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Induced breeding attempt of vulnerable freshwater mud eel, *Monopterus cuchia* (Hamilton 1822)

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Abstract
A breeding attempt was conducted during 2012-13 for breed a vulnerable freshwater fish species *Monopterus cuchia*. Broods were reared in the pond of BFRI, FPSS, Santahar, Bogra, Bangladesh. For induced breeding purposes average 350g injected female and average 230g injected male of *M. cuchia* were stocked and after injection transferred into the three habitats such as Cistern, Hapa and Pond. Different doses were applied. Only Pond Habitat had shown the desired result by using Pregnyl. Pond was dried up and collects total three-thousand (3000) cuchia Larvae from six holes (Pit). Larvae were shifted into two habitats (a. Cistern and b. Tray). From four months results, it was observed that the mean final length and weight of fry under Tray habitat were 10.68±1.89 cm and 1.09±0.24g. Besides Cisterns habitat were 10.78±0.32 cm and 1.02±2.35g respectively. Percentage of survival rate was 80 and 77.

Keywords: Induced breeding, larval rearing, *Monopterus Cuchia,* Mud eel, Bangladesh

1. Introduction
Inland waters of Bangladesh are rich with wild fishery resources and many fish species are going to be disappeared from the inland waters of the country due to man-induced and natural factors. *Monopterus cuchia*, locally known as Kuchia/Kuichaa, is freshwater fish in Bangladesh [1]. International union for Conservation of nature (IUCN) Red List of Bangladesh [2] has enlisted mottled that fish. *M. Cuchia* in their red list of vulnerable fishes of Bangladesh. It is a freshwater air-breathing swamp mud eel found in Bangladesh, India, and Burma [1]. It has developed specialized pharyngeal pouches for bimodal gas exchange [3, 4]. It often spends the day hidings under crevices, water hyacinth, stones and mud [5]. The habitat or microenvironment plays an important role for the better growth and survival of some fish species [6]. It is a carnivorous fish and nocturnal in nature. It prefers live food like small fishes, earthworms, tubifex and other aquatic organisms. *M. cuchia* is a nutritious and tasty fish having medicinal value. Generally, low earning groups of people especially tribal people take it as food. Peoples of South Asian countries like Korea, Philippines, China, Vietnam, Cambodia, Laos, and Japan also take it as food, especially during the religious festival. As a result, it has a high demand in foreign countries. Density and diversity of *M. cuchia* are decreasing day by day due to indiscriminate harvest from nature by using various pesticides and by drying up of water bodies. Consequently, broods of *M. cuchia* are decreasing alarmingly and breeding grounds became squeezed. It is rational to conserve the fish and rehabilitate in nature. Therefore, research should be undertaken on breeding biology, induced breeding and nursing techniques, and survey on the availability of *M. cuchia* to overcome the adverse conditions related to losing of natural habitats, spawning grounds, over-harvesting, indiscriminate catching, and improper export rules. The situation could only be reversed by culturing the species in closed water bodies that will help to prevent it from extinction. Artificial propagation is the primary means of getting juveniles for aquaculture. Therefore, it is necessary to develop artificial breeding and nursing techniques of *M. cuchia*. For this reason, brood development of *M. cuchia* had been done to know the breeding time, yolk sac absorption period of larvae and finally induced breeding status. Moreover, export policy for this species should be developed by the Government to prevent the indiscriminate killing of this species.

2. Materials & Methods

2.1. Study site

This study was conducted in the pond complex of the campus of Bangladesh Fisheries Research Institute (BFRI), Floodplain Sub-station (FPSS), Santahar, Bogra, Bangladesh during 2012-2013.

2.2. Collection of Cuchia brood fish

Females averaging 280g and males averaging 190g were collected from the Raktadaha Beel of Santahar, Bogra and Chalan Beel of Pabna, Bangladesh.

2.3. Pond preparation

A pond having an area of five (5) decimal was prepared by sun drying followed by liming at the rate of 1 Kg/Decimal. After drying up the pond, Urea, TSP and cow-dung were applied at the rate of 0.100, 0.075 and 8 Kg/Decimal, respectively. After Cuchia stocking, fertilizers were used fortnightly depending on the primary production of the pond. Water depth was maintained at about 1-1.25m. The pond was protected by fencing with nylon net, bamboo, and rope. Fencing was done to stop entering of different fish-eating animals like snake, otter, and other reptiles. Water hyacinth, Helencha (*Enhydra fluctuans*) and PVC pipes were provided to create suitable and safe shelter.

2.4. Rearing of brood Cuchia in prepared pond

Cuchia broods were reared in a prepared pond of BFRI, Floodplain Sub-Station for three months from January to March 2013. Live foods like earthworms (Fig.1), moribund fingerling fish were provided at the rate of 3% body weight twice daily. Guchi (*Mastaecmbelus puncalus*), Carpio (*Cyprinus carpio*), Taki (*Channa punctatus*) and Puia (*Lepidocephalus guntea*) were released in the pond for natural breeding because cuchia will take those breeding-after larvae as food.

2.5. Experimental steps

The experiment was conducted into Induced breeding technique. In this experiment, various doses of cPGE, HCG, Cuchia PG, Ovaprim and Pregnyl considered as three steps: 1.Cistern, 2. Hapa and 3. Pond.

2.6. Maturity stages indication

Identification of male & female is very difficult but some external characteristics and GSI mean to ensure the differentiation of male & female. The mean gonad somatic index of the female was minimum (2.78) in the month of December and January and the mean gonad somatic index of the female was maximum (5.00) in the spawning stage of maturation with one pick in April to June. The variation of mean GSI occurred in mature fishes and the peak would be indicative of the peak period of spawning [7]. Sexual maturity of broods was confirmed by observing the body color and shape of the genital organ [7]. (Table 1, Figure 2 and 3)

Table 1: Male, female and maturity stages identification of *M. Cuchia.*

Sl. No.	Indication key	Characteristics	
		Male	Female
1	Body Size	Thin cylindrical Shape	Thick cylindrical Shape
2	Color	Slight blackish, few black spots	Goldish, few deep spots
3	Size (Length)	Small	Large
4	Abdomen	Slight Ass-black	brownish abdominal skin
5	Tail part	short	long
6	Ana's and Genital papillae	Tubular, Sunken	Round shape, Swollen
7	Mature Ovary and Testes	i) Testes enlarged somehow, flat, ribbon like, yellowish white ii) Testes creamy white, soft, occupying 2/3 of the ventral cavity	i) Increase in volume, eggs distinctly visible with naked eye, yellowish in colour ii) Ovary with thin wall, eggs completely round, translucent, yellow in colour
8	Genital Pressure	Slight Transparent, whitish liquid milt	Yellowish/ brownish fluid
9	Ripe and gravid condition	whitish milt from Genital papillae	Yellowish egg from Genital papillae

2.7. Induced breeding

Broods were collected from rearing pond. A total fifty-two (26 Male & 26 Female) were selected for induced breeding. Collected female & male brood's weights were average 335g and 245g respectively. Female and male broods were kept for a few hours in separate cisterns for conditioning. Before induced breeding broods were taken out from the cistern and caught with a wet soft cloth. When female were injected twice while male received a single dose. In case of the female, the time interval between two injections was maintained at 6-12 hours. The male was given a single dose at the time of second dose of the female. After that, the injected broods were transferred to the Cistern, Hapa and Pond. Two pairs of injected *M. cuchia* were stocked at a ratio of 1:1 in each Cistern, Hapa and in Pond.

2.7.1. Cistern, Hapa and Pond preparation

Cemented cisterns (9×6×2.5ft) were used for the experiment. Floor and sidewalls of cistern were covered by soil to create natural habitat like as pond bottom. Due to borrowing nature, M. cuchia survive well in contact with soil. Water hyacinth and PVC pipe were provided for creating shelter and hiding place. Five black Hapas made of smooth and soft cloth were used for creating spawning habitat of *M cuchia*. Hapa was installed in the experimental cisterns. Water depths of each cistern were 1.25 fit. Continuous water flow was maintained in cisterns and Hapa to make sure regular oxygen circulation and proper water quality. Excess water passes out through an outlet after maintaining the mentioned volume of water. A pond having an area of five (5) decimal was prepared by sun drying followed by liming and after drying up the pond, Urea, TSP and cow-dung were applied. Fertilizers were used for the primary production of the pond. Water depth was maintained at about 1-1.25m. For stop entering of fish-eating animals like snake, otter, and other reptiles, the pond was protected by fencing with nylon net, bamboo, and rope. Water hyacinth, Helencha (*Enhydra fluctuans*) and PVC pipes were provided to create suitable and safe shelter. According to need, pond water was exchanged regularly.

2.7.2. Injected Cuchia transferring in Cistern

Different dosages of cPGE, Cuchia PG (Pituitary Gland), Ovaprim and Pregnyl were applied in different cisterns (Table 2). Six (6) Cisterns were used for six (6) type's doses. Earthworm, dead/moribund Guchi, Taki and Puia were supplied as a food of injected *M. cuchia*.

Table 2: Different doses of hormone used for breeding of *M. cuchia* in Cistern

Treatment	Fish Weight (g)		Fish Length (cm)		Hormone and Dose			
	Female	Male	Female	Male	Female	Dose	Male	Dose
T_1 (Cistern)	346	220	64	58	50 mg cPG and 20 mg cPG/kg Bw	2	35 mg cPG/kg BW	1
	342	240	67	53	40 mg cPG and 30mgcPG/kg BW	2	40 mg cPG/kg BW	1
	310	230	77	65	Ova prim 0.8 mg/kg BW	1	Ova prim 0.3 mg/kg BW	1
	340	280	68	52	25 mg Cuchia PG/kg BW	1	10 mg Cuchia PG/kg BW	1
	350	240	75	58	**Triple dose** 1st day 80mg/kgBW 2nd day 1000 IU Pregnyl/kgBW 3rd day 500 IU Pregnyl/kg BW)	3	3rd day 500 IU Pregnyl/kgBW	1
	340	250	68	60	40 mg cPG and 1000 IU HCG/kg BW	2	40 mg cPG/kg BW.	1

2.7.3. Injected Cuchia transferring in Hapa

After injection, ten pairs of injected male-female Cuchia were stocked in the Hapa. Water hyacinth and PVC pipe were provided for creating shelter. Different dosages of cPGE, HCG and Ovaprim were applied in different Hapas (Table 3). Earthworm, dead/moribund Guchi and Puia were supplied as a food of injected *M. cuchia.*

Table 3: Different doses of hormone used for breeding of *M. cuchia* in Hapa

Treatment	Fish Weight (g)		Fish Length (cm)		Hormone and Dose			
	Female	Male	Female	Male	Female	Dose	Male	Dose
T_2 (Hapa)	320	230	66	50	10mg cPGE and 20 mg cPG/kg BW	2	10 mg cPGE/kg BW	1
	310	240	70	48	40 mg cPGE /kg BW	1	15 mg cPGE/kg BW	1
	335	250	75	58	20mg cPGE and 1000 IU HCG/kg BW	2	20 mg cPGE/kg BW	1
	345	245	70	60	25 mg cPGE &1500 IU HCG/kg) and 40 mg cPGE/kg BW	2	25 mg cPGE/kg BW	1
	310	280	75	55	Ovaprim 0.7 mg/kg BW and 20 mg PG/kg BW	2	**Double dose:** 30 mg cPGE and 1000 IU HCG/ kg BW	1

2.7.4. Injected Cuchia transferring in Pond

After preparing the pond, fifteen (15) pairs of injected Cuchia were stocked in the Pond in 1st week of April 2013. Selected dose of Pregnyl was applied to the fish in Pond (Table: 4). Injected Cuchia were fed with spawn of Carpio, dead/moribund fry of Taki, Guchi, Puia and Earthworms thrice in a week. Feeding rate was 2-3 percentage of body weight.

Table 4: Doses of hormone used for breeding of *M. Cuchia* in Pond

Treatment	Fish Weight Range (g)		Fish Length Range (cm)		Hormone & Dose			
	Female	Male	Female	Male	Female	Dose	Male	Dose
T_3 (Pond)	300-350	230-250	60-75	45-60	Pregnyl 1000 IU/kg BW	1	Pregnyl 500 IU/kg BW	1

2.8. Water quality measurement

Physicochemical water quality parameters were measured weekly. A centigrade thermometer measured the temperature of air & water. HACH water test kit (Model-FF-2, USA) was used to measure pH, dissolved oxygen (DO) and alkalinity. Water quality of Cistern, Hapa and Pond was measured regularly following standard methods [8].

3. Results

3.1. Breeding performance of *M. Cuchia*

3.1.1. Cistern

After injection, observations were made to record the effect of hormone treatments. Total l9 dosages were applied; female & male received those doses. Stripping was done to collect eggs but the eggs were found in liquid conditions. Damage of eggs was probably due to a high dose of hormones. Females and males did not respond to the hormone treatments and few broods have died. The only female was responded by showing unfertilized egg in cistern (Figure 4 and 5, Table 2)

3.1.2. Hapa

Observations were made on the effect of such hormone treatments. Ovulation was not observed. Any brood fishes did not respond to another hormones and doses. Few brood fishes have died. The death of brood fishes was probably due to high doses of cPGE. (Table 3)

3.1.3. Pond

A few days later of releasing the injected Cuchia, a regular observation was made to check the Water hyacinth's root for identification the presence of Cuchia larvae which will be a screw with water hyacinth. Ensure the screw with water hyacinth symptom and the early-first rain of rainy season (third week of May 2013/after 21 days); pond was dried up to collect larvae of *M. Cuchia*. Spawning nests (Pit) were identified in the pond near the dyke and larvae were collected

from those nests. The spawning nest (Pit) was like a flat hole (Figure 6). During collection of larvae, spent broods were found in every hole. Mother Cuchia was kept a guard there. Three-thousand (3000) larvae were collected from six holes (Pit) (Figure 7). Between these six holes, two holes larvae were newly hatched which is assured by observing eggshell remaining in the head of larvae. (Table 4, Figure 8 and 9)

3.2. Water quality

During the experimental period, the water quality parameters of Cistern, Pond & Hapa such as temperature (°C) dissolved oxygen (mg/L), pH and total alkalinity (mg/L) were measured regularly (Table 5). There was more or less same water quality parameters presented in all the three (3) conditions.

Table 5: Water quality parameter of *M. Cuchia* breeding in Cistern, Pond and Hapa

Sl. No.	Water Quality Parameter	Cistern	Pond	Hapa
1	Air Temp. (°C)	30.91±1.05	31.10±0.11	30.62±1.25
2	Water Temp. (°C)	28.81±0.15	30.29±1.52	28.92±1.39
3	pH	7.97±0.42	7.91±0.39	7.98±0.31
4	DO (mg/l)	6.41±1.05	6.98±1.02	6.02±1.10
5	Alkalinity (mg/l)	190.12±2.46	192.00±2.22	190.18±1.06

3.3. Larval rearing and nursery development of *M. cuchia* larvae

Collected larvae were stocked in two rearing units. Larvae were stocked at the rate of 1000/m^2 in a Cistern having a dimension of 2.74×1.82×1.00m (Figure 11). A Tray having a dimension of 1.0×0.5×0.12m was also stocked with the larvae at the rate of 1000/m^2 (Figure 10). Before releasing larvae, floor and sidewalls of tray and cisterns were covered by smooth clay soil. Water hyacinth was provided to create suitable shelter for the larvae. Water depth was kept always at 0.45 and 0.12m in the cistern and tray, respectively. Water showers were provided over the cisterns and tray to increase dissolved oxygen in the water and to keep clean the cisterns and tray. After 12-15^{th,} day's yolk sac of larvae has been completely absorbed. For 1st 3 days, the larvae were fed thrice daily with boiled poultry egg yolk in the morning and next two times with earthworm's juice at the rate of 80-90% of estimated body weight. After 3 days, earthworm pest, zooplankton, and chopped Tubifex were provided in the morning, noon, and afternoon, respectively. The feeding rate was 5-10% of estimated body weight. Initial mean length (cm) and weight (g) were 4.86±0.24, 0.07±0.00 in Tray while it was 4.16±0.28, 0.07±0.00 in Cistern, respectively. After four months of rearing, it was observed that the final mean length and weight of fry in Tray were 10.68±1.89cm and 1.09±0.02g while the final mean length and weight were 10.78±0.28cm and 1.02±2.35g in Cistern. Survival rate was 80 and 77 percentage (%) in tray and cistern, respectively (Table 6).

Table 6: Growth performance and survival percent of *M. Cuchia* at different habitats

Growth parameters	Tray	Cistern
Initial length (cm)	4.86±0.24	4.16±0.28
Initial weight (g)	0.07±0.00	0.07±0.00
Final length (cm)	10.68±1.89	10.78±0.28
Final weight (g)	1.09±0.02	1.02±2.35
Weight gain (g)	1.02±0.24	0.95±0.23
(%) Weight gain	1460.00	1364.28
Survival (%)	80	77
Specific growth rate (%)	2.29	2.23

Fig 1: Earthworm caught by *Monopterus Cuchia*

Fig 2: Genital papilla of male Cuchia

Fig 3: Genital papilla of female Cuchia


Fig 4, 5: Unfertilized egg of female Cuchia in cistern

Fig 6: Spawning nests (Pit) of *M. cuchia*

Fig 9: Cuchia larvae with yolk sac condition

Fig 7: Collected larvae of *M. Cuchia*

Fig 10: Releasing of *M. Cuchia* larvae in Tray

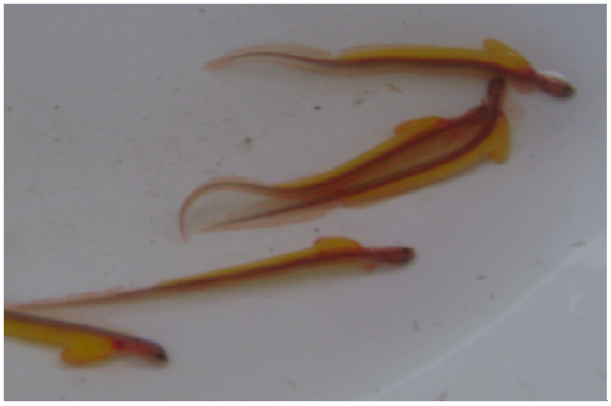

Fig 8: Day-old larvae of *M. Cuchia*

Fig 11: Releasing of *M. Cuchia* larvae in Cisterns

4. Discussion

Breeding in the Captive condition of *M. Cuchia* has not been yet successful. Banerji SR [9] reported that after breeding, Cuchia larvae's yolk absorption period is completed among 22-24 days and some young larvae actively engaged feeding and adult characters are almost attained in about a month's time. In this study after induced breeding, 21 days left, three-thousand (3000) larvae were collected from six holes (Pit). At the time of collection larvae of two holes, yolk sac was not absorbed. Absorption of yolk sac was finished within 12-15th days. This result is approximately similar to the result of Banerji SR [9].

Miah MF [10] was taken an attempted to a breeding trail of *M. Cuchia*. Total three treatment were applied. Only third trail (Induced breeding by PG, HCG, GnRH and Ovulin) had made a result where mature eggs in the oviduct were reduced, absorbed and some eggs were found in spoiled conditions. At least spawning activities were not shown by inducing agents. Shuvra TM [11] also found same results. In the study, three treatments were run; first two treatments had shown negative result but among all doses of first treatment, only female were responded by showing unfertilized egg in the cistern (Table: 2). Third treatment had a result that shown successful breeding by pregnayl hormone and many larvae were collected after breeding (Table 4).

Yamamoto K and Yamauchi K [12] first succeeded in obtaining fertilized eggs and larvae of the Japanese eel (*Anguilla japonica*) by hormonal treatments. They reared pre-leptocephalus larvae for two (2) weeks, which reached 7mm TL. Yamauchi K, Ohta H [13, 14] had been succeeding to induce breeding of Japanese eel by using salmon pituitary extract. The Japanese eels had been stimulated to spawn by hormonal treatments [15], but the larvae were extended their life only six days long. Thereafter, many researchers succeeded in obtaining eel larvae [16, 17]. In other eel species, for example, the European eel *Anguila. Anguilla* [18] and the New Zealand freshwater eels *Anguilla dieffenbachia* and *Anguilla australis* [19], experimentally produced larvae which survived only for a few days and like the Japanese eel, did not develop into leptocephali. However, artificial fertilization of the European eel has not been yet succeeded. On the other hands, in this study after breeding, the survival rate of cuchia larvae were 80 and 77 percentage (%) which were stable for the next research steps (Table 6).

5. Conclusion

Limited success on induced spawning of *M. cuchia* was achieved. The experiments were conducted with a limited number of broods. Some limiting factors were made the zigzag condition on this research. Being a bottom dweller, it is difficult to recover the fish from the pond bottom and difficult to check all over the condition of the pond. It prefers to live in bottom mud and usually hide under aquatic vegetation. Being a carnivore, *M. cuchia* do not accept artificial feed. Therefore, it was difficult to develop brood for artificial breeding. It has cannibalistic character and feeds on smaller one during the scarcity of suitable food. Current experiments on induced breeding of *M. cuchia* were conducted with a trial. Hence, different hormones with different doses were tried to breed the fish. Therefore, details and specific studies of artificial breeding, seed production, and rearing techniques are needed for establish a development programmed for artificial breeding and develop culture practice of Cuchia. This could be providing the sustainable production of Cuchia to increase the economical source. These studies ensure the further extensive research for breeding of Cuchia.

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