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ARTICLE

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FIRST REPORT ON SUCCESSFULLY INDUCED BREEDING AND LARVAL REARING OF ONE STRIPE SPINY EEL *MACROGNATHUS ARAL* FOR ARTIFICIAL PROPAGATION AND CONSERVATION

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In this study, wild Macrognathus aral were collected from different floodplain wetlands in the lower Gangetic basin. These fish are significant both for their nutritional value and as ornamental fish, resulting in high market demand. To induce spawning under captive conditions, mature fish were injected with the synthetic hormone Spawn Pro, which contains GnRH. Different doses of the synthetic hormone (ranging from 0.03 to 0.05 ml/kg body weight) were administered via intramuscular injection. Each set of fish was placed in a glass aquarium with hollow tubes covered with 0.6mm mosquito netting. Spawning behavior varied with different hormonal doses, and no spawning was observed in the control set. Fertilization rates ranged from 25.80% to 72.62%, with each female depositing between 85 to 135 eggs. The fertilized eggs, measuring 2460µm to 2650¼m, were attached to the net in the breeding tank. The eggs hatched 30-32 hours after fertilization, with a hatching rate of 40.50% to 77.86%. Newly hatched larvae measured 2.8 ± 0.03 mm, with a yolk sac diameter of 590.94 ± 10µm. The survival rate of hatchlings after the first week ranged from 5% to 10%. This study provides essential baseline data for breeding, seed production, conservation, and further propagation of these ornamental fish.

Introduction

Among the freshwater Eel species available in India *Macrognathus aral* has high demand as an ornamental fish in domestic as well as in the export market due to its playful behavior, brilliant coloration with vibrant ocelli and eye-catching body shape[1]. The species is commonly available in lowland habitats and at moderate elevations in all larger river systems of the Indian subcontinent and Bangladesh, Myanmar, Nepal, Thailand, Pakistan and Sri Lanka[1,3-6]. However, due to the degradation of natural habitats and environmental changes, the wild population is declining rapidly[7]. Since the supply of the fish is fully dependent on the wild collection, its supply become highly fluctuating and the price is increasing alarmingly. The local market price of one stripe One Stripe Sping Eel as food fish varies between Rs. 750-1000/kg depending upon size. While as ornamental fish the species are valued Rs. 55-75/piece. The species also have an export value of 1-2 US$ per piece. Biodiversity conservation gains momentum when it is implemented through preaching and practice, involving common people concerning their livelihood. The reproduction biology of the fish from wild collection was recorded by several

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researchers from a different region[8-15]. It has been recorded that the reproducing period of the species stretched out from September to October. The information on maturity size, sex proportion, maturity stages, fecundity, ova diameters, etc. from different aquatic habitats is also reported[10,13]. The review of the literature indicates that the first induced breeding of *M. aral* with pituitary extract was carried out by Chakraborty[16] in Bangladesh with partial success but the details of the record of breeding protocol, larval development survivality, hatchling and larval growth of the species were not systematically documented. So, there is a data deficiency on the complete protocols of successful captive breeding and large-scale seed production of the fish. In India so far, our knowledge no reports are available on the induced breeding and captive propagation of *M. aral* in the Gangetic floodplain areas and the supply of ornamental fish is purely dependent on natural stocks. Hence, the research aimed to investigate and gather wild *M. aral* stocks from Gangetic floodplain wetlands, acclimatize them, and raise brooders under controlled conditions. This involved selecting mature brooders for induced breeding, rearing the larvae, and producing seeds of this valuable species.

Material and Methods

Study Area and Sample Collection: About 235 live samples of *M. aral* were collected from the freshwater pond and different wetlands i.e., Beledanga; Chamta; Duma; Sashadanga; Panchpota; Panchitaat Bongaon in North 24 Parganas, Khalsi wetland in Nadia, Ganga River in Balagarh and Tribeni at Hooghly district, Diamond harbor khal at Diamond harbor in South 24 Parganas district, West Bengal during September, 2019 to August, 2020 (Table 1). The natural habitat preference of One Stripe Sping Eel is slow-moving shallow open water bodies thorough in Gangetic floodplains. The fish are collected using local bamboo traps like Ghunni, Bitti, Dori, etc., and also employing cast net. Sometimes fishes are also collected by dewatering shallow water pockets. Fishes were mainly collected in pre-monsoon and winter when the water level of the wetlands was reduced.

Brood Stock Maintenance and Development: Natural habitat study reveals that the fish prefer to stay in slow-moving or stagnant vegetated water bodies. In captive conditions the fish reared under aquatic vegetation and bamboo hideouts. After procurement, the fish are disinfected with 0.5% $KMnO_4$ solution for 30 seconds. To trigger maturity in captive conditions different types of feed are offered to them viz. tubifex, mixed plankton, Mosquito larvae, dry feed (commercial dry feed by CP), chicken waffle mixed with rice bran and mustered oil cake, commercial dry feed mixed with vitamin E and mineral. The feed was given @ 1.5% of total body weight for 60 days to observe the feed acceptance and growth of the fish. After that, the fish was fed with tubifex mixed with vitamin and mineral mixer for quick gonadal maturation of the fish. After 8-9 months of captive rearing wild collection the fishes become matured in captive condition.

Reproductive Biology of Fish: The seasonal changes of gonadosomatic index (GSI) were calculated by the formula:

$$GSI = \frac{\text{Weight of gonads}}{\text{Total weight of fish}} \times 100$$

Fecundity estimation of total 15 *M. aral* females were observed during the breeding season. As recommended

Table 1. The details of the wild collection sites of *M. aral* in West Bengal, India

Name of Water Body	Location	Coordinates	Type of Water Body	District
Natidanga Baor	Beledanga	23.08° N, 88.76° E	Connected with Ichhamati River	North 24 Parganas
Panchita Chanda Baor	Panchita	23.09° N, 88.81° E	Closed	
Panchpota Baor	Panchpota	23.10° N, 88.76° E	Connected with Ichhamati River	
Sashadanga Baor	Sashadanga	22.93° N, 88.86° E	Connected with Ichhamati River	
Duma Baor	Duma	22.98° N, 88.83° E	Connected with Ichhamati River	
Chamta Baor	Chamta	23.11° N, 88.70° E	Closed	
Khalsi Baor	Khalsi	22.99° N, 88.64° E	Closed	Nadia
Hooghly River	Balagarh	23.11° N, 88.46° E	River	Hooghly
Hooghly River	Tribeni	22.99° N, 88.39° E	River	
Diamond Harbor Canal	Diamond harbor	22.19° N, 88.18° E	Connected with Hooghly river	South 24 Parganas

by Clark[17] and Qasim and Qayyum[9] ova diameter study occurred. The formula used for absolute fecundity calculation is:

$$\text{Absolute Fecundity (F)} = \frac{\text{Number of ova in specimen} \times \text{total weight of overy}}{\text{Weight of specimen}}$$

Brooder Selection and Stock Development: Fully matured and healthy *M. aral* were selected by using primary sexual dimorphic characters for captive breeding experiments. The length of the mature female varies between 12.7 to 15.8 cm and weight varies from 6.8 to 12.5 g respectively. Males are matured at comparatively smaller in size than females. The length of mature males varies between 10.4 to 15.2 cm and weight from 4.6 to 11.6 g respectively. After maturation males and females are segregated in separate aquariums. The tank water was covered with aquarium plants and bamboo hideouts. At this time tubifex @1.5% body weight was given as food. Water quality management was made in every 7 days interval. The breeding experiments were done in July 2021.

Table 2. Different doses of hormone used in Induced breeding

Treatment	Female: Male	Sex	Length (cm)	Weight (g)	Dose (mg/10g)
T1	1:2	Female	15.8	13.1	0.05
		Male	11.4	8.5	0.025
		Male	14.7	7.7	0.025
T2	1:1	Female	13.3	9.5	0.05
		Male	10.3	7.6	0.025
T3	2:3	Female	13.2	8.2	0.05
		Female	12.7	8.1	0.05
		Male	15.1	11.6	0.025
		Male	12.6	5.3	0.025
		Male	13.6	6.2	0.025
T4	1:2	Female	13.1	6.5	0.04
		Male	13.9	7.2	0.025
		Male	15.2	10.7	Nil
T5	1:2	Female	11.8	7.4	0.03
		Male	11.5	4.6	0.025
		Male	12.1	7.5	0.025
T6	1:2	Female	12.4	7.6	0.05
(Rainwater)		Male	10.8	5.2	0.025
		Male	11.5	5.7	0.025
Control	1:2	Female	11.6	6.9	No
		Male	10.5	4.8	injection
		Male	11.9	6.2	

For induced breeding glass aquarium of 2.5×1.5×1.5ft was used for both breeding and rearing of fish. The aquarium was supported with filter, continuous air-pump and breeding mops (nylon threads) etc. The breeding tank is covered with 0.6 mm mosquito nets and the walls are covered with dark sheets. Nylon threads are used as a breeding mop as the sticky eggs are attached to the threads. All substances were disinfected with KMnO4 solution before the breeding tank setup. Rainwater was also used in one tank for the breeding experiment.

Experimental Design: In this experiment for induced breeding synthetic Salmon GnRH analog (Spawn Pro) was injected to the brooders. Spawn Pro is a product of P. R. Organics, Mumbai. The hormone consists of Salmon GnRH Analogue 20 mcg, Domperidone BP 10 mg, and Propylene Glycol IPQ.S. Male and female both were given a single dose. The experiments were designed to have 6 different treatments and one control. The details of the experimental setup including sex ratio and hormonal doses used in the two different sexes are listed in Table 2. A control set was maintained where no hormonal injection was administered.

Estimating Effectiveness of Spawn Pro at Different Doses: Latency period of the fish spawning estimated as time interval between first dose of injection and starting of ovulation. After two hours of ovulation the eggs were examined to observe the fertilization rate. The fertilization rate was determined following the formula:

$$\frac{\text{No. of fertilized eggs}}{\text{Total No. of eggs}} \times 100$$

To determine hatching rate the number of hatchlings were counted by visual observations. Then hatching rate was determined following the formula:

$$\frac{\text{No. of hatchlings}}{\text{Total number of fertilized eggs}} \times 100$$

Embryonic Developmental Study: The samples of eggs after fertilization at every 30-minute interval observation were recorded under a stereoscopic microscope. The developmental stages were divided into embryonic and larval development. The embryonic stage occurs inside the chorion and ends in hatching. Water temperature, dissolved oxygen, and pH of water were measured daily by a multiparameter water quality analyzer[18]. The embryonic development period was defined as the

period at which 50% of fertilized eggs were hatched. The egg hatching and larval rearing was taken up in the same tanks that were used for spawning and the parent fish was removed.

Results

Breeding Behaviour and Estimating Effectiveness of the Hormone: In this present experimental trial breeding behaviour and spawning were observed in every treatment group after hormone injection. No spawning was observed by females in the control set. The spawning behavior of the fish starts after 3-8 hours of hormone induction. Male shows more active movement than females. There is no change in prominent colouration pattern in either sex. During courtship, the male chases the female after that both the fishes were entangled with each other around the bushy mop for spawning.

The breeding duration lasts upto 8-10 hours then the transparent greenish fertilized eggs are observed in the bottom and breeding mop of the tank. The spawning efficacy of *M. aral* with different doses and sex ratios are described in table 3.

Table 3. Optimum dose and spawning efficacy of Spawn Pro for *M. aral* breeding

Treatment	Dose (Mg/10g)	Sex Ratio	% Spawning
T1	0.05	1:2	75-90
T2	0.05	1:1	35-45
T3	0.05	2:3	55-65
T4	0.04	1:2	60-70
T5	0.03	1:2	40-50
T6 (Rainwater)	0.05	1:2	30-45
CONTROL	0.0	1:2	No spawning

The water quality parameters play an important role in successful breeding and seed production. In the present study, the water quality parameters were recorded for each experimental breeding set (Table 4). The water temperature was maintained at about 31.7±±0.7°C in different treatments. Slight alkaline water with a pH of 7.5±0.4 was maintained for the fish breeding. In the rainwater breeding tank the pH was highly acidic. The sticky fertilized eggs are attached to the nylon tread or bushy substratum. The latency period, incubation period, fertilization rate, and hatching rate of the fish are shown in Fig. 1.

The fertilization time or latency period varies up to 12-14 hours from the injection. The latency period significantly varied with the hormone's doses. The low latency period was observed in T1, followed by T3, T4, and T6. The prolonged latency period was observed in T2, T3, and T5. The fertilized eggs are transparent, thin, demersal, and spherical in character. The unfertilized eggs were pale and opaque.

After fertilization, the embryonic developmental study starts and continues up to the hatching period at every 30 min interval. Treatment-wise fertilization rate of the fish is given in. A significantly higher fertilization rate was observed at T1 (72.62%) followed by T4 (71.42%). The lowest rate of fertilization was observed in T6 (40.5%). The results showed that the fertilization rate was significantly higher with a dose of 0.05 ml/10g of body weight than other treatments and the female and male ratio was kept at 1:2.

The differential incubation period was observed in different treatments, varying from 30-32 hours after fertilization. The hatching rate was significantly higher in T1 (77.86%) as compared to other treatments and the rate of hatching in T6 was significantly lower. The study showed that 0.05mg/10g (uniform unit should be) of fish hormone induction (synthetic hormone /fish hormone make it uniform) and with a 1:2 female and male ratio *M. aral* shows the highest fertilization and hatching rate.

Embryonic Development: The fertilized eggs of *M. aral* are green in colour and swollen egg size varies between 2460¼m to 2650¼m (Fig. 2). Due to the slightly sticky nature of the egg, considerable debris adhered to the outer capsule of the egg. The embryonic development from fertilization to hatching was classified into different stages. The details of each stage with their timing described in table 5 and the images of different embryonic stages showed in Fig. 2.

Table 4. The water quality parameters at the time of breeding

Parameters	T1	T2	T3	T4	T5	T6	Control
Temperature(°C)	30.2±0.7	32.0±0.7	32.3±0.7	32.1±0.7	32.5±0.7	31.5±0.7	31.5±0.7
pH	7.8±0.4	7.6±0.4	7.8±0.4	7.6±0.4	7.8±0.4	6.6±0.4	7.4±0.4
Dissolved Oxygen (ppm)	9.2±0.3	9.1±0.3	9.5±0.3	9.2±0.3	8.9±0.3	8.4±0.3	8.8±0.3
Alkalinity (mg/L)	148±26	144±26	148±26	146±26	150±26	78±26	146±26

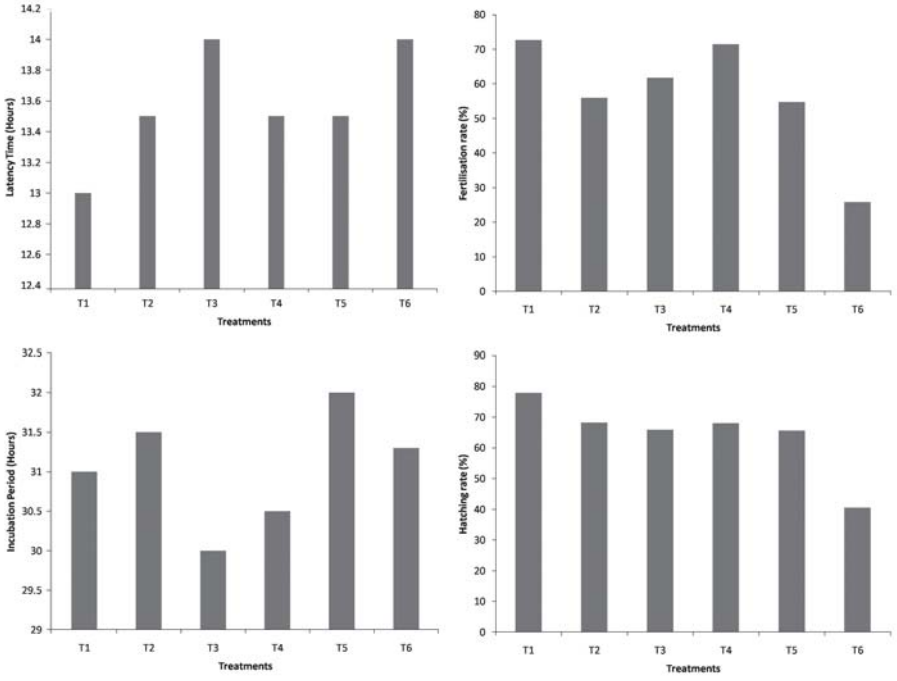


Fig. 1. Spawning parameters of M. aral under different

Morula Stage: the cleavage of the egg starts after 40-45 minutes of fertilization. Gradually the cleavage furrow is restricted to the animal pole of the cytoplasm. The segregation of blastodisc from the clearer yolk granule-rich vegetal cytoplasm continues during early cleavage stages. When the protoplasm moved the blastoderm became thicker. The morula stage continues up to 2 hours 50 min.

Blastula Stage: after cleavage, the developing embryo of fish is further divided into numerous cells and it forms a layer called blastoderm. This time a narrow space formed between the blastodisc and yolk. Epiboly was initiated and continued during the gastrulation period. At about 7 hours after fertilization, the blastoderm flattens up and starts to wrap around the yolk sac and then the blastula stage ends up and enter to gastrula stage.

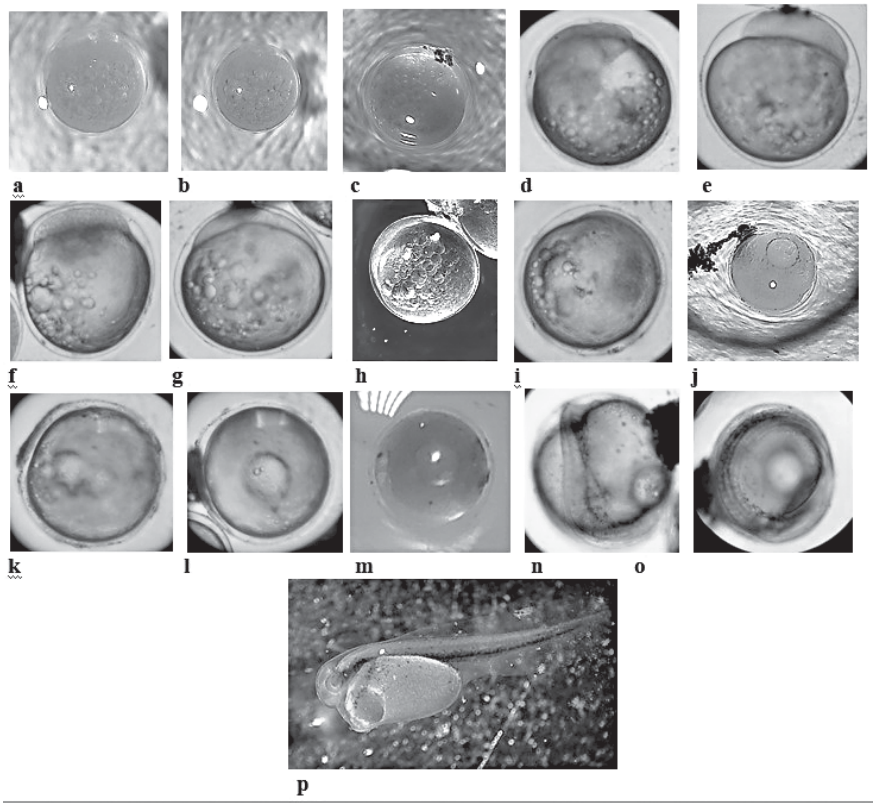
Gastrula Stage: during this stage, the blastomeres of *M. aral* were extended towards the vegetal pole. The lower rim of the blastodisc thickened and formed a germ ring. Optic rudiment was visible in this stage. Approx. 15 hours of post-fertilization, blastoderm covered about 3/4 of the yolk, which is the marking of the end of the gastrula stage.

Somite Stage: During this stage, the development of somites came into sight and mesoderm developed into bone and muscle. The rudiments of the primary organs became visible; the tail bud became more prominent and the embryo elongated. The body movement starts in this stage. At the end of this stage, tail was completely detached from the yolk and the yolk sac was restricted to the head region.

Hatching Stage: After the embryonic development is completed, the twitching movement starts vigorously and the embryo hatches out within 30-32 hours at a water temperature 30-33°C. The vigorous movement of a fully

Table 5. Stages of embryonic development of *M. aral*

Stage	Duration after fertilization	Division	Plates under Figure 7
Morula	45 min to 2 hour 50 min	8 cell	a
		16 cell	b
		32 cell	c
		64 cell	d
Blastula	2 hour 50 min to 7 hours 30 min	Early blastula	e
		Mid blastula	f
		Late blastula	g
Gastrula	7 hours 30 min to 15 hours 20 min	Early gastrula	h
		Germ ring	i
		80% epiboly	j
		Blastophore closed	k
Somite	15 hours 20 min to 30 hours	Somite appearance	l
		Optic vesicle appearance	m
		Muscle function phase, C shapes embryo	n
		Heartbeat phase	o
Hatching	30 hours to 32 hours	Just hatched larvae	p



a. 8 cell; b. 16 cell; c. 32 cell; d. 64 cell; e. Early blastula; f. Mid blastula; g. Late blastula; h. Early gastrula; i. Germ rig; j. 80% epiboly; k. Blastophore closed; l. Somite appearance; m. Optic vesicle appearance; n. Muscle function phase, C shapes embryo; o. Heartbeat phase; p. Just hatched larvae.

Fig. 2. Different stages of embryonic development of *M. aral*

a. 24 hours after hatching; b. Just before yolk sac absorption (48 hours after hatching); c. After yolk sac absorption; d. 15 days after hatching; e. 30 days old fry; f. 4 months old fingerling.

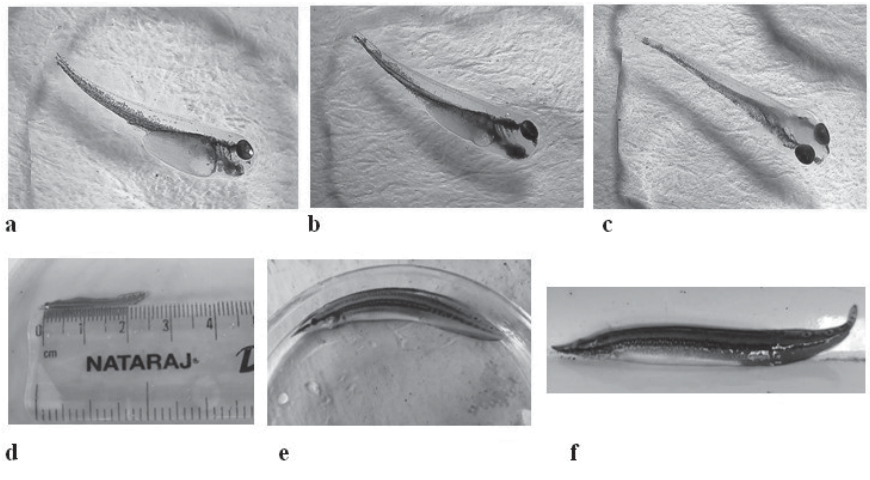


Fig. 3. Larval development of *M. aral*

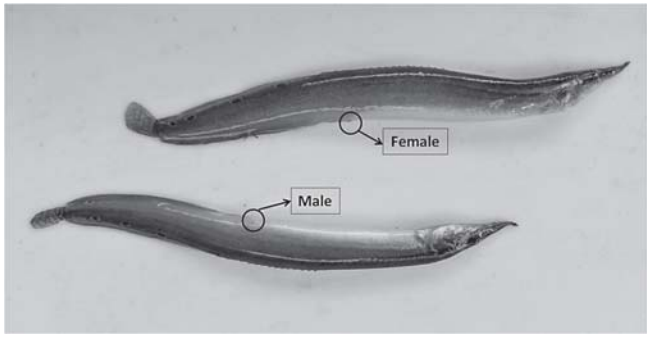


Fig. 4. Sexual dimorphism of *M. aral*

developed embryo ruptured the egg's thin shell and the larva hatched out.

Larval Development and Maintenance: The newly hatched larvae of *M. aral* ranged between 2.5 mm and 3mm in length. After hatching, the larvae were transferred to a small aquarium 1×5×5ft filled with aerated water and Hydrilla plant. The yolk sac is absorbed about 55-60 hours after the hatching. After yolk sac absorption the larvae fed with mixed plankton, Infusoria, and Artemia larvae for 15-20 days. The larvae reached 10-25 mm in length within 15 days (Fig. 3). After 15 days the larvae fed with small, cleaned Tubifex. In this larval development, water qualities are checked every day and exchange water is exchanged every 3 days. The water temperature varies between 31-33°C and the water was slightly alkaline. Ocelli were visible after 25-30 days of hatching. After 4 months the fingerling size on average was 7.5cm in length and 3.4g in weight.

Table 6. Seasonal changes of GSI of *M. aral*

Seasons	Avg. GSI
Pre-monsoon (April to June)	4.32
Monsoon (July to September)	8.3
Post-Monsoon (October-December)	1.75
Winter (January to March)	0.75

Reproductive Phenology of M. aral: The male and female fishes were maintained separately for breeding purposes and identification rapidly through external characters. The fish can be easily distinguished by the greenish female dorsal parts and darker stripes in male fish (Fig. 4). Among the 550 specimens studied, 375 and 175 have been observed to be female and male respectively. The average ratio of female to male has been observed to be 2:1. The matured gonad of the female is elongated, dark green in colour and has asymmetric lobes. The male testis is milky white coloured, elongated and asymmetric lobbed. The breeding season of the fish continues from July to September. The gonadosomatic index (GSI) fluctuated from 0.75 to 8.30. The seasonal changes of mean GSI are shown in table 6. The average ova diameter recorded 0.94 mm in a fully matured ovary. The absolute fecundity of the fish varies between 775 to 2250 numbers during the breeding season.

Discussion

The present investigation demonstrated the artificial propagation and documented the early development of *M. aral*. The review of the literature shows the variation in the breeding periodicity of fish in the different studies. The peak reproducing period of the species was observed during July to September whereas Qasim and Qayyum[9] recorded the breeding season from September to October. Dutta and Banerjee[15] observed the highest value of GSI in the month June. In the present study highest GSI value was observed in July to September. This might be due to climatic-environmental changes[19,20]. The sex proportion of the fish was reported near 1:1[11,23,26] but in the present study, the sex ratio was recorded 2:1 female and male from the floodplain wetlands of West Bengal. The ova diameter increased from 0.3mm (March) to 1.4 mm (May) as per Abujam and Biswas[13] and almost similar results were observed in the present study where in the month of June the ova diameter recorded 0.94mm. However, there is a noticeable change in the fecundity of fish. Sharma and Lavanya[10] recorded 1065 to 4961 absolute fecundity while Abujam and Biswas[13] recorded 833.43±248.26 to 3027.57±1689.66 absolute fecundity, but in the present study the absolute fecundity was comparatively lower ranged from 775 to 2250. The reason might be due to environmental changes and climatic variability. It was previously hypothesized that simulation of natural environmental conditions under confined captive conditions may trigger spawning. The present study partially supported the theory as the fish successfully matured under captive conditions. However, it failed to spawn naturally under captivity[21]. At present no reports are available on captive breeding and larval developments of fish in India. Das and Kalita[1] demonstrated the spawning success of. *M. aculeatus* under captivity, where spawning was triggered using Ovaprim. In another attempt Borah et al.[21] described the breeding success of *M. pancalus* with the Ovasis hormone. Many authors[22,24,25] have also reported captive breeding and seed production of *M. aculeatus* and *M. pancalus* in the agro-climatic condition of North-Eastern Hill region and Bangladesh. But there are no detailed reports are available on the induced captive breeding of *M. aral* by any of the synthetic inducing agents except carp pituitary hormone used in Bangladesh[16]. In the present study, the hormonal dose (low, medium, and high) of Spawn Pro was selected considering the doses used in earlier reports. The results obtained from the present study show distinctly higher fertilization and hatching success with 0.05mg/10g of fish Spawn Pro dose and 1 female and 2 male ratio. The present observation on the reproductive biology of *M. aral* shows that the breeding of this species is seasonal and extended from July to September. The optimum water quality parameters during the breeding time recorded are water temperature 30.2-32.5°C, pH 7.6-7.8, alkalinity 144-150mg/L, and Dissolved Oxygen 8.9-9.5ppm. It is also observed that

rainwater with a high acidic value reduces the hatching rate so it can be concluded that alkaline water is preferable for *M. aral* breeding. In another study, Borah[21] also reported a similar water pH (7.30±0.05) during the breeding period for successful spawning activity. In the case of *M. aculeatus* the preferable water pH was 7.6-7.8 for breeding[1]. The fish is a batch spawner and the spawning process continues for up to 2 hours. Approx. 85-135 numbers of eggs deposited per female. The fertilized egg diameter varies from 2460¼m to 2650¼m. The differential hatching period was recorded even among the eggs fertilized at the same time and developed under the same conditions. Mostly the fish hatched after 30-32 hours of fertilization whereas Chakraborty[16] recorded hatching done 40-42 hours after fertilization with pituitary gland hormone. The success of induced spawning depends on many factors such as optimal breeding conditions, brooder selection and experimental setup, which is also confirmed from the present study. Approximately 62-95 hatchlings were procured from 1 female fish. The fertilization rate and hatching rate vary depending upon hormone dose. It was observed that the yolk sac was completely dissolved after 4 days of hatching. Das and Katlia[1] observed similar result for *M. aculeatus* in the agro-climatic condition of Assam. After yolk sac absorption the larva started self-feeding with plankton, Infusorians, and Artemia larvae. As per previous study, double dose of hormone was used for captive breeding of *M. aral* and *M. aculeatus*[1,16] but in this study single dose also gave good results for *M. aral*. The main objective of the present study was fulfilled and maximum egg output, and hatching rate which was observed in the single dose of 0.05ml/10g of body weight with Spawn Pro. Further extensive studies especially on breeding may be able to throw more light on the subject and hopefully present endeavour will form the basis of future studies.

Conclusion

The results of our study demonstrate that the synthetic fish hormone, Spawn Pro, can be effectively used to induce spawning and seed production in the native ornamental species *M. aral*. A dosage of 0.05 ml/kg body weight for females and 0.025 ml/kg body weight for males of Spawn Pro showed promising results for induced spawning and hatching. This dosage may be considered as a standard for future breeding of *M. aral*. The breeding protocols developed in this study could help reduce the fishing pressure on natural stocks of *M. aral* and be used for seed production by ornamental fishers. The cost-effective nature of these protocols makes them an option for small farmers, marginal farmers, and women fishers for income generation and sustainability. However, further research is needed to refine the techniques for mass-scale production in various eco-regions.

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