

Breeding Biology and Induced Breeding Status of Freshwater Mud Eel, *Monopterusuchia*

M. F. Miah, H. Ali, E. Zannath, T. M. Shuvra, M. N. Naser, M. K. Ahmed

Abstract—In this study, breeding biology and induced breeding of freshwater mud eel, *Monopterusuchia* was observed during the experimental period from February to June, 2013. Breeding biology of freshwater mud eel, *Monopterusuchia* was considered in terms of gonadosomatic index, length-weight relationship of gonad, ova diameter and fecundity. The ova diameter was recorded from 0.3 mm to 4.30 mm and the individual fecundity was recorded from 155 to 1495 while relative fecundity was found from 2.64 to 12.45. The fecundity related to body weight and length of fish was also discussed. A peak of GSI was observed 2.14 ± 0.2 in male and 5.1 ± 1.09 in female. Induced breeding of freshwater mud eel, *Monopterusuchia* was also practiced with different doses of different inducing agents like pituitary gland (PG), human chorionic gonadotropin (HCG), Gonadotropin releasing hormone (GnRH) and Ovuline-a synthetic hormone in different environmental conditions. However, it was observed that the artificial breeding of freshwater mud eel, *Monopterusuchia* was not yet succeeded through inducing agents in captive conditions, rather the inducing agent showed negative impacts on fecundity and ovarian tissues. It was seen that mature eggs in the oviduct were reduced, absorbed and some eggs were found in spoiled condition.

Keywords—Breeding biology, induced breeding, *Monopterusuchia*.

I. INTRODUCTION

THE Synbranchid freshwater mud eel, *Monopterusuchia* [1] is a tasty, nutritional and highly economically valuable fish. This freshwater mud eel are generally available in open water resources including mud holes, paddy field, pits and swamps, etc. The fish lay their eggs in the March to June each year. In Bangladesh, the tribal people and a few of other castes eat this fish while the annual landing of the freshwater mud eel is about 1.85 Metric tons [2]. This fish can play a unique role for socio-economic welfare of the area which will be potential to develop extension of the fishery in that area [3]. This fish has tremendous demand in foreign countries and are exported from Laos, Cambodia, Vietnam and Indian, and now a scope has been estimated to export this fish from Bangladesh. Due to present it's highly modified accessory respiratory organs this

fish can survive 90 to 132 days without food and can be stored large number in a small sample container [4], [5] and this facility play a unique role to export as live. It has been reported from the local fishermen, the population of this freshwater mud eel has been declining from the natural water bodies caused by several reasons. Due to overfishing this fish is now a risk species in Bangladesh [6] which is completely depended on natural spawning. Thus, it is deemed important to extend proper management to improve this fishes that may offer tremendous contribution to the national economy of the country. There is no culture system yet to operate for freshwater mud eels in Bangladesh. To develop a scientific mud eel culture system, it is necessary to observe their growth, foods, survival rate as well as production and habitat ecology. Besides this, breeding biology and artificial seed production of this fish is very important for their rearing, production and conservation. Though limited research has been conducted on this mud eel in Bangladesh [7]-[11], however, so far no data available on breeding biology and induced breeding of this fish is available, while unsuccessful induced breeding was also observed without spawning responds [10]. Therefore, to maintain the natural mud eel resources and to obtain reliable seed of this mud eel for aquaculture, examine the breeding biology, which mainly involved the study of reproduction system and sex organs of freshwater mud eel and development of induced breeding technique for eels have been eagerly desired. Thus, the specific objectives of this research are to observe the breeding biology of mud eel, *M.uchia* during natural conditions as well as after hormonal treatments and to observe breeding performance through inducing agents as well as in captive conditions.

II. MATERIALS AND METHODS

A. Observation of Breeding Biology of Freshwater Mud Eel

The breeding biology of freshwater mud eel, *M.uchia* was observed by analyzing twenty fish specimens (ten males and ten females) where fishes were collected randomly during the period of February to June 2013 and collected fish was identified through observing taxonomic characteristics [12]-[14]. Total length and weight of fish, length and weight of gonads, egg diameter, fecundity, etc. were measured monthly by using lifting balance, measuring scale, measuring tape and forceps, etc. The individual gonad weight was divided by the individual body weight to give the gonadosomatic index (GSI). The GSI of male and female was estimated separately by using the formula of GSI is $[\text{Gonad weight (g) / Fish weight (g)}] \times 100$, and the relative fecundity was calculated by the formula of Relative fecundity is Individual fecundity / Body

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length (cm). Mean of egg diameter was calculated by:

$$\sum XiFi/N$$

Besides these periods of natural observations, on the other hand, breeding biology was also observed with 20 fish specimens after one week of hormonal treatments as the same process where different doses of HCG, PG, Ovulin, GnRH were induced and fish specimens were selected randomly.

B. Observation of Artificial Breeding in Different Settings

Experimental Fish

Matured fish were collected from flood plains by helping local fishermen and identified the fish through observing morphological characteristics [12]-[14]. Male fish was considered with 60-65cm length and 450-550g weight whereas the length and weight of female fish were 70-75cm and 750-850g respectively. Due to difficulty to determine the sex the male and female fish was observed by [11].

Observation of Breeding in Cemented Tanks without Hormonal Treatment

Brood fish was collected from nature by a fisherman and stocked in a cemented tank as 1:1 ratios with recirculatory water in a hatchery for 20 days. During this observation lives tilapia fry, small dead fish, prawns and pellet feed were supplied up to 15 days by three day a time in the evening. No feed was given for last week of the experiment. Water temperature and pH were maintained with 26-30°C and 7 respectively. Aerator was used in the tank to maintain dissolved oxygen.

Observation of Breeding through IVF Technique

In this study female fish was dissected to removal of oviduct membrane carefully for taking out of eggs and kept them in a petridish after harvesting from the oviduct. Male gonad/testis with sperm duct was taken out carefully and kept it in a petridish. Cut it in small pieces and kept them in 2.5% saline water. Eggs were poured immediately in sperm petridish and mixed very well with a spoon. After five minutes some water was given and again mixed them very well. Finally egg-sperm mixture was transferred into a small glass aquarium with filter water and aerator where temperature was maintained from 27-30°C and kept it for three days, and it was checked daily.

Observation of Breeding through Inducing Agent

Three cemented tanks with 2m² were filled with water of 25cm of water with hyacinth. Three doses of PG such as 100mg/kg, 120mg/kg and 150mg/kg respectively were used for this first trial. First dose was conducted at 2: 3 ratio of male and female where second dose was 3: 3 ratios and third dose was 3: 2 ratio of male and female. Dorsal muscle was used as a suitable place for the injection. The injected fish were kept in three cemented tanks separately. Trial with HCG was conducted in three cemented tanks with three doses such as 2500 IU/kg, 2000 IU/kg and 1500 IU/kg where 2: 3 ratio,

2:2 ratio and 2:2 ratio of male and female fishes were considered respectively. Induced breeding of *M. cuchia* was trialed with GnRH in three cemented tanks with three different doses. 1st dose was injected with 1ml/kg fish with 3:3 ratio of male and female, and 2nd dose was given with 1.2/kg fish with 2:2 ratio of male and female while 3rd dose was given 1.5/kg fish with 2:2 ratio of the male and female fishes. Finally, trial with ovulin was 0.6 ml ovulin/kg of fish with 2:2 ratio while 0.8 ml/kg and 1 ml/kg was injected considering 2:3 and 3:2 ratios respectively. In this case both male and female fish were observed in the environments of cemented tank with sufficient water and in hapa on a pond. During these observations small live fish, earth worms and small dead fish were supplied as food. The water temperature and pH were considered between 28°C-31°C and 7.2-7.5 respectively. In breeding house, the water was changed after three days interval.

III. RESULTS

A. Breeding Biology of *M. cuchia*

Breeding Biology of Natural and Treated Fish

Breeding biology of freshwater mud eel, *Monopterus cuchia* was observed where the length of male fish ranged from 40 cm to 82 cm and the lowest length was recorded 40 cm in male fish during February to March and higher length was found 80cm during May-June in natural fish while highest length 82 cm was recorded in treated fish (Table I). The body weight of experimental male fish was 90g to 330g in natural fish while lowest 90g was found during February to April and 330g was found during May to June, however, hormonal treated fish weight was observed 550g. The length of testis with sperm duct was found lowest 11cm during February to March and highest 16.5cm in natural fish during May-June while highest length was observed 20cm in treated fish. In addition, observed weight of testis with sperm duct was found lowest 0.5g in February to March and highest 6.7 in natural fish during May to June where 8.1 g was found in treated fish. On the other hand, testis was also measurement without sperm duct where the lowest length was found 4cm during March-April and highest was observed 8.75 during May-June. The weight of testis was found without sperm duct 0.35g (February-March) to 4.2g (May-June) in natural fish while highest 4.7g was recorded in treated fish. In case of female fish, the body length and weight varied from 50 to 90 cm and 150g to 520g respectively whereas the length and weight of female ovary were found 5.9cm to 12cm and 2.4g to 24.5g respectively (Table I). Highest length and weight of ovary were found 12cm and 24.5g respectively during May-June and usually the female gonad weight was found higher than the male. The individual fecundity was recorded 203 to 1495 while lowest number of eggs was observed 203 during the month of March to May and highest was observed 1495 during May to June. However, in case of treated fish the number of eggs was found 155 to 998. The mean egg diameter 1.075mm was observed during February-March and 2.49mm during May-June, and 2.195mm was recorded in treated.

TABLE I
MEASUREMENT OF GONAD AND OVA DIAMETER

MEASUREMENT OF GONAD AND OVA DIAMETER													
Study option	Male Fish		Female Fish		Testis				Ovary		No. of eggs individually	Egg Diameter (mm)	
					With sperm duct		Without sperm duct						
	Wt (g)	Lt (cm)	Wt (g)	Lt (cm)	Wt (g)	Lt (cm)	Wt (g)	Lt (cm)	Wt (g)	Lt (cm)		Range	Mean
Feb15-Mar14	90-280	41-70	150-500	50-83	0.5-1.9	11-16	0.35-1.60	4.5-7.2	2.4-6.75	6-10.5	280-621	0.3-2.2	1.075
Mar15-Apr14	90-250	40-75	170-300	65-77	1.4-3.5	12-16	1-2.4	4-7	4.4-11	6-7.6	203-603	0.4-3.2	1.805
Apr15-May14	120-280	43-78	150-400	55-82	3-4.4	13.2-16	1.8-3.2	4.25-7.2	6.1-17.6	6-8	203-742	0.5-4	2.5
May15-June14	180-330	55-80	220-400	60-82	4.6-6.7	12.7-16.5	2.4-4.2	5.75-8.75	9.8-24.5	8-12	385-1495	0.5-4.3	2.49
Treated fish	90-550	44-82	140-520	52-90	2.1-8.1	12-20	1.45-4.7	5-8.2	6.9-20.4	5.9-11.3	155-998	0.5-4	2.195

WT = weight, LT = length

TABLE II
MONTHLY CHANGES OF GSI IN MALES AND FEMALES

Trials	Months	Mean GSI (male)	Mean GSI (female)
1	15 Feb - 14 Mar	0.64±0.14	1.6±0.36
2	15 Mar - 14 Apr	1.42±0.24	2.86±0.67
3	15 Apr - 14 May	1.9±0.25	4.87±0.96
4	15 May - 14 June	2.14±0.2	5.1±1.09
5 (Induced)	15 Apr - 14 May	2.16±0.43	4.72±1.65

TABLE III
MONTHLY VARIATIONS IN BODY LENGTH, BODY WEIGHT AND FECUNDITY

Period	Mean body length	Mean body weight	Absolute fecundity	Relative fecundity
Feb 15-Mar 14	63.9±10.28	276.5±113.02	411.8±118.75	6.48±2.76
Mar 15-Apr 14	71.1±3.65	230±43.53	411.1±102.42	5.79±1.38
Apr 15-May 14	71.8±7.5	255±70.57	376.6±134.14	5.31±2.08
Apr 15-May 14 (Induced)	67.5±11.44	267±108.54	472.7±264.58	7.02±3.55
May 15-June 14	67±6.49	300.5±57.12	572±216.97	8.41±2.61

TABLE IV
OBSERVATION OF GSI IN BOTH HORMONAL INDUCED AND NON-INJECTED FISH

Fish	Treatment	No. of fish	Max.	Min.	Mean ± SD
Male	Control	5	3.2	1.7	2.26 ± 0.52
	5 days	5	3.5	1.3	2.32 ± 0.71
	10 days	5	3.6	2.1	2.86 ± 0.57
Female	Control	5	5.1	3.3	4.26 ± 0.65
	5 days	5	4.8	3.3	4.1 ± 0.31
	10 days	5	5.5	3.8	4.74 ± 0.65

Monthly Changes of GSI in Male and Female

The gonadosomatic index (GSI) is an indicator of the seasonal development of the gonad. The monthly variation in GSI of the male was found from 0.64±0.14 to 2.14±0.2 while GSI of the female was observed from 1.6±0.36 to 5.1±1.09 (Table II). In case of male fish specimens, it was found that the mean GSI of hormonal treated fish (2.16±0.43) was higher than the untreated fish (1.9±0.25) during the same time but in case of female the mean GSI of hormone induced fish (4.72±1.65) specimens lower than the untreated fish (4.87±0.96).

Monthly Variations in Body Length, Weight, and Fecundity

Estimation of body length, body weight and fecundity are the prerequisite in successful breeding program. In the present study monthly mean body length varied from 63.9±10.28 to

71.8±7.5 and monthly mean body weight was found from 230±43.53 to 300.5±57.12, where the highest mean body length was found in April-May while the highest mean body weight was found in May-June. In the present study the individual fecundity recorded from 1495 (65 cm / 310g) to 155 (240 cm / 69g) while relative fecundity ranged from 2.64 to 12.45. The average monthly absolute fecundity for female *M. chuchia* was found 376.6±134.14 to 572±216.97 while relative fecundity varied from 5.31±2.08 to 8.41±2.61. The absolute high fecundity and relative fecundity was found in May-June. The mean body length, mean body weight, mean absolute fecundity and relative absolute fecundity of the hormonal induced fish were found 67.5±11.44, 267±108.54, 472.7±264.58 and 7.02±3.55 respectively (Table III).

GSI in both Treated and Non-Treated Fish (Control)

The GSI values showed significant difference in both males and females. In male fish pituitary gland hormone (PG) as compared to control group (2.26± 0.52) significant differences were seen in the group injected for 5 days (2.31 ± 0.71) and 10 days (2.86 ± 0.57) while in case of females as compared to control group (4.26 ± 0.65) significant differences were seen in group of injected fish for 5days (4.1 ± 0.31) and 10 days (4.74 ± 0.65) (Table IV).

B. Observation of Induced Breeding

Breeding in Cemented Tank without Hormonal Treatment

In this experiment for breeding in cemented tank without hormonal treatment, no spawning respond was seen during the 20 days of observation.

Breeding through IVF Technique

Unsuccessful trial was done for artificial breeding through IVF technique. Every day checked it and finally the decision was taken that no fertilization was seen there.

Induced Breeding by Hormonal Treatment

In this experiment different types of techniques were used for induced breeding of freshwater mud eel, *M. chuchia*. After six months experimental analysis, it was seen that fish is unable to spawn through inducing agents. Chronologically three separate experiments were done in both house tanks and in hapa for each inducing agent (PG, HCG, GnRH and Ovulin). During one to two weeks of observation for each

treatment no spawning behavior were seen within the treated fish while checking regularly. After five to seven days, some fishes were become weak and injected area was infected. At the end of first, second and final trials, it was seen that mature eggs in the oviduct were reduced, absorbed and some eggs were found in spoiled conditions. Therefore, it was concluded that all experiments of the induced breeding were failed and no positive results were found.

IV. DISCUSSION

In the present study the experiment was described for the observation of breeding biology and induced breeding of freshwater mud eel, *Monopterus albus* in cemented tanks, hapa and in the laboratory condition. The freshwater mud eel, *M. albus* [1] is a rare species of air breathing teleostean fish in Bangladesh and India with spawning period confined down to peak summer [8]. Biological and reproductive aspects in synbranchids are also poorly studied, except by a few papers dealing with aspects of reproductive biology, larval development and gas exchange in a few species. Observation of breeding habits and larval development were also provided for the Asian synbranchid *M. albus* [15] and *M. albus* [16]. It was observed on that the reproductive biology of air-breathing freshwater mud eel, *M. albus* from Bangladesh by [17] and [7] where the ova diameter was found between 0.3 mm to 4 mm but in the present study almost same result on the ova diameter was found with the range of 0.3 mm to 4.3 mm. In the present study the average ova diameter was recorded 1.07, 1.81, 2.5 and 2.49 but in case of hormonal induced fish the egg diameter was comparatively lower (2.19) than the same duration of non-injected fish (2.5). It has shown that the egg diameter was gradually increased during the breeding season of freshwater mud eel, *M. albus*. Eggs were spherical and uniform in diameter. Similar findings were also reported by [18] in *Macrognathus aculeatus* and [19] in *Macrognathus pancalus*.

The peak of Gonadosomatic Index (GSI) recorded during April to June for female (7.52 ± 1.15) and male (5.50 ± 1.25) by [7] but in present study the peaks of Gonadosomatic Index (GSI) was recorded differ during February to June for female (5.1 ± 1.09) and male (2.14 ± 0.2). In case of spiny eel, *Macrognathus aral*, the peak value of GSI attained during May for male (1.3) and August for female (12.4) [20], which was far from the present findings. In the present study it was also found that the mean of GSI for the Induced fish was higher in case of male both in 5days (2.32 ± 0.71) and 10days (2.86 ± 0.57) experiment than the control fish (2.26 ± 0.52) while in case of female the GSI of the control fish (4.26 ± 0.65) was higher than a 5day treated fish (4.1 ± 0.31) but lower than a 10day treated fish (4.74 ± 0.65). Therefore, it may be said that the induced hormone influence the gonad development.

Estimation of body length, body weight and fecundity are the prerequisite in successful breeding program. Bagenal and Braum had reported that fecundity in fish species characteristically varied among individually of the same size and age and the present findings are agreed with this statement

[21]. Fagede et al. had also suggested that fecundity variation may be due to differential food [22]. In the present study the individual fecundity recorded from 1495 (65 cm / 310g) to 155 (240 cm / 69g) while relative fecundity ranged from 2.64 to 12.45 but for this air-breathing freshwater mud eel, individual fecundity recorded from 5480 (88.5cm / 650g) to 260 (41.8cm / 88g) by [7]. Nasar reported the fecundity of *M. albus* ranging from 118-687 eggs [23] but in present study it was found much higher than that. The fecundity also varied with the seasons, climate conditions and environmental habitat, nutritional status and genetic potential [24]. It was observed during present study, the same length and weight fish does not possess same amounts of eggs.

The result of induced breeding was successfully observed in different eel fish such as *M. albus*, *Anguilla anguilla*, *Anguilla japonica*, etc. Though rice field eel, *M. albus* were spawned by the injection of HCG (1000, 1500 and 2000 UI/ kg fish) and LH-RH α (50, 100 and 150 μ g / kg fish) however, the spawning rate was found highest (75%) in case of 150 μ g LH-RH α / kg fish and 2000 UI HCG / kg fish by [25]. Also, it was found that the *M. albus* can breed in captivity without using chemical stimuli, natural reproduction is considered the most optimum solution [26], but the success rate was very low and it is not commercially usable. In case of *M. albus*, induced breeding was observed and found unwilling to spawn by hormonal treatments through different doses of inducing agents and same result was found without using chemical stimuli in captive condition. Same results were also found by [10] where spawning activities were not seen by inducing agents.

Artificial propagation of eels has long been attempted not only to address fundamental questions on the reproductive biology of these fish, but also from a fisheries management and eel aquaculture perspective. Increasing fishing pressures on eels have led to reduced recruitment of juveniles of several anguillid species, limiting the industry and potentially leading to ecological impacts. Despite these research efforts, however, captive breeding of eels has not yet been achieved. Sexually development in freshwater eels is halted when these fish are held in captivity [27], but this arrest can be overridden by hormone treatment. Same results were found in this experiment when PG, HCG, GnRH and ovulin were used in captive condition.

Boethius and Boethius were able to mature males of European eel and the species was injected weekly with carp pituitary and maintaining them in sea water at a temperature of about 14°C [28]. Several other workers have subsequently succeeded in maturing the male, as well as obtaining the release of mature eggs from females, but artificial fertilization of the European eel has not been succeeded. Same results were found in this experiment where induced breeding was not succeeded. But, The Japanese eels *Anguilla japonica* have been stimulated to spawn by hormonal treatments and by keeping the brood fish in sea water at a temperature of about 23°C [29], but the larvae could be reared only until the sixth day. Although induced breeding was not performed successfully in freshwater mud eel, *M. albus*, however,

successful results were recorded for induction of vitellogenesis in female European eels. But in the present study eggs and oviduct tissues were found damaged in almost all the induced female fish.

V. CONCLUSION

The freshwater mud eel have great economic and food value in the different part of the world and due to reducing this fish from nature by several reasons, so, now time to establish a development programme for artificial breeding as well as develop culture practice is important. Hence, breeding biology is very important for a successful artificial breeding which is already studied in this experiment. For the purpose of the seed production this experiment was studied to breed using inducing agents in captive conditions. Though unsuccessful results were found in induced trial but it will be consequent progress for future study. It may have several causes to unsuccessful spawning responds such as probably imbalance of environmental conditions, physical stress etc. Another reason can be considered that its need more time with different inducing agents with different doses. Special consideration should be taken for tanks, hapa, high quality water, recycling system, good aeration, sufficient food supply, avoid crowding etc.

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