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First Record of Induced Spawning of Magur (*Clarias batratus*) Without Sacrificing Male Fish in Bangladesh

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ABSTRACT

Background and Objective: *Clarias batrachus* is an important freshwater fish of Bangladesh and is locally known as Magur. It has great commercial value due to its taste and economic aspect. This study was undertaken to establish the natural spawning of *Clarias batrachus* in captive conditions. **Materials and Methods:** The experiment was conducted at the hatchery of Bangladesh Fisheries Research Institute, Freshwater Sub-Station, Saidpur to determine the reproductive response of *Clarias batrachus* using different types of hormones. The average length and weight of brood used for the breeding trial was (26±2.0 cm an average weight of 160±4 g) and female (average length of 24±3.0 cm and an average weight of 210±6.0 g) *Clarias batrachus*. To detect ovulation, fertilization, spawning, hatching and survival different doses of the hormone were used due to optimizing the hormone doses. **Results:** After 24-28 hrs of injection, natural spawning was found successful in all cases except in T_1. The case of PG (20 mg kg^{-1} for females and 10 mg kg^{-1} for males with ovuhom 1 mL kg^{-1} for females and 0.5 mL kg^{-1} for males) shows better results in terms of ovulation, fertilization and hatching and survival in T_3 rathe than T_2. In the higher dose, almost 100% of fishes spawned naturally and fertilization and hatching rates were also higher. Hatching of fertilized eggs occurred between 30-35 hrs of incubation at 27 to 28°C and the larvae started to feed within 72 hrs after hatching. **Conclusion:** This is considered a landmark in the strategy of saving this male from sacrificing, by establishing this technique for mass production of fry *Clarias batrachus*.

KEYWORDS

Clarias batrachus, chasing, hooking, natural spawning, ovulation, hatching

INTRODUCTION

The small fishes from natural water bodies, such as rivers, canals, paddy fields and channels connecting with rivers contribute around 15.65% to the household income of fishermen in Bangladesh[1].

Zeol fish, particularly major fish, have a long history of being regarded as a prestigious and well-liked fish in Bangladesh. Because it's delightful to eat and has fewer bones, this fish is notably in demand among all socioeconomic strata, particularly youngsters and patients. Due to its deliciousness, high protein

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content (15.0%) and iron content (710 mg/100 g tissue), notably low-fat value (1.0%) and medicinal use, it is of significant commercial importance in China, India, Bangladesh, Thailand, Philippines, Myanmar and Bangladesh[2-5]. This fish's market value is higher because it can be sold alive and in its native habitat, it spawns once a year[6]. The lack of high-quality seeds in the available natural resources is the main barrier to the development of magur production. Catfish were once found in natural waters, but today fish are captured in rivers while using current nets to fish and when fishing in reservoirs and rivers during the dry season. Siltation is interfering with magur fish reproduction. Additionally, fishing with poison in agricultural lands and water bodies is causing these native catfish to disappear day by day. Male fish had their guts removed and their testicles removed in the past to produce local magur. The male fish's life had to be given up, which forced the reduction of the male catfish population and the need to purchase fry at a higher cost. Until now production of magur fish fry was not possible in a natural or easy way, so the fish farmers did not get enough fry and lost interest in magur fish farming. As a result, in 2021, researchers at the Bangladesh Fisheries Research Institute Freshwater Sub-Station, Saidpur, Nilphamari, became the first in the nation to produce magur fry naturally without sacrificing male fish. This achievement has expanded the possibilities for the acquisition and growth of magur fish fry. However, there is no published material available on the growth of the *C. batrachus* brood stock, induced breeding, or seed production. Thus, successful artificial reproduction in captivity will aid in taking action to return the biodiversity of fish to its prior state in both aquariums and their natural habitats. To optimize the dose and obtain higher ovulatory efficiency, the present study was designed to promote breeding in the *C. batrachus* utilizing various doses of synthetic hormone and carp pituitary gland (carp PG). These initiatives will save the fish from extinction while also giving rural residents a chance to sample the fish and access a source of protein for their diet.

To address the needs of small and medium-sized businesses and local farmers, it becomes vital to start producing and rearing magur larvae under controlled conditions. Few researchers have successfully employed induced spawning methods for *C. batrachus* to produce seeds utilizing a variety of natural and synthetic agents, including fish pituitary gland extracts, Human Chorionic Gonadotropin (HCG), ovaprim and ovatide, among others[7-11]. Although *C. batrachus-induced* breeding is not a tough task, the main issue is fertilization, hatching success and early fish development. After the yolk sac has been absorbed, there is widespread mortality in the early stages of development[12]. The study of the breeding performance of *C. batrachus* by various hormones with different doses is poor[13,14]. Information on the effects of temperatures and the latency period on the breeding efficiency of *C. batrachus* is very rare. Therefore, this study was asses to determine the ovulation and natural spawning (and produce fry and fingerlings production of *Clarias batrachus* in the captive environment without sacrificing the male population by using natural and synthetic hormones.

MATERIALS AND METHODS

Study area: The research work was conducted in the hatchery unit of Bangladesh Fisheries Research Institute (BFRI) Freshwater Sub-Station, Saidpur, Nilphamari. The induced breeding trials were done during the months of May-July, 2021.

Brood fish collection and rearing: After collection from the natural sources (Chikli and Atrai Rivers), the fish were transported to the BFRI Sub-Station, Saidpur by using the traditional method with plastic dram. The fish were transported most often in the early morning and sometimes during sunset. As magur is a hard fish little mortality was found during transportation and stocking. The collected semi-adult broods (50-70 g) were stocked in research ponds with a stocking density of 150 nos per decimal. In Freshwater Sub-Station, Saidpur, Nilphamari, the stocked fish were fed with a floating catfish feed (30% protein: 7% lipid) at 5% of body weight. Fishes were given feed two times per day during morning and evening.

Selection of brood fish and maturity: The gravid fishes were selected based on their external characteristics. In the breeding seasons, female fishes were identified with their swollen belly and eggs were come out by gentle pressure in the abdomen. On the other hand, mature males were identified with their streamlined bodies and elongated genital papilla with pointed reddish tips.

Experimental design: Five pairs of magur (*Clarius batratus*) used for this experiment were collected from the earthen pond of BFRI, Saidpur, Nilphamari. These fishes were transported to the hatchery complex in June 2021 and kept in a cistern with the provision of aeration and a water flow-through system for acclimatization. The body weight (g) and total length (cm), of brood fishes, sex ratio and type of hormone used in this experiment were described in (Table 1).

Breeding operation:
Spawning hapas were placed inside the cemented cistern tank by keeping the water depth at 75 cm with mild aeration facilities. A single dose of intramuscular injection of ovuhom and PG were injected just underneath the muscle in the evening Fig. 1(a-b). The doses were PG 30 mg kg^{-1} and ovuhom 1 mL kg^{-1} body weight in the case of females on the other hand PG 15 mg kg^{-1} and ovuhom 0.5 mL kg^{-1} were used in males (Table 2). After injection, previously prepared spawning hapas (placed inside the cemented tank) at the ratio of [(Male: Female) (1:1)] (Table 1) with continuous water supply through shower and keeping them in a salient environment free from noise. A maximum of two males and two females were released into each set of breeding hapa (1.2×0.9×0.9 m). Immediately after administering the hormone spawners were released into breeding hapa settled in the concrete tanks of the hatchery (capacity: 500 L) containing dechlorinated tap water (temperature: 28-31°C, DO: 5.9-6.5 ppm and pH: 7.8-8.2). To create a natural environment some water hyacinth was also used in the breeding hapa. Reproductive activities including nudging, dozing, circling, ovulation and finally, spawning were observed in the spawning tank. The female usually lays their eggs naturally 24-28 hrs after hormone injection. When the whole egg is laid, the brood fish has to be removed from the hapa. The eggs were transferred to the tray carefully. Artificial aeration was run by perporus plastic pipe. After 30-35 hrs hatchlings come out from the eggs and the hatchlings

Table 1: Name of hormone application and sex ratio for the induced breeding of magur (*Clarius batratus*)

Trial	Types of hormone	Dose female (♀)	Dose male (♂)	Sex ratio
T_1	PG (mg kg^{-1})	20	10	1:1
T_2	PG (mg kg^{-1}) and	30	15	
	Ovuhom(mL kg^{-1})	1.0	0.5	
T_3	PG (mg kg^{-1}) and	40	20	
	Ovuhom(mL kg^{-1})	1.0	0.5	

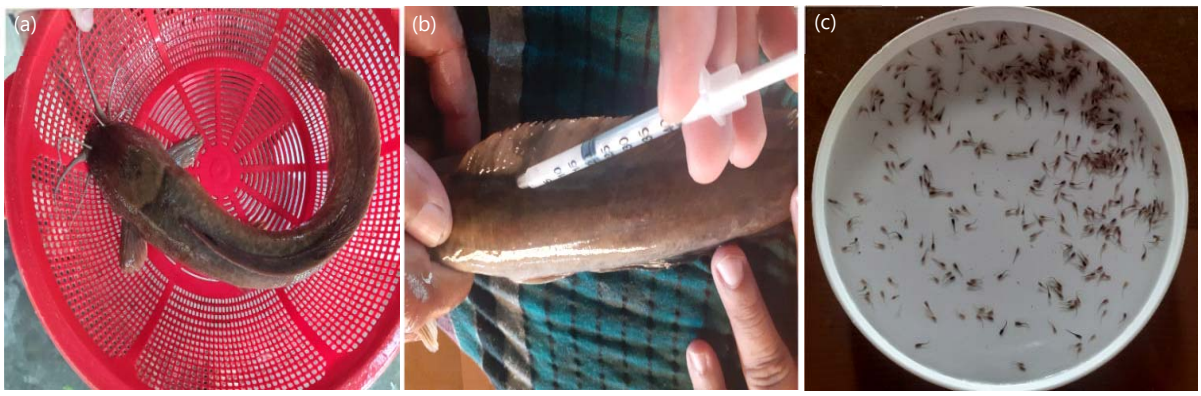

Fig. 1(a-c): Mature (a) Female of *C. batrachus*, (b) Injecting hormone dose of male *C. batrachus* and (c) Seven days old hatchlings of *C. batrachus*

are fed egg yolk/tubifex worm. Eggs yolk should be applied 04 times a day at 06 hrs intervals. Thus, after keeping in the tray for 72 hrs, the hatchlings have to be transferred to the pre-prepared nursery pond at Freshwater Sub-Station Saidpur, Nilphamari.

Calculation of breeding parameters:
Percent ovulation was calculated using the following formula[15]:

Ovulation (%)

Percentage fertilization and hatching were calculated using the following formula[15]:

Fertilization (%) = ×100

Hatching (%) = ×100

For calculating percent fertilization, several egg samples (about 50 eggs) were taken from each group and the number of fertilized and unfertilized eggs was counted under a microscope:

Survival rate (%) = ×100

Statistical analysis: The values were given with Means±SD and differences were considered significant at the subset for alpha is 0.05 ($p \leq 0.05$). The SPSS 20 (Chicago, USA) conducted a One-way Analysis of Variance (ANOVA). Duncan's Multiple Range Test to detect significant differences among the treatments at a 5% significance level.

RESULTS AND DISCUSSION

The experiment was conducted at the hatchery of BFRI, FSS, Saidpur to determine the reproductive response of *Clarias batrachus* using different types of hormones. The average length and weight of brood used for the breeding trial was (26±2.0 cm with an average weight of 160±4 g) and female (average length 24±3 cm and average weight 210±6 g) *Clarias batrachus*. Detailed doses of the pituitary gland and ovuhom used in the different trials were shown in Table 2 and breeding performances and outcomes were shown in Table 3. Ovulation occurred 24-26 hrs after the injection at 27 to 28°C[16].

Nandus perform their breeding activities at the temperature of 27-28°C, other scientists also reported the same temperature in the case of induced breeding of *Anabas testudineus* 28-29°C[17]. In this temperature, the most indigenous fish species of Bangladesh including *Clarias batrachus* performed their breeding activities.

Determine the reproduction response of *Clarias batrachus* to different doses of natural and synthetic hormones in captive conditions. The variable doses of the hormone were used to concentrate to

Table 2: Summary of results of ovulatory performances of *Clarias batrachus* at carp PG and synthetic hormones trial

	Hormone dose			
Treatment	M	F	Latency period (hrs)	Temperature (°C)
T_1	PG 10 (mg kg^{-1})	PG 20 (mg kg^{-1})	-	25.73±0.2
T_2	PG: 15(mg kg^{-1})+ Ovuhom: 0.5 (mL kg^{-1})	PG: 30 (mg kg^{-1})+ Ovuhom:1.0 (mL kg^{-1})	26-28	26.86±0.2
T_3	PG 20 (mg kg^{-1})+ Ovuhom: 0.5 (mL kg^{-1})	PG 40 (mg kg^{-1})+ Ovuhom: 1.0 (mL kg^{-1})	24-26	24.97±0.2

Values are means of data obtained±Std, Deviation (Mean±SD) of monthly determinations, Values in the same row with the same superscripts are not significantly different (p>0.05) and Absence of superscripts indicates no significant difference between treatments

Table 3: Spawning response and performances of *Clarias batrachus* under natural method (1♂: 1♀)

Treatment	Ovulation rate (%)	Of egg release (%)	Of fertilization (%)	Of hatching (%)	Survival in last 03 days	Remarks
T_1	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	No ovulation
T_2	90±0.0^a	84±14^b	76±10^b	72±08^b	70±12^a	occurs in T_1
T_3	100±0.0^b	95±10^a	86±5.0^a	80±6.0^a	74±10^a	

Values are means of data obtained±Std, Deviation (mean±SD) of monthly determinations. Values in the same row with the same superscripts are not significantly different (p>0.05) and Absence of superscripts indicates no significant difference between treatments

determine ovulation, fertilization, hatching and survival to yolk sac absorption. It was observed that both the female and male fish ejected eggs and milt at contemporary times when the fish were treated with a single injection mixed with both hormones at T_3 and T_2. After 24-28 hrs of injection, natural spawning was found successful in all cases except in T_1. The case of PG (20 mg kg^{-1} for females and 10 mg kg^{-1} for males with Ovuhom 1 mL kg^{-1} for females and 0.5 mL kg^{-1} for males) shows better results in terms of ovulation, fertilization and hatching and survival in T_3 and T_2. No spawning occurred in T_1 when single PG doses (40 mg kg^{-1} for females and 20 mg kg^{-1} for males). A larger latency period was noticed for the natural spawning of *Clarias batrachus* when PG (40 mg kg^{-1} for female and 20 mg kg^{-1} for male with Ovuhom 1 mL kg^{-1} for female and 0.5 mL kg^{-1} for male) were used. The latency period of the present study was 24-26 hrs. The scientist also mentions that in the case of *Clarias batrachus* smooth stripping and ova without clusters were identified and at a higher dose of pituitary gland extracts (120 mg kg^{-1} b.wt., of female) when the latency period was maintained for 14 or 15 hrs irrespective of temperatures for *Clarias batrachus*[18]. The highest average ovulation rate 100 and 90% was recorded in T_3 and T_2. On the other hand, % of eggs released was higher in T_3 (95%) which was significantly higher (p<0) than in T_2 (84%). This is because they were treated in different hormone doses. In highest average ovulation rate indicate that the concentration of hormone dose was accurate and maintained once water quality. The brood fishes were highly mature in the better broodstock management practice with a special diet[18]. Observed that the highest ovulation rate was 90% in the cemented cistern. He recorded May and June, as the peak season for the induced breeding of the specimen[19]. It was observed the fertilization rate of *M. pancalus* was 82%. Maximum and minimum fertilization and hatching of *Clarias batrachus* were found in 86 and 80% in T_3 which is significantly higher with a higher dose (p<0.05) than in T_2 (76 and 72%) spawning largely depends on the synchronization of ova and sperm release[20,21]. Comparatively lower fertilization and hatching rates with regard to the larger carp were found when compared with small indigenous fish species[22] but the findings of the present study were satisfying in the sense of comparatively better breeding success means fertilization and hatching rates. Fertilization and hatching rates indicate the status of the well-being of the broods used in breeding and the quality of hatchery management. In some cases, hormone doses 8 mg kg^{-1} for females and 4 mg kg^{-1} for males provided the best result in the sex ratio 2♂: 1♀[23]. Survival of the 03 day old larvae was not significantly varied with T_3 and T_2. The survival rate of 03 days of hatchling was 74% in T_3 and 70% in T_2. found a 90% fertilization rate that yielded hatchings in about 80% of fertilized eggs in *Mystus gulio*[24]. Although, some workers reported higher doses of PG for inducing ovulation as 40 mg carp PG kg^{-1} body recommended for ovulation[14] and *in vitro* fertilization of *Clarias batrachus* eggs. Some research also found 10.0 mg PG kg^{-1} b.wt., the in first dose and 45.0 mg in the second dose causes ovulation in *C. batrachus*[3]. This was attributable to the fact in the present study of PG and ovuhom doses (30 mg kg^{-1} and 0.5 mL kg^{-1}) gives lower results than dose at high doses of PG (40 mg kg^{-1} and ovuhom 1 mL kg^{-1}) for female fish getting weaker and releases a smaller number of eggs. After absorption of the yolk sac (72 hrs), the spawn was transferred into metallic trays and fed on semi half boiled eggs yolk and renu gold (commercial fish feed containing 36% protein) for up to 03 days to optimize the rearing condition of larvae. The survival rate of 03 days old hatchling (Fig. 1c) of *Clarias batrachus* varied from 70-74% in 2 different treatments[25,26]. In the present study, *C. batrachus* also showed a similar pattern of survival rate like as another tangra species *Mystus gulio*.

This study is very much applicable in the field of induced breeding techniques. Hatchery owners or fish farmers can easily produce fry and fingerlings if they follow this technique and maintain a good quality of mature males of *Clarias batrachus*. However, strictly maintaining the inbreeding-free brood in pond conditions if they are succeeding in the natural spawning of *C. batrachus*. Prior to making the solid decision different ages of males need to try for induced breeding and found out the suitable age of brood for natural spawning without scarifying the male population. Optimization of natural and synthetic hormone doses also needs to assess for better success and economic aspects of hatchery owners.

CONCLUSION

This study showed that *Clarias batrachus* was successfully induced to ovulate without sacrificing the male brood *C. batrachus* for the first time in Bangladesh. The best outcomes were obtained using a carp pituitary with a mix of ovuhom doses PG 40 (mg kg^{-1}) +ovuhom: 1.0 (mL kg^{-1} b.wt.) for females. Meanwhile, when males received a total dose of 20 mg kg^{-1} of carp pituitary gland and ovuhom 0.5 mL kg^{-1} b.w.t., there was a high incidence of egg release, fertilization and hatching and survival of larvae were found in this experiment. This is regarded as a turning point in the plan to produce enough fry in large quantities to save this highly cultural and delicious species without sacrificing male brood stock.

SIGNIFICANCE STATEMENT

Clarias batrachus was a very popular catfish in Bangladesh on the basis of higher yield, consumer demand and higher market profit. Hatchery owners tried to develop a method to produce fry and fingerlings by natural spawning but they failed because male fish were not released milt that's why they scarify the male fish. So, the purpose of the study was to conserve the male population in nature. After various trials, the natural spawning of *C. batrachus* was obtained by maintaining the natural conditions in the fish hatchery and the quality of a well-mature inbreeding-free brood by injecting natural and synthetic hormones with long latency periods.

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REFERENCES

1. Lahiri, T., M.A. Rahman and A.A. Mamun, 2023. Reassessing the food security implications of export-oriented aquaculture in Bangladesh. Aquacult. Int., 31: 1143-1162.
2. Srivastava, P.P., S. Raizada, R. Dayal, S. Chowdhary and W.S. Lakra *et al*., 2012. Breeding and larval rearing of asian catfish, *Clarias batrachus* (Linnaeus, 1758) on live and artificial feed. J. Aquacult. Res. Dev., Vol. 3. 10.4172/2155-9546.1000134.
3. Hossain, M.Y., Z.F. Ahmed, P.M. Leunda, S. Jasmine, J. Oscoz, R. Miranda and J. Ohtomi, 2006. Condition, length-weight and length-length relationships of the Asian striped catfish *Mystus vittatus* (Bloch, 1794) (Siluriformes: Bagridae) in the Mathabhanga River, Southwestern Bangladesh. J. Appl. Ichthyol., 22: 304-307.
4. Kamal, D., A.N. Khan, M.A. Rahman and F. Ahamed, 2007. Biochemical composition of some small indigenous fresh water fishes from the river Mouri, Khulna, Bangladesh. Pak. J. Biol. Sci., 10: 1559-1561.

5. Devaraj, K.V., T.J. Varghese and G.P.S. Rao, 1972. Induced breeding of the freshwater catfish *Clarias batrachus* (Linn.) by using pituitary glands from marine catfish. Curr. Sci., 41: 868-870.
6. Kumar, R., U.L. Mohanty and B.R. Pillai, 2021. Effect of hormonal stimulation on captive broodstock maturation, induced breeding and spawning performance of striped snakehead, *Channa striata* (Bloch, 1793). Anim. Reprod. Sci., Vol. 224. 10.1016/j.anireprosci.2020.106650
7. Sahoo, S.K., S.S. Giri, S. Chandra and B.C. Mohapatra, 2008. Evaluation of breeding performance of Asian catfish *Clarias batrachus* at different dose of HCG and latency period combinations. Turk. J. Fish. Aquat. Sci., 8: 249-251.
8. Khan, A.B.S., M. Akhter, A. Bosu, S. Ahmmed and M.M. Ali, 2021. Optimization of hormone for artificial breeding and seed production of *Clarias batrachus* (Linnaeus, 1758) under captive condition. Asian J. Fish. Aquat. Res., 14: 35-43.
9. Sahoo, S.K., S.S. Giri and A.K. Sahu, 2005. Effect on breeding performance and egg quality of *Clarias batrachus* (Linn.) at various doses of ovatide during spawning induction. Asian Fish. Sci., 18: 77-83.
10. Sahoo, S.K., S.S. Giri and A.K. Sahu, 2005. Induced spawning of Asian catfish, *Clarias batrachus* (Linn.): Effect of various latency periods and SGnRHa and domperidone doses on spawning performance and egg quality. Aquacult. Res., 36: 1273-1278.
11. Tilahun, G., K. Dube, C.S. Chtruvedi and B. Kumar, 2016. Assessment of reproductive performance, growth and survival of hybrids of African Catfish (*Clarias gariepinus*) and Indian Catfish (*Clarias batrachus*) compared to their parental lines crosses. Turk. J. Fish. Aquat. Sci., 16: 123-133.
12. Ferosekhan, S., S.K. Sahoo, K. Radhakrishnan, M. Gokulakrishnan, S.S. Giri and B.R. Pillai, 2022. Standardisation of weaning protocol for larvae of *Clarias magur* (Hamilton, 1822). Asian Fish. Sci., 35: 68-75.
13. Assan, D., K. Anane, E.D. Abarike, E.H. Alhassan and A. Ampofo-Yeboah, 2022. Evaluation of induced breeding of catfish (*Clarias gariepinus*), using different doses of normal saline diluted ovaprim. J. Appl. Aquacult., 34: 456-468.
14. Goswami, U.C. and N.N. Sarma, 1997. Pituitary dose optimization for induced ovulation, *in vitro* fertilization and production of normal fry of *Clarias batrachus* (Linn.). Asian Fish. Sci., 10: 163-167.
15. Dhara, K. and N.C. Saha, 2013. Controlled breeding of Asian catfish *Clarias batrachus* using pituitary gland extracts and ovaprim at different temperatures, latency periods and their early development. J. Aquacult. Res. Dev., Vol. 4. 10.4172/2155-9546.1000186.
16. Pal, S., H. Rashid, M.A.K. Tarafder, N.T. Narejo and M. Das, 2003. First record of artificial spawning of *Nandus nandus* (Hamiltom) in Bangladesh using carp pituitary gland: An endangered species bred in captivity. Pak. J. Biol. Sci., 6: 1621-1625.
17. Hossen, M.A., M.A. Hossain, A.K.M. Munzurul Hasan, B. Das, S. Mian and M.M. Iqbal, 2021. Observation of embryonic and larval developmental stages in endangered nona tengra (*Mystus gulio*) induced with S-GnRHa. Punjab Univ. J. Zool., 36: 91-99.
18. El-Hawarry, W.N., S.H. Abd El-Rahman and R.M. Shourbela, 2016. Breeding response and larval quality of African catfish (*Clarias gariepinus*, Burchell 1822) using different hormones/hormonal analogues with dopamine antagonist. Egypt. J. Aquat. Res., 42: 231-239.
19. Hasan, M.R., M.S. Islam, A. Afroz, P. Bahadur and S. Akter, 2016. Captive breeding of striped spiny Eel, *Mastacembelus pancalus* (Hamilton, 1822) considering the various hormonal responses. Int. J. Fish. Aquat. Stud., 4: 7-11.
20. Kamei, M., S. Munilkumar, C. Basudha, S. Dasgupta, P.B. Sawant and W.R. Mangang, 2023. Breeding and larval rearing of juvenile of Burmese loach, *Lepidocephalichthys berdmorei* (Blyth, 1860): A new candidate species for aquaculture. J. Exp. Zool. India, 26: 11-18.
21. Sayeed, M.A., S. Akter, A.K. Paul, M.R. Ahashan, M.M.H. Miah and M.A.R. Hossain, 2009. Development of artificial breeding technique of gutum, *Lepidocephalichthys guntea* (Hamilton, 1822) using carp pituitary gland. J. Agrofor. Environ., 3: 195-197.
22. Hoque, A.S.M.M. and M.R. Rahman, 2008. Reproductive ecology of mola (*Amblypharyngodon mola*). J. Agric. Rural Dev., 6: 165-174.

23. Islam, S.S., M.S. Shah and M.L. Rahi, 2011. Study of fecundity and induced breeding of *Mystus vittatus*. Bangladesh J. Zool., 39: 205-212.
24. Kumar, P., G. Biswas, T.K. Ghoshal, M. Kailasam, L. Christina and K.K. Vijayan, 2019. Current knowledge on the biology, captive breeding and aquaculture of the brackishwater catfish, *Mystus gulio* (Hamilton, 1822): A review. Aquaculture, 499: 243-250.
25. Sarker, P.K., H.K. Pal, M.M. Rahman and M.M. Rahman, 2002. Observation on the fecundity and gonado-somatic index of *Mystus gulio* in brackishwater of Bangladesh. Online J. Biol. Sci., 2: 235-237.
26. Alam, M.J., M. Begum, M.A. Islam and H.K. Pal, 2006. Spawning behaviour and induced breeding of an estuarine catfish, *Mystus gulio* (Ham.). Bangladesh J. Fish. Res., 10: 101-109.