and 50% water was replaced by freshwater every two days.

3. RESULTS

3.1 Response of Inducing Hormone

Effects of various doses of ovaprim on *Clarias* spp. and *Heteropneustes fossilis* for gonadal maturation are given in Table 2. The results indicate that a single dose of ovaprim was sufficient enough to induce both male and female of all species. In case of *Clarias gariepinus* female, a dose of 1.5 ml/kg whereas in *Clarias batrachus* and *Heteropneustes fossilis* 2.0 ml/kg weight of fish were sufficient for proper free ovulation after 12-14 hours of injection. However, the lower dose of 1.0 ml/kg of female fish was not effective enough for oocytes maturation in all the species. In *Clarias batrachus* and *Heteropneustes fossilis*, even higher dose of 1.5 ml/kg body weight was not sufficient enough. Free or natural spawning was observed in *Clarias gariepinus* at a dose of 2.0 ml/kg of body weight of female after 7-8 hours of injection. A minimum dose of 0.5 ml/kg of male fishes of all the species was required for proper excitement of the sperms and also for getting proper fertilization during artificial insemination.

3.2 Hybridization

Table 3 represents the artificial fecundation of pure cross of *Clarias batrachus* and the interspecific and intergeneric hybridization between *Clarias* spp. and *Heteropneustes fossilis*. Latency period was found to be varied between 12 to 14 hours after injection in all the species with water temperature 28-30ºC (Mean 29.0ºC), pH 7.5-8.0 (Mean 7.9) and DO 4.0-6.5 mg/l (Mean 5.2 mg/l) (Table 4). The percentage of fertilization varied from 40 to 85% in all cross. The pure cross of *Clarias batrachus* showed the highest fertilization percentage (85%) followed by 80% in the cross of *Clarias gariepinus* ♂ x *Clarias batrachus* ♀. The lowest fertilization percentage i.e. 40% was observed from the cross of *Clarias gariepinus* ♂ x *Heteropneustes fossilis* ♀. After hybridization, eggs received from cross of *Clarias batrachus* ♂ x *Clarias gariepinus* ♀ and *Heteropneustes fossilis* ♂ x *Clarias gariepinus* ♀ were found to be more adhesive than the others.

3.3 Incubation and Hatching

Table 5 also reveals that the hatching percentage ranged from 35% to 75% in different crosses with water temperature 27.0 to 29.0ºC, pH 8.0-8.2, and DO 5.0-8.0 mg/l. But in case of cross III i.e. male *Clarias batrachus* ♂ x *Clarias gariepinus* ♀ and cross IV i.e. male *Clarias gariepinus* ♂ x *Heteropneustes fossilis* ♀, hatchlings were not observed though the fertilization success was 40-50%. First hatching from fertilized eggs was found in cross V (*Heteropneustes fossilis* ♂ x *Clarias gariepinus* ♀) after 21 hours 10 minutes of fertilization and complete hatching was observed within 26 hours with 35% success, which was lowest among the different crosses. Maximum hatching success was exhibited in cross I (Pure) i.e. 75% and complete hatching occurred between 25 to 34 hours of post-fertilization. In case of cross II, first hatching was noticed after 23 hours 10 minutes of fertilization with 65% hatching and completed within 33 hours. During hatching water temperature ranged from 27.0 - 29.0 ºC, pH 8.0 - 8.2 and DO 5.0 - 8.0 mg/l in different incubation bowls of 'Flow-through System'.

3.4 Embryonic Development (Table 5)

In all the cases, fertilized eggs were demersal, adhesive and spherical in shape. However, colour and size differences were observed.

Yellowish with copper tinge colouration was observed for both cross I (Pure) and cross II (*Clarias gariepinus* ♂ x *Clarias batrachus* ♀). However, the size varied from 1.5 to 2.0 mm, and they were less adhesive with large yolk sac. In case of cross III, cross IV and cross V, the eggs were greenish to pale greenish with copper tinge colour, much adhesive or sticky in nature, but comparatively smaller in size (0.7 to 1.0 mm) however, the size of the yolk sacs was also smaller in all the crosses here. The absorption of yolk sacs in all crosses was observed within 4-5 days time of development.

It was interesting to note that before absorption of yolk sac i.e. 2 days of development of post hatching all the hatchlings of hybrids and pure cross started feeding on very minute zooplankton viz., *Brachionus* spp., *Keratella* spp., *Filinia* spp., *Moina micrura, Bosmina tripurae, Bosminopsis* sp. and Nauplius. On the other hand, in cross III and IV, the embryonic developments were completely stopped after 23 h and 6 h of post-fertilization, respectively. All the adult characters appeared within 15-16 days of development in all the crosses.

3.5 Larval Rearing

After feeding the larvae with natural food and formulated feed, it was found that maximum average survival rates in different stages of cross II were 65%, 56% and 47% after 2 days, 5 days and 15 days respectively. Next to this, was in cross I (Pure cross). Growth performance was found to be higher in cross II i.e. after 15 days of rearing the length varied from 15.5 to 20.5 mm whereas in cross I, it was 10.5 - 12 mm and cross in V, 8.5-9.5 mm (Table 4). Water quality during larval rearing revealed that temperature ranges from 29 – 31ºC (Mean 29.5ºC), pH 8.0 - 8.4 (Mean 8.2) and DO 5 - 6 mg/l (mean 4 mg/l).

4. DISCUSSION

4.1 Response of Inducing Hormone

The effective dose of ovaprim for artificial fecundation of female *Clarias batrachus*, and *Clarias gariepinus* was found to be 2.0 ml/kg and 1.5 ml/kg, respectively. In the case of *Heteropneustes fossilis*, the responding dose was same as in *Clarias batrachus*. The results of the present study coincided with the findings of [10,4]. For males of all the species, only 0.5 ml/kg ovaprim was required for the excitement of the sperm or to enhance the fertilization rate of the stripped eggs as suggested by [10,4]. So, a single dose of ovaprim was sufficient for inducing the *Clarias* spp. and *H. fossilis*.

Latency period was found to vary between 12 to 14 hours of post-injection in all the species at a temperature 28-30ºC, which roughly same with the findings of [10,4]. [11] observed the peak stage of ovulation between 15 hours 45 minutes to 16 hours of post-injection in *Clarias batrachus* and in the same species the latency period for proper spawning was reported as 14 hours at temperature 27-28.5ºC [12]. However, in *Clarias gariepinus*, it was reported to take less time to responds i.e. 9-14 hours [3]. Similarly, [13] reported latency period of 11±2 hours with temperature 25-28ºC.

4.2 Artificial Fecundation and Hybridization

[14,15] discussed the possibilities of hybrid vigour in fish. But, the hybridization in air-breathing catfishes is still in infancy. In artificial fecundation of pure cross of *Clarias batrachus*, 85% of fertilization was achieved. [4] observed more than 80% fertilization. However, at different centres of All Indian Co-ordinated Research Project partial success was achieved in fertilizing stripped eggs by homo and heteroplastic injection [16]. In the present study, 80% fertilization in F_1 hybrid II i.e. *Clarias gariepinus* ♂ x *Clarias batrachus* ♀ and 65% in F_1 hybrid V i.e. *Heteropneustes fossilis* ♂ x *Clarias gariepinus* ♀ was achieved. However, in case of hybrid III and hybrid IV, the fertilization success was found to be 50% and 40% respectively. Previously, [10] reported that the fertilization varies from 40-80% in different crosses of *Heteropneustes fossilis*, *Clarias batrachus* and *Clarias gariepinus*.

The pure cross I of *Clarias batrachus* showed maximum hatching rate (75%) in comparison to the other F_1 hybrids (crosses). However, in further developmental phases F_1 hybrid II (*Clarias gariepinus* ♂ x *Clarias batrachus* ♀) higher survival and growth rates were observed. Previously, the lower hatching rate was observed from 35-49% in *Clarias batrachus* [4]. In case of F_1 hybrid II (*Clarias gariepinus* ♂ x *Clarias batrachus* ♀) and F_1 hybrid V (*Heteropneustes fossilis* ♂ x *Clarias gariepinus* ♀), the hatching rates were found to be 65% and 35%, respectively. However, the fertilized eggs of their reciprocal crosses were failed to hatch out. A

more or less similar observation was drawn by [10].

Complete hatchings were observed in between 25 to 34 h, 23 to 33 h and 21 to 26 h of post spawning for pure cross I, F_1 hybrid II and F_1 hybrid V, respectively. The present findings regarding incubation period and temperature were also supported by the findings of [10,4]. The incubation periods and temperatures for different air-breathing catfishes and their F_1 hybrids are given in Table 6. The present study indicates that for *Clarias batrachus*, the incubation period varies from 18-34 hours with water temperature 24-40ºC. In *Heteropneustes fossilis*, it ranges from 16-24 hours with temperature 24-30ºC reported by different workers [23,32,33,35,34]. However, in *Clarias gariepinus* the incubation time varies from 12-48 hours with 20-29ºC water temperature. [17] claimed that at lower temperature, incubation for *Clarias gariepinus* takes longer duration.

On the contrary, [7] have shown that in case of *Heteropneustes fossilis* ♂ x *Clarias batrachus* ♀ cross, the incubation period varied from 24 to 26 hours with water temperature 29-30ºC, however, in *Clarias batrachus* ♂ x *Heteropneustes fossilis* ♀, it took less time (18-20 hours). In the present study, the reciprocal crosses between male *Clarias batrachus* and *Heteropneustes fossilis* female meet with success only up to hatching stage. It indicates that in intergeneric hybridization, the incubation time was lesser for both the crosses at same temperature which has also been agreed by present authors and the previous work of [4].

The present study was undertaken to develop strains with stable desirable characters like growth rate, body shape and colour, height-length ratio, fecundity, size and age at first maturity, resistance to disease, fat content, intramuscular bones, viability, response to pond manure and/or fertilizers and productivity. Though, it was the first step in this context.

Further, some deformities were found in the present F_1 hybrids like rudimentary caudal fin, etc. but the percentage was very negligible.

However, the present study revealed that the scarcity of males of all the species were a great problem during artificial breeding/hybridization of these catfishes. In this regard, works of [18] assured the availability of milt during artificial breeding of *Heteropneustes fossilis* and *Clarias batrachus* by using a cryopreservation protocol for spermatozoa. However, the technique needs to be practically and economically viable for use at farmers' field.

4.3 Embryonic Development and Larval Rearing

Observations on embryonic development of the *Clarias batrachus* egg and F_1 hybrid eggs of *Clarias gariepinus* ♂ x *Clarias batrachus* ♀, *Clarias batrachus* ♂ x *Clarias gariepinus* ♀, *Clarias gariepinus* ♂ x *Heteropneustes fossilis* ♀ and *Heteropneustes fossilis* ♂ x *Clarias gariepinus* ♀ in the present study were similar to the findings of [10,4]. [7] reported that the fertilized eggs of F_1 hybrid(*Heteropneustes fossilis* ♂ x *Clarias batrachus* ♀) were adhesive, demersal, round, yellowish-brown measuring 1.82 mm and the newly hatched larva measured 2 mm in size. In another F_1 hybrid (*Clarias batrachus* ♂ x *Heteropneustes fossilis* ♀), the fertilized eggs were also adhesive, demersal, but greenish in colour measuring 0.95 mm. It was clear from the present study that the egg colour of the F_1 hybrids was just like their mother's egg. The absorption of yolk sac in *Clarias batrachus* and in different F_1 hybrids varied from 4-5 days. This finding was also supported the previous results of [19,20,21,4,10,7].

In the present study, hatchlings were found to start feeding on minute zooplankton vigorously from 2nd day onwards of larval rearing. Earlier studies by [19,20,17,21,22,10,4] revealed that the minute live mixed zooplankton were preferred as diet during the early larval rearing of *Clarias batrachus*, *Clarias gariepinus* and their F_1 hybrids, and the F_1 hybrids of Singhi and African magur. [23] were also reported that the survival of 3 days old hatchlings of singhi was improved by feeding them with minute zooplankton. However, they also opined that the survival rate of Singhi hatchlings were enhanced by 40% after adding cobalt chloride (1 mg/l) and 80% after adding yeast (0.05 mg/l) singly or simultaneously.

While comparing the overall performance of the hatchlings, spawns and early fry of three crosses, it was found that the F_1 hybrid of *Clarias gariepinus* ♂ x *Clarias batrachus* ♀ exhibit better performance than *Clarias batrachus* and F_1 hybrid of *Heteropneustes fossilis* ♂ x *Clarias gariepinus* ♀.

Different sizes of hatchlings were reported by different scientist [24,21,10,4]. The variability in the size may be due to the differences in the brooders' management.

Table 1. Disinfectant, micronutrient, and larval feed used during larval rearing

Property	Larval stage		
	During egg rearing & up to 2 days old	**From 2 days to 5 days old**	**From 6 days to 15 days old**
Feed Provided	---	5% Glucose-D 5 mg Yeast/1 lt. of water + LIVE FEED: *Brachionus* spp. *Keratella* spp. *Filinia* spp. *Moina micrura* *Bosmina tripurae* *Bosminopsis* sp. Nauplius	LIVE FEED: *Brachionus* spp. *Keratella* spp. *Filinia* spp. *Moina micrura* *Bosmina tripurae* *Bosminopsis* sp. Nauplius + Formulated feed @ 5% body wt. or as demand food
Disinfectant / Micronutrient used	0.01 mg/l $KMnO_4$ 2 mg/l dispersible Amoxycilin in per litre of water (time to time)	0.01 mg/l $KMnO_4$ 2 mg/l dispersible Amoxycilin in per litre of water(if required) 100 mg Multiplex/lt. of water (once after 2 days)	0.01 mg/l $KMnO_4$ 2 mg/l dispersible Amoxycilin in per litre of water(if required) 100 mg Multiplex/ lt. of water (once after 2 days).

Table 2. Effect of different doses of OVAPRIM on gonadal maturation of *Clarias* spp. and *H. fossilis*

Date of experiment	Species	Number and sex	Weight (in g)	Length (in cm)	Ovaprim Dose (ml/kg)	Ovaprim required (ml)	Response
		1. ♀	150	16.0	1.0	0.15	Not responded (after 12-14 h injection)
		2. ♀	175	16.5	1.5	0.26	Partial ovulation (after 12-14 h injection)
		3. ♀	200	17.0	2.0	0.40	Free ovulation (after 12-14 h injection)
	Clarias batrachus	1. ♂	200	17.5	0.5	0.10	Excitement of testis observed
		2. ♂	150	16.5	1.0	0.15	Excitement of testis observed
		3. ♂	100	15.0	-	-	No excitement observed
		1. ♀	250	22.5	1.0	0.25	Partial ovulation (after 12-14 h injection)
		2. ♀	300	25.0	1.5	0.45	Free ovulation (after 12-14 h injection)
26-5-97	*Clarias gariepinus*	3. ♀	275	23.5	2.0	0.55	Free spawning (after 7-8 h injection)
6 pm		1. ♂	250	21.0	0.5	0.13	Excitement of testis observed
to		2. ♂	325	26.5	1.0	0.33	Excitement of testis observed
7 pm		3. ♂	200	20.0	-	-	No excitement observed
	Heteropneustes fossilis	1. ♀	100	14.0	1.0	0.10	Not responded (after 12-14 h injection)
		2. ♀	095	13.0	1.5	0.14	Partial ovulation (after 12-14 h injection)
		3. ♀	150	15.5	2.0	0.30	Free ovulation (after 12-14 h injection)
		1. ♂	100	14.5	0.5	0.05	Excitement of testis observed
		2. ♂	080	12.5	1.0	0.08	Excitement of testis observed
		3. ♂	075	11.0	-	-	No excitement observed

Table 3. Artificial fecundation of *Clarias batrachus* and interspecific and intergeneric hybridization between *Clarias* spp. and *Heteropneustes fossilis*

Date & time of injection	Species	Number and sex	Weight (in g)	Length (in cm)	Ovaprim dose (in ml)	Number of cross	Date and time	Eggs stripped (No.)	Nature and size of egg (mm)	Fertili-sation (%)	Hatching (No.)	Average hatching (%)
30-05-97 6.30 to 7.00 pm	*C. batrachus*	1. ♀	250	17.5	0.50[a]	I] Pure Cross of *C. batrachus*	31.5.97 7.0 am	4000	Not so sticky (1.5-2 mm)	85	3400	75 (hatching 25 to 34 hrs)
		2. ♀	300	18.0	0.60[a]							
		3. ♀	275	17.0	0.55[a]	II] ♂*gariepinus* X *batrachus*♀		2050		80	1640	65 (hatching 23 hrs to 33 hrs)
		1. ♂	200	15.0	0.10[b]		31.5.97 7.30 am		Not so sticky (1.5-2 mm)			
		2. ♂	195	14.0	0.09[b]	III] ♂*batrachus* X *gariepinus*♀		825		50	No hatching	Dev. stop after 23 hrs of Fertilisation
							31.5.97 8.00 am		Sticky (0.8-1.0)			
	C. gariepinus	1. ♀	300	19.0	0.45[c]	IV] ♂ *gariepinus* X *fossilis* ♀		950	Not so sticky (0.7-0.9)	40	No hatching	Dev. stop after 6 hrs of Fertilisation
		2. ♀	350	19.5	0.53[c]		31.5.97 8.45 am					
		1. ♂	250	17.0	0.13[b]							
		2. ♂	325	19.0	0.16[b]							
	H. fossilis	1. ♀	150	14.5	0.30[a]			1250	Sticky (0.8-1.0)	65	813	35 (hatching 21 to 26 hrs.)
		2. ♀	180	15.0	0.36[a]	V] ♂ *fossilis* X *gariepinus*♀						
		1. ♂	080	11.0	0.04[b]							
		2. ♂	100	13.0	0.05[b]							

'a' @ 2.0 ml/kg of fish; 'b' @ 0.5 ml/kg of fish; 'c' @ 1.5 ml/kg of fish

Table 4. Water quality during experiment

Parameter	During brooders incubation	During egg rearing in hatchery	During larval rearing
Water temperature (ºC)	28.0 - 30.0 (Mean 29.0)	27.0 - 29.0 (Mean 28.0)	29.0 - 31.0 (Mean 29.5)
pH	7.5 - 8.0 (Mean 7.9)	8.0 - 8.2 (Mean 8.0)	8.0 - 8.4 (Mean 8.2)
Dissolved oxygen (mg/l)	4.0 - 6.5 (Mean 5.2)	5.0 - 8.0 (Mean 7.0)	5.0 - 6.0 (Mean 4.0)

Table 5. Rearing of *Clarias batrachus* hatchlings and hybrid's hatchlings in laboratory

Cross	Reared in hatchery		Reared in aquarium	Reared in plastic pool/cistern
	Hatchlings (No.) and size (mm)	Survival rate (%) upto 2 days *	Survival rate (%) after 5 days and size (mm)	Survival (%) after 15 days size (mm) & Number
I] Pure cross of *Clarias batrachus*	3400 nos. (3.5 mm-4.5mm)	56 - 65 % (mean 62 %) (5.5 - 6.5 mm)	50 - 56 % (mean 53 %) (8.5mm - 9.5mm)	36-42 % (mean 40 %) (10.5 - 12.0 mm) 1020 nos. (13%[a])
II] F_1 Hybrid : ♂*Clarias gariepinus* x *Clarias batrachus*♀ = *Gariepinus batrachus*	1640 nos. (4.5mm - 5.0mm)	60 - 69 % (mean 65 %) (7.5 - 8.5 mm)	55 - 60 % (mean 56 %) (10.5mm-12.5mm)	42 - 49 % (mean 47 %) (15.5 - 20.5 mm) 281 nos. (17%[a])
V] F_1 Hybrid : ♂ *Heteropneustes fossilis* x *Clarias gariepinus*♀ = *Fossilis gariepinus*	813 nos. (3.0mm - 3.5mm)	54 - 62 % (mean 60 %) (4.5 mm - 5 mm)	38 - 45 % (mean 40 %) (6 mm - 7.0 mm)	30-36 % (mean 34 %) (8.5 - 9.5 mm) 66 nos. (8%[a])

'' start feeding on minute mixed zooplankton; 'a' % on the basis of hatchlings number*

Table 6. Incubation period of different air-breathing catfishes and their F_1 hybrids as reported by various authors

Species / Hybrids	Time (h)	Temp. (℃)	References
C. batrachus	18.30	-	[24]
C. batrachus	26 - 32	24 - 28	[19]
C. batrachus	21 - 24	26 - 29	[21]
C. batrachus	18 - 24	25 - 30	[1] [12]
C. batrachus	25 - 34	27 - 29(28)	[4]
C. batrachus	25 - 34	27 - 29(28)	Present Study
C. gariepinus	24	-	[27]
C. gariepinus	48	20	[17]
C. gariepinus	12 - 22	27 - 29	[3]
C. gariepinus	22 - 26	27 - 29(28)	[4]
C. gariepinus	>87 % hatching	25 - 28	[13]
C. macrocephalus	20	25 - 32.2	[28]
H. fossilis	within 24	25	[29]
H. fossilis	18 - 20	26 - 29	[30]
H. fossilis	20 - 24	27 - 30	[31]
H. fossilis	24	24 - 26	[32]
H. fossilis	18 - 24	26 - 29	[33]
H. fossilis	14 - 20	26	[34]
H. fossilis	19 - 32	28 - 29	[35]
F_1 hybrids :			
♂*H. fossilis* x *C. batrachus*♀	24 - 26	29 - 30	[36]
♂*C. batrachus* x *H. fossilis* ♀	18 - 20	29 - 30	[36]
♂*C. gariepinus* x *C. batrachus* ♀	23 - 33	27 - 29(28)	[10]
♂*C. gariepinus* x *C. batrachus* ♀	23 - 33	27 - 29(28)	Present Study
♂*H. fossilis* x *C. gariepinus*♀	21 - 26	27 - 29(28)	[10]
♂*H. fossilis* x *C. gariepinus*♀	21 - 26	27 - 29(28)	Present Study

The growth and survival rates were observed for each cross after 2 days, 5 days and 15 days of rearing period and the best performance was found in F_1 hybrids of *Clarias gariepinus* ♂ x *Clarias batrachus* ♀. Almost all the adult characters were attained within 15 days of development in both the F_1 hybrids and *Clarias batrachus*. The present findings more or less were in agreement with the previous works of [21,10,4] though [2] mentioned that it ranges from 15-20 days and [4] noted within 18 days.

On the contrary, [25] reported that the post- yolk fry of F_1 hybrid (*Clarias gariepinus* ♀ x *Heterobranchus longifilis* ♂) grew significantly faster than the parents with mixed zooplankton or pond green water.

During later stage of larval rearing, it was observed that survival rates have declined in all the cases. Earlier works were also supported these findings. [26,21] and the present study have opined that the survival rate of *Clarias* spp. during larval rearing are related with the water depth. The dependence on the water depth as they have aerial respiration and 10-18 cm water depth is good for them to respire easily.

Floating weeds were used in the larval rearing units to provide resting place for fish. However, feeds have effect on the better survival of these larvae.

5. CONCLUSION

In conclusion, the hybridization of available two varieties of magur and singhi is possible through

artificial propagation. The larval rearing process in controlled conditions by using mixed minute zooplankton and formulated feed can yield encouraging results.

From the present study it can be suggested that the setting up of mass scale commercial hatchery to produce assured quality seeds of local magur and F_1 hybrids of (*Clarias gariepinus* ♂ x *Clarias batrachus* ♀) and (*Clarias gariepinus* ♂ x *Heteropneustes fossilis* ♀) in Tripura will be a feasible technology in terms of survivability of fish. Along with that, the F_1 hybrids can be brought under aquaculture practices as a representative species after giving proper cultural trial in the ponds on experimental basis to evaluate whether they are eco-friendly or compatible to our native species. Later it can be demonstrated in farmers' field and can be adopted by the farmers of Tripura in particular and North East in general for earning their livelihood as well as for the upliftment of their soci-economic status.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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