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Original Article

Induced breeding, embryonic and larval development of *Macrognathus pancalus* (Hamilton, 1822) under captive condition

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Abstract: The present study was carried out to enumerate induced breeding technique and larval development of *Macrognathus pancalus* (Hamilton, 1822) reared under captivity. Five different doses of Ovasis hormone (T_1, T_2, T_3, T_4, and T_5) with 3 replicas each were administered to the matured brooders to standardize the breeding performance of the target species. The results indicated variation in fertilization rate, latency period, egg output and hatching rate in response to different treatments. Spawning was occurred between 20-24 hrs of injection in all the experiments at 26.33±0.88°C water temperature. Among all the experimental trials, the highest fertilization rate was observed in T_3 (96.15±0.60) of E_2 and the highest hatching rate was observed in T_3 (92.49±1.00) of E_2. The present work elucidated the viability of seed production of *M. pancalus* reared under confined condition which will useful for aquaculture and conservation.

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Introduction

Macrognathus pancalus (Hamilton, 1822) or barred spiny eel, a member of the family Mastacembelidae, is well-distributed in the Asian countries (Talwar and Jhingran, 1991; Froese and Pauly, 2006). This fish due to their sturdy nature, inhabits in a variety of habitats, including beels, small rivers, streams, canals, inundated fields, river plains and estuaries (Bhuiyan, 1964, Rahman, 1989; Talwar and Jhingran, 1991; Galib et al., 2009). This species commands good market value when sold alive as food and ornamental fish in domestic and international markets, including northeastern region of India (Serajuddin and Ali, 2005; Suresh et al., 2006; Abujam and Biswas, 2011; Raghavan et al., 2013). In recent years, impact of anthropogenic activities such as habitat modification, dam construction, introduction of invasive species, overexploitation etc. has had profound impact on population dynamics in freshwater fishes around the world including *M. pancalus* (Maitland, 1995; Suresh et al., 2006; Abujam and Biswas, 2011). Mitigation of such man-made adversities on these ichthyofaunal resources necessitates time bound action to stop further decline in their natural stocks.

In this regard, aquaculture which is the fastest growing production globally represents a viable alternative to boost productivity of fishes (FAO, 2011). Despite its contentious implications on fish biodiversity, aquaculture has made significant contribution in management and conservation of many extant fishes by reducing pressure on the depleted stocks and sustainable utilization of aquatic resources (Diana, 2009; De Silva, 2012). Captive breeding and reintroduction of different species into the wild have shown positive outcomes in different regions of the world (Philippart, 1995). Successful propagation of fishes through aquaculture requires thorough studies on their taxonomy, distribution, feeding and reproductive dynamics of brood stocks etc. (Meffe, 1990; Maitland, 1995). Many authors have carried out studies pertaining to sexual dimorphism, reproductive strategies, feeding habits etc. upto some extent in *M. pancalus* (Swarup et al., 1972; Karim and Hossain, 1972; Serajuddin and Ali, 2005). Similar studies have also been carried out in other members of Mastacembelidae family (Karim and Hossain, 1972;

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Table 1. Doses of hormone (Ovasis) applied to both the sexes of *Macrognathus pancalus* brood.

Treatments	Hormonal doses (ml/kg)	
	Male	Female
(control)	No inducement	No inducement
T_1	0.2	0.4
T_2	0.4	0.6
T_3	0.6	0.8
T_4	0.8	1.0
T_5	1.0	1.2

Umezawa et al., 1991; Serajuddin and Mustafa, 1994; Das and Kalita, 2003; Suresh et al., 2006; Oliveira and Hable, 2010; Rahman et al., 2011). Notwithstanding, limited information on breeding performance of *M. pancalus* still persists that merits immediate interventions to bridge the existing shortcomings. Proper supervision of natural resource or to ventilate artificial propagation or culture is obligatory to conserve the species. With this background information, the present investigation aimed to enumerate information on breeding and rearing feasibility of *M. pancalus* reared under captivity that might provide vital inputs in management and conservation in near future. Standardization of captive breeding would help to diminish the spawning interval and intensify the production of more seeds within a short duration for commercial enslavement and thereby conserve the natural population.

Materials and Methods

Collection and rearing of broodstock: Samples of *M. pancalus* (n=162) were collected from different natural habitats (beels, oxbow Lake, rivers etc.) of Dibrugarh district, Assam during August 2016 to August 2019. The wild collected specimens were acclimatized in laboratory conditions and maintained in facilities at Department of Life Sciences, Dibrugarh University, Assam, India. Fishes were fed *ad libitum* with formulated feed containing all necessary ingredients at 5% of total body weight on daily basis and were kept in fiber tanks of 500 L capacity and aquariums (120 × 40 × 40 cm) with flow through systems. Due to their burrowing and bottom feeding habits, sand substratum was provided in the experimental set ups to simulate their natural environmental condition. Different aquatic plants such as *Eichhornia crassipes, Ceratophyllum demersum*, *Sagitlaria guayayensis*, *Ludwigia repens* etc. were also grown to mimic the natural environment. Water parameters such as pH, water temperature, dissolved oxygen (DO), free carbon dioxide (FCO_2) and hardness were regularly monitored following APHA (1998).

Brood fish selection: The healthy and matured male and female brood fish were selected by visual and physical examination for secondary sexual characters. The females were comparatively larger in size with dark greenish brown colour on the dorsal side and light whitish coloration ventrally. They possessed a soft and swollen abdomen with fleshy translucent protruding genital papilla with oviduct. The males were comparatively smaller in size with greenish brown dorsal and yellowish ventral side with smaller genital papilla. Mature males were observed to release milts through genital papilla on applying subtle pressure on the posterior abdomen region.

Experimental design: For induced breeding of *M. pancalus*, the brooders were collected from rearing tanks and separated and released in plastic containers for acclimatization prior to hormone administration. To standardize the hormonal doses and breeding performances, three experimental trials were employed: (E1) 1:1 male and female ratio (E2) 2:1 male and female ratio (E3) 3:1 male and female ratio. Five different doses of ovasis hormone treatments (T_1, T_2, T_3, T_4 and T_5) were used with three replications of each during experimentation (Table 1).

Hormone administration: Synthetic Ovasis hormone doses were administered near the base of the dorsal and pectoral fin at 45° with the body. Prior to hormone administration, doses of hormone were calculated and prepared according to the body weight of the brooders.

Table 2. (E1): Captive breeding experiment of *Macrognathus pancalus* (M: F=1:1).

Size of males		Size of females		Hormonal doses (ml/kg body weight)		Latency period (hrs)	Fertilization rate (%)	Hatching rate (%)
W (g)	**TL (cm)**	**W (g)**	**TL (cm)**	**M**	**F**			
8.21±0.29	12.38±0.59	13.87±0.39	15.27±0.53	control	control	--	--	--
7.58±0.14	12.06±0.84	12.21±0.82	14.14±0.58	0.2	0.4	--	--	--
6.41±0.09	10.65±0.58	11.68±1.12	13.23±0.64	0.4	0.6	--	--	--
6.13±0.21	11.97±0.46	13.56±0.80	14.48±0.67	0.6	0.8	24	69.86±1.41	74.25±3.50
7.81±0.08	12.1±0.34	12.59±0.56	14.32±0.52	0.8	1.0	--	--	--
6.70±0.25	12.25±0.13	12.92±1.01	14.81±0.38	1.0	1.2	--	--	--

W, fish weight in gram; TL, total length in cm, Data expressed as mean±SE; n=3.

Table 3. (E2): Captive breeding experiment of *Macrognathus pancalus* (M: F=2:1).

Size of males		**Size of females**		**Hormonal doses (ml/kg body weight)**		**Latency period (hrs)**	**Fertilization rate (%)**	**Hatching rate (%)**
W (g)	**TL (cm)**	**W (g)**	**TL (cm)**	**M**	**F**			
8.54±0.29	12.65±0.62	12.33±0.77	14.94±0.13	control	control	--	--	--
6.91±0.07	11.39±0.44	11.87±1.08	14.13±0.52	0.2	0.4	24	84.31±2.19	87.92±3.59
6.08±0.05	10.98±0.58	12.02±0.78	13.57±0.49	0.4	0.6	22	88.56±1.03	86.67±1.83
6.46±0.14	11.30±0.32	14.56±0.32	14.48±0.10	0.6	0.8	22	96.15±0.60	92.49±1.00
7.47±0.09	12.43±0.17	12.26±0.86	13.98±0.18	0.8	1.0	20	92.94±1.72	88.96±2.63
6.33±0.21	11.92±0.46	12.25±1.06	13.81±0.38	1.0	1.2	--	--	--

W, fish weight in gram; TL, total length in cm, Data expressed as mean±SE; n=3.

After hormone administration, the fishes were released at the sex ratio of 1:1, 1:2 and 1:3 in separate fiber tanks with provision of continuous air and water flow system. Water hyacinths were placed as a substratum to hold the sticky eggs after ovulation.

Breeding performance: After ovulation, the eggs of *M. pancalus* were found attached to the roots of water hyacinth. Microscopic observation revealed that the fertilized eggs were transparent with intact nucleus whereas the unfertilized were dark brownish. Effective fecundity of each female after spawning was determined through random sampling of released eggs. The total eggs count and fertilization rate was determined following Behera et al. (2010). Developmental stages of fertilized eggs were monitored and documented under Leica DM 750 microscope. After hatching, the hatchlings were maintained in circular fiber reinforced plastic (FRP) tanks. Fertilization rate and hatching rate was counted visually following the formula:

$$\textbf{Fertilization rate: } \frac{\text{Number of fertilized eggs}}{\text{Total number of eggs}} \times 100$$

$$\textbf{Hatching rate: } \frac{\text{Number of hatchlings}}{\text{Total number of fertilized eggs}} \times 100$$

Statistical analysis: The results were calculated as mean±standard error (SE) in all the experiments. The experimental calculations were statistically analyzed using SPSS software (version 17.0 for windows). One-way analysis of variance (ANOVA) was used to compare significant level of differences between the experimental observations. Significant results (P<0.05) were additionally tested using Duncan's New Multiple Range Test (DMRT) to evaluate significance differences among means.

Results

A total 162 fish specimens (male=108; female=54) were used for captive rearing and induced breeding of *M. pancalus*. The fishes attained maturity during June to August. Fertilization rate, latency period, egg output and hatching rate in response to different treatments and sex ratios have been summarized in Tables 2, 3 and 4. No fertilization took place in the control (without hormonal inducement) set of experiment.

Table 4. (E3): Captive breeding experiment of *Macrognathus pancalus* (M: F=3:1).

Size of males		Size of females		Hormonal doses (ml/kg body weight)		Latency period (hrs)	Fertilization rate (%)	Hatching rate (%)
W (g)	TL (cm)	W (g)	TL (cm)	M	F			
8.87±0.57	12.71±0.64	14.20±0.38	15.93±0.46	control	control	--	--	--
8.24±0.35	12.06±0.26	12.54±0.63	14.14±0.58	0.2	0.4	--	--	--
7.08±0.28	10.98±0.58	12.35±0.45	13.56±0.49	0.4	0.6	24	74.02±3.86	76.47±1.70
7.12±0.15	12.30±0.77	12.56±0.32	13.81±0.31	0.6	0.8	22	77.22±1.47	83.17±2.48
7.47±0.09	12.76±0.41	12.59±0.02	13.98±0.74	0.8	1.0	22	75.31±2.60	86.17±1.15
6.99±0.25	11.58±0.38	13.58±0.56	14.48±0.65	1.0	1.2	--	--	--

W, fish weight in gram; TL, total length in cm, Data expressed as mean±SE; n=3.

Table 5. Different physicochemical parameters of breeding and rearing tanks.

Parameters	Breeding and hatching tank	Rearing aquarium
pH	7.30±0.05	7.43±0.02
Water temperature (^{0}C)	25.33±0.88	26±0.47
Free CO_2 (mg/l)	4.15±0.22	3.30±0.23
DO (mg/l)	11.13±0.41	9.30±0.49
Total alkalinity (mg/l)	55.74±0.91	54.33±0.72

(Mean ± SE); values of the parameters in each column differ significantly (P<0.05).

Breeding behaviour: After hormonal administration, the brooders showed varied mating behaviour in all the treatments (except control) after 10-14 hrs of injection. Each female was paired with male and the mating was preceded by courtship behaviour. Latency period varied significantly in different hormonal doses and in different experimental set up. Spawning took place between 20 to 24 hrs of injection in different experiments as shown in Tables 2, 3 and 4.

Fertilization rate: Fertilization rate in E_1 was 69.86±1.41 in T_3 and no fertilization took place in all the other doses of E_1 (Table 2). In E_2, a significantly higher fertilization rate was observed in T_3 (96.15±0.60) and lowest was in T_1 (84.31±2.19) and no fertilization took place in control and T_5 (Table 3). In E_3, the fertilization was observed only in T_2, T_3 and T_4 where in T_3 fertilization rate was highest (77.22±1.47) (Table 4). In all the experiments, the highest fertilization rate was observed in T_3 (96.15±0.60) of E_2 (P<0.05). The fertilization rate of E_2 is significantly higher (P<0.05) in all the treatments compared to E_1 and E_3.

Hatching rate: A contorted movement of the embryos was observed within 14-16 hrs of spawning and hatching within 20-22 hrs of fertilization. Calculated hatching rate was significantly higher (P<0.05) in E_2 compared to E_1 and E_3 and the highest hatching rate was observed in T_3 (92.49±1.00) with male and female ratio of 2:1.

Water parameters: Different water parameters such as pH, water temperature, Free CO_2, DO, total alkalinity in experimental tanks and aquarium under different treatments of *M. pancalus* were monitored and are presented in Table 5. The means values of water parameters were not significantly (P<0.05) different among the different experimental treatments.

Embryonic development: The development of embryo of *M. pancalus* were categorized into eight stages viz. zygote, cleavage, blastula, gastrula, segmentation, pharyngula, hatching and larval stages were described as follows (Figs. 1, 2):

Zygote stage: The fertilized eggs were adhesive, dark greenish brown in colour and the diameter measured was 1.3-1.4 mm. Cytoplasmic movements were observed after fertilization.

Cleavage stage: The first cleavage observed 30-40 min after fertilization i.e. formation of blastomere was occurred. After the first cleavage simultaneously the blastomeres divided at an interval of 20-30 min and completed 64 cell divisional stages within 2-3 hrs.

Blastula stage: With the entire formation of 128 cell blastula period initiate. At this stage, the cell

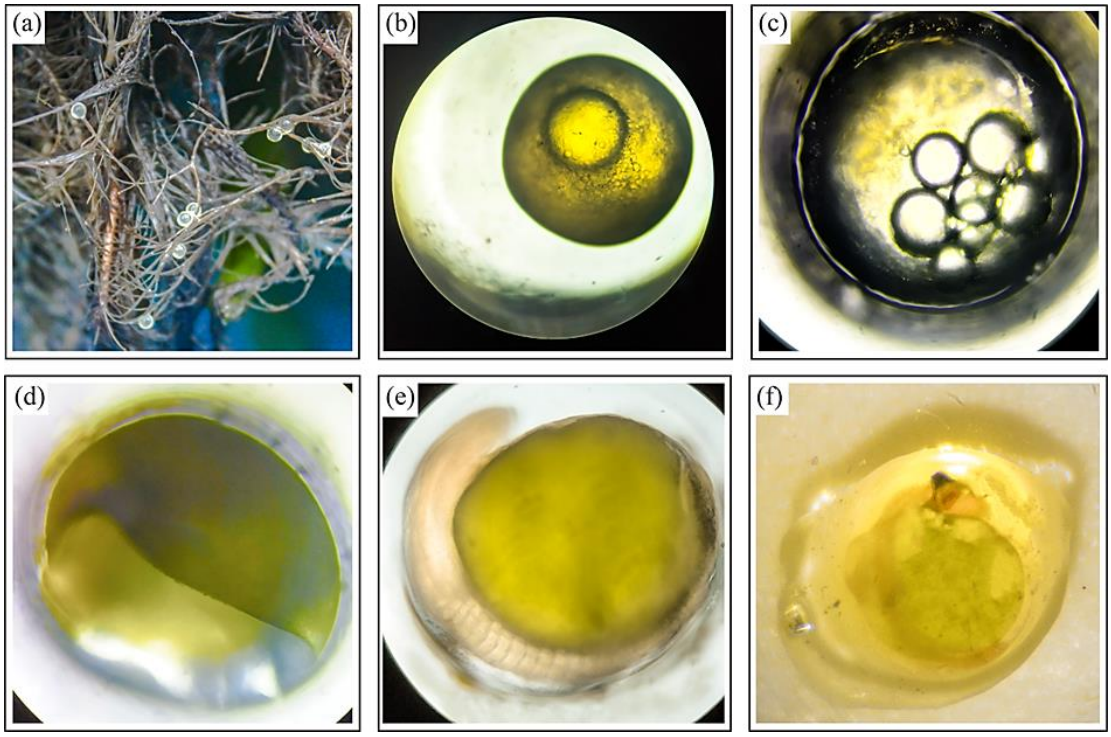

Figure 1. Fertilized eggs with different developmental stages. (a) Fertilized eggs, (b) Zygote stage, (c) Cleavage stage, (d) Blastula stage, (e) Gastrula stage, and (f) Segmentation stage.

rearrangement took place and this period is observed to be 2-5 hrs and ended with the beginning of the gastrulation. At the end of this phase, the embryo has reached 50% epiboly stage.

Gastrula stage: In this period, substantial cell movements were observed simultaneously with convergence, involution and extension producing three germs layers and embryonic axis. Gastrula period ranged 5-10 hrs.

Segmentation stage: The first morphological cells differentiated and first body movements become visible in segmentation period. Sequential formation of the somites was also observed in this stage and this period continued prior to hatching. Segmentation period was observed to be 10-18 hrs.

Pharyngula stage: The embryo was bilaterally organized in this period observing in 18-24 hrs. The notochord was developed gradually and complete set of the somites was formed extending to the end of a post anal tail.

Hatching stage: After 20-24 of fertilization, the larva emerged from the egg membrane and the newly hatched larva was 2.8±0.37 mm in length. The hatchling was appeared slender and transparent with a voluminous yolk with large oil globules and the yolk sac is measured to be 1.63±0.03 mm. Half of the body of the hatchling was covered by the yolk sac. Newly hatched larva was not very active and remained in resting condition within the aquatic plants and the wall and bottom of the aquarium. After 10-12 hrs, they became very active and came to the surface of water after a while continuously for gulping air. The heart is not prominent but heartbeat was observed as 108-113 beats/ min.

After 24 hrs, the hatchlings were characterized by large pigmented eyes and olfactory pits and the yolk sac was decreasing gradually. Two-day old (DPH) hatchlings were characterized by prominent heart and was seen pulsating located between the head and yolk sac, ventral to the eyes. 3 dph hatchlings were observed with reduced yolk mass and the size of the yolk sac reduced to 0.83±0.03. The forehead region which attached to the yolk sac, observed free from the yolk mass. Four-day old hatchlings were characterized by large and highly pigmented eyes. During this period, the mouth cleft appeared although not with any marked movement.

Five-day old hatchlings were characterized by

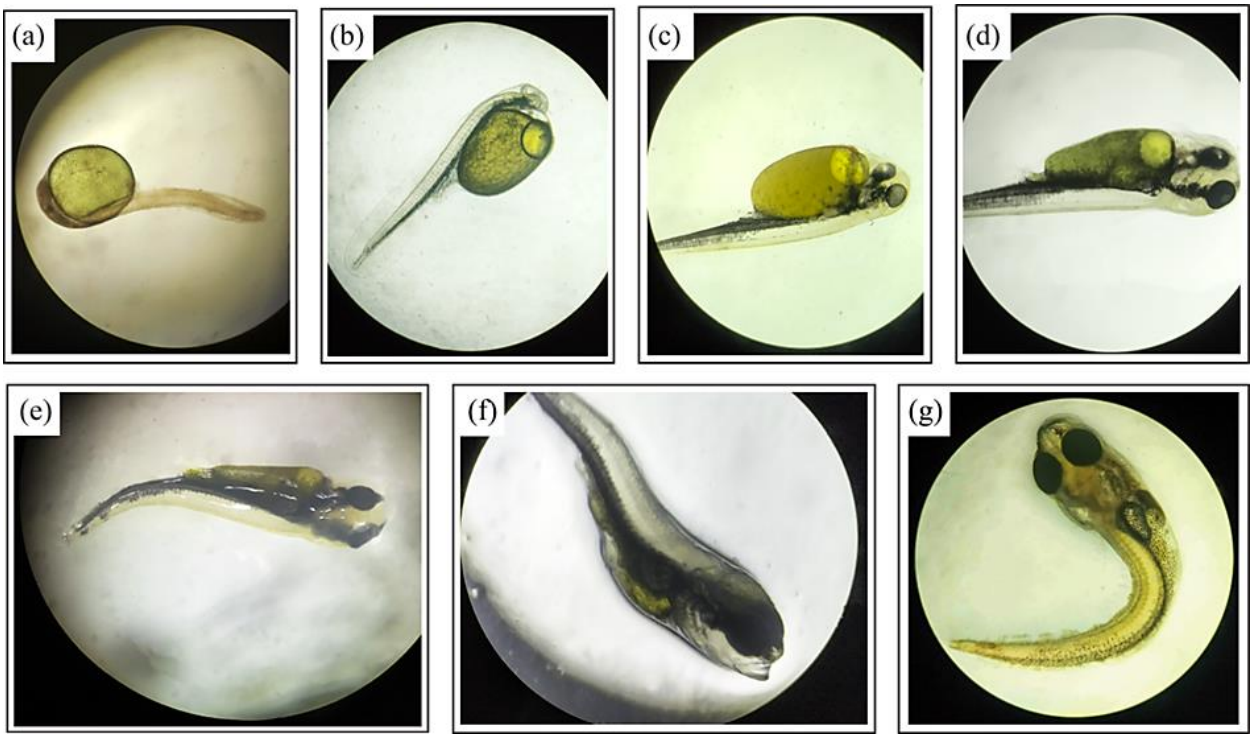

Figure 2. Different developmental stages of newly hatched larva (a) 1-day old larva, (b) 2-day old larva, (c) 3-day old larva, (d) 4-day old larva, (e) 5-day old larva, (f) 6-day old larva, and (g) 7-day old larva.

spherical heart appeared pulsating and the yolk sac became reduced to half. Six-day old larva was observed with two chambered heart located ventrally anterior to the globular yolk and the streaming larval blood circulation perceptible ventrally. Conspicuous melanophore was observed on the body. Dorsal and caudal fins developed with continuous fin rays. Gill movements and jaws movements were also visible. Pectoral fins were developed. 7 dph hatchlings were characterized with completely absorbed yolk and it began wondering in search of food. The fully developed larva was measured to be 6.8±0.23. Pigmentation on head and trunk region became remarkable. Well-developed upper and lower jaw with movements was noticed. Clear and functional fins and fin rays were observed. After one week of hatching, the spawns were released into another tank providing with continuous feed for growth.

Discussions

The present investigation demonstrated the captive feasibility and artificial propagation of barred spiny eel, *M. pancalus*. It was hypothesized that simulation of natural environmental conditions in confined aquarium may trigger spawning. The present observation partially supported the theory as the fish successfully matured under captive conditions. However, it failed to naturally spawn under artificial stimulant. Das and Kalita (2003) described spawning success in peacock eel, *M. aculeatus* under captive environmental conditions, where spawning was triggered using synthetic hormone (Ovaprim). Contemporary investigation pertaining to the use of ovasis (synthetic spawning hormone) as a spawning inducer in captive breeding of *M. pancalus*, showed significant variation in breeding performance. The variation in their spawning regime might be attributed due to the different hormonal doses and respective sex ratios. The basic success of induced breeding is governed by the appropriate administration of specific hormone, hormonal doses, condition of the brood fish as well as the environmental conditions (Miah et al., 2008; Afroz et al., 2014). In *M. pancalus*, the hormonal doses of 0.6 and 0.8 ml/kg in males and females, respectively were found to be effective for inducing the species with optimum spawning success. Das and Kalita (2003) and Alam et al. (2009) did a comprehensive evaluation on the efficacy of different

hormonal doses to trigger spawning activity in peacock and spiny eel, respectively. Their findings highlighted differential hormonal doses in inducing spawning which are in conformity to the present study. In this regard, Atz and Pickford (1959) reported that females generally require higher doses as compared to males and further suggested the use of small multiple doses giving better results than the single dose. Among all the experimental set up, the highest fertilization and hatching rate was observed with ovasis hormone at the sex ratio of M: F=2:1. The sex ratio M: F=2:1 was found effective in induced breeding experiment compared to other ratio maintained which was strongly supported by Islam et al. (2011) and Nagarajan (2012).

Latency period ranged 20-24 hrs in *M. pancalus* with different doses of Ovasis in different experimental set up. Contrary to the present findings, Das and Kalita (2003) reported relatively low latency period whereas Rahman et al. (2011) found much higher latency period in some members of the Mastacembelidae family. In *M. pancalus*, the lowest latency period (20 hrs) was observed at the experiment E_2 where the hormonal dose was 0.8 and 1.0 ml/kg of male and female, respectively. The highest latency period (24 hrs) was observed at the hormonal doses of 0.2 and 0.4 ml/kg at 2:1 as well as 0.4 and 0.6 ml/kg of 3:1 male and female, respectively. Variations observed in latency period might be due to use of different hormonal doses and their mode of action in that particular fishes. Peter et al. (1986) also established similar result whereby differential level of dopamine activity in fishes is influenced by the dose concentration of a particular hormone.

Physico-chemical parameters of water in the experimental tank might also contribute to the increased fertilization rate, successful hatching etc. The mean water temperature (25.33±0.88ºC), pH (7.30±0.05) and DO (11.13±0.41 mg/l) during the breeding period in the experimental tank coincided with the successful spawning activity. According to Behera et al. (2007), *Labeo bata* administered with Ovaprim and ovatide showed high hatching rate induced under optimum environmental conditions. Similar inferences have also been opined by Alam et al. (2009) on the influence of water parameters for successful fertilization and quality hatching rate.

In this experiment, hormonal doses administration apparently affected the fertilization and hatching rate. Low dosing of the inducing hormone caused late inducement in species, whereas overdosing caused early milting. The results are in conformity to the findings of Routray et al. (2007) and Pandey et al. (2002b) regarding efficacy of different synthetic hormone doses in triggering successful fertilization and hatching rate. The fertilized eggs observed were transparent whereas unfertilized ones were opaque and dark in colouration. Kimmel et al. (1995) in *Danio rerio*, Udit et al. (2014) in *Puntius sarana*, Dey et al. (2014) in *Devario aequipinnatus*, and Malla and Banik, (2015) in *D. aequipinnatus* reported similar morphological development/changes in oocyte structure after fertilization.

The first cleavage i.e. formation of blastomere occurred 30-40 min after fertilization. Udit et al. (2014) reported similar findings where cleavage occurred 30 min after fertilization in *P. sarana*; Kimmel et al. (1995) reported 40 min in *D. rerio* whereas Dey et al. (2014) reported 45 min in *D. aequipinnatus*. Following successful fertilization, the incubation period reportedly varied from 20-24 hrs in this particular species. Earlier reports on incubation period by Kimmel et al. (1995), Udit et al. (2014), and Dey et al. (2014) revealed 48 hrs of incubation in *D. rerio*, 15-17 hrs in *P. sarana,* 36 hrs in *D. aequipinnatus*, respectively. In the present work, the newly hatched larva measured 2.8±0.37 mm with voluminous yolk sac after 20-24 hrs of fertilization. The yolk sac and mass of the hatchlings showed gradual decreasing trend coupled with organ development till the 7th day. It was observed that the yolk sac was completely dissolved after 1 week following which the larva started self -feeding in the rearing tank. Similar studies were reported by Alam et al. (2006) and Arockiaraj et al. (2003).

Conclusion

Maturation and seed production of *M. pancalus*

through captive breeding technique can be consummated through the administration of inducing hormone (Ovasis) at variegated doses. However, it was observed that different factors viz. rate of stimulation, sex ratio, as well as optimum environmental conditions, acts synergistically for the success of viable seed production. Post spawning period is a critical phase, which requires scrupulous monitoring and care as it determine the reproductive success of a particular species. This study gives an elaborative overview of the reproductive dynamics, feeding preference, environmental congeniality of the potential ornamental spiny eel *M. pancalus* in captive condition. A stepwise optimization of the involved techniques will offer an impetus to the aquaculturist and conservationist to comprehend proper management and further framing conservational strategies for this valued species.

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