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Breeding and embryonic development of an indigenous ornamental fish *Trichogaster lalius* (Hamilton, 1822) in captive condition

Shibam Saha, S. Behera, Dibakar Bhakta, Abhrajyoti Mandal, Sanjeev Kumar and Anandamoy Mondal

Abstract
The present study was conducted to perform the captive breeding and embryonic development of dwarf gourami *Trichogaster lalius* (Hamilton, 1822) in control condition at the Laboratory of Faculty of Fishery Sciences, WBUAFS, Kolkata, West Bengal between May and June, 2016. A total 10 sets of experiment were conducted by keeping one pair of healthily fish (male and female 1:1 ratio) for each set. The absolute fecundity was ranged from 1000 to 1350. The fertilization rate was found to be 63±0.50% and incubation period was recorded 23 to 26 hours at 29.15 ± 0.95 °C. The fertilized eggs were not adhesive, golden in colour and optically transparent and size was ranged from 0.60 ± 0.05 to 0.69 ± 0.08 mm. The present findings established that *T. lalius* can easily bred in captive condition by maintaining suitable environmental parameters which is prerequisite to conserve the species in natural water bodies.

Keywords: *Trichogaster lalius*, captive breeding, embryonic development, conservation

1. Introduction
Trichogaster lalius, commonly known as Dwarf gourami and locally known by 'Khalisa or Lal Kholse' is an indigenous ornamental fish of West Bengal [1]. The fish have both food and ornamental value and fetches a market value of Rs. 150 to 200/ kg while sold as food fish and Rs. 10-20 per pair as ornamental [1]. The species, mainly the male is having high domestic as well as foreign demands to the aquarium traders due to its sparkling, translucent blue colour with red or dark orange stripes in the body. They are well known for their interesting reproductive behaviour which includes bubble nesting and parental care [2]. The species used to collect from the wild and exported to the foreign countries like USA, Singapore, Japan, Republic of Korea, Sri Lanka, Germany, UK, Hong Kong, Taiwan, Thailand, Netherlands, Bangladesh, Malaysia and China [3].
Over exploitation and indiscriminate destruction of breeding and feeding ground by applying pesticides and insecticides in nearby agricultural fields, reason to be the threatened for ornamental fishes [4]. To meet up the demands for both domestic and foreign fish traders and regain the fishes in natural environment artificial propagation in captive as well as natural condition is prerequisite. Embryonic development in fishes depends on many environmental factors and embryonic stage occurs inside the chorion and ends at hatching [5]. Embryonic development is a complex process where cellular differentiation and proliferation occurs simultaneously [6]. Knowledge on breeding, embryonic and larval development is of important parameters to understand the basis biology of a species and their dietary needs [7, 20]. Studies on embryonic and larval development are very much important for successful larval development and mass scale aquaculture [8]. Earlier studies on this species mainly focused on sexual dimorphism, gonadal development [1, 4] but details study on breeding and embryonic development is rare. In this context, the present study was conducted to observed the captive breeding and embryonic development of native dwarf gourami.

2. Materials and methods
2. 1 Brood stock collection and acclimatization
The present study was carried out in the Laboratory, Department of Fisheries Resources Management, Faculty of Fishery Sciences, WBUAFS, Kolkata, West Bengal between May and June, 2016.

Adults *Tricogaster lalius* male (5.75 ± 0.25 cm, 1.55 ± 0.12 g) and female (4.50 ± 0.19 cm, 1.50 ± 0.09 g) were procured from local ornamental fish market (Galef Street), Kolkata. Live fish sample were transported to the laboratory by plastic bags with oxygen packing. In the laboratory the fishes were given a short treatment with 3 ppm $KMnO_4$ solution for 3 to 5 minutes for disinfection. Subsequently, they were released carefully to the aquarium (60 × 30 × 30 cm) at a density of 30 fish per aquarium in 35 l of chlorine free tap water, for acclimatization in laboratory condition. Both male and female adults were kept in rectangular tanks (60 ×30 × 30 cm) with continuous aeration for a period of 15 days before starting the experiment.

2.2 Preparation of experimental aquariums

For the experiment, glass aquarium were used which were cleaned, disinfected with $KmnO_4$ and dried. The brood fish (both male and female) were kept in two aquariums (60 × 60 × 30 cm) filled with tap water with continuous aeration. Another six numbers of aquarium were also kept (60 × 60 × 30 cm) filled with tap and aeration to facilitate nest building by male. Both the aquariums were decorated with floating weed Hydrilla (*Hydrilla verticulata*) along with thermocol plate to provide them natural breeding environment as well as to make their own bubble nest.

2.3 Water quality parameters for brood stock management

The water quality parameters are very important for rearing and breeding of *T. lalius* in control condition. Fresh, de-chlorinated and well aerated water was used for the whole experiment. The physico-chemical parameters *i.e.* temperature, pH, dissolved oxygen alkalinity and hardness were measured by using the standard methods of APHA [9].

2.4 Feeding of experimental fish

During the experiment fishes were fed with artificial feed at the rate of 2 to 3% of their body weight daily *i.e.* during morning and live feed (*Tubifex* worm) at the evening hours. Left out feed and accumulated faecal materials was siphoned out daily morning in order to maintain healthy condition in the aquarium. The acclimatized fishes were very active, healthy and normal in their body colour as well as with their behaviour. After 15 days of acclimatization the fishes were taken for experiment.

2.5 Selection of breeders

For the breeding purpose, acclimatized fishes both male and female were selected at the ratio of 1:1. Matured fishes were observed by their secondary sexual characters [1]. When male became mature; they started making bubble nest on the surface of the water. Male became aggressive in nature, their body colour became more prominent with vertical diagonal stripes of alternating red to dark orange that encountered into the fins and look more attractive. A deep lucent colour band was seen over the operculum in case of mature male. In case of female, their body colour became silvery or a dull silvery blue to gray colour and also the belly portion of the matured female looked bulge.

2.6 Breeding

The breeding experiment was conducted by natural breeding method. Ten (10) set of experiment were conducted by maintaining male and female (1:1 ratio) in the breeding tank (30 × 30 × 30 cm). Before placing individuals in the breeding tank, length and weight of all male and female fish were measured using standard scale and electrical balance. Following this method, the weight of the individual fish was taken before and after spawning, so that decreased in the weight of that individual gave gonad weight.

2.7 Study of embryonic development

During study the embryonic developmental stages; eggs were collected from the spawning tank with a pipette as they dropped from the female. Then they were put on glass slide and observed under Trinocular phase contrast research microscope. Eggs from the same spawn were observed for the entire developmental period. Temperature in the aquarium was maintained at 26-29 ºC. Finally, the photographs were taken between (1-26) hours.

2.8 Estimation of fecundity and fertilization rate

Once fertilisation was confirmed spent brooders were removed from the tank. The fecundity and fertilization rate were calculated by using the following formula:

Fecundity = Number of eggs in sub sample × weight of the gonad / Weight of sub sample and

Fertilization rate (%) = Number of fertilized eggs / Total number of eggs in the sample × 100

2.9 Statistical analysis

All data presented are expressed as mean ± standard deviation subjected to two way analysis of variance (ANOVA).

3. Results

3.1 Water quality parameters of brooders tank

The water quality parameters during the study period are depicted in Table 1.

Table 1: Water quality parameters recorded during breeding of *Trichogaster lalius*.

Parameters	Value (± SD)
Water temperature (^{O}C)	29.15 ± 0.95
pH	7.25 ± 0.23
DO (mg l^{-1})	6.10 ± 0.13
Total alkalinity (mg l^{-1})	137.12 ± 6.32
Hardness (mg l^{-1})	467 ± 25.39

3.2 Breeding

The breeding experiment was conducted by natural breeding method by maintain ten set of experimental fishes. Matured female with the length of 39.67-43.53 mm and weight 1.24-1.70 g were kept for eggs collection.

3.3 Natural breeding

Eight to ten days after introduction of both male and female (1:1) in the breeding tank (30 × 30 × 30 cm), male started making bubble nest under the hiding place (thermocol). Male started chasing towards female and during this period male added few more bubbles to the nest. It was examined that, male chased the female when she came to the surface for aerial respiration. But if female returned to bottom and stay at the corner of the tank, male will not disturbed her. Sometimes, male chased the female aggressively, when the female tired to come near to the nest. After 24 hours of chasing behaviour when female showed response, male stop chasing and engaged for making the nest for spawning. The pair will become ready for spawning when the nest formation was completed.

After every mating the released egg were collected by male in

his mouth for a few seconds and adds saliva on outer surface of eggs to make them sticky. At this time male produced more air bubbles with its mouth just to encode this eggs into the bubble and help to float them in the nest. As soon as the spawning was over, male guarded the eggs which also care hatchlings for next 2 days and did not allow female to come near to the nest. During this breeding time water quality was maintained and partial removal of water was made for better breeding response among the brooders.

Table 2: Results of *Trichogaster lalius* breeds naturally in control condition

Breeding Set	Parameters					
	Length of female (mm)	Weight of female (gm)	Fecundity (Nos.)	Incubation period (hours)	Fertilization rate (%)	Size of eggs (mm)
Set-1	43.03	1.695	1350	24	66.14	0.68 ± 0.09
Set-2	40.25	1.300	1040	23	58.55	0.67 ± 0.08
Set-3	41.11	1.360	1120	25	55.98	0.63 ± 0.10
Set-4	42.65	1.501	1345	24	68.34	0.65 ± 0.06
Set-5	43.53	1.701	1345	26	55.16	0.61 ± 0.08
Set-6	41.23	1.356	1100	24	67.00	0.60 ± 0.05
Set-7	42.70	1.571	1265	23	66.16	0.62 ± 0.07
Set-8	40.51	1.301	1060	25	62.83	0.64 ± 0.08
Set-9	39.67	1.241	1000	24	66.00	0.69 ± 0.08
Set-10	41.46	1.380	1156	26	60.46	0.64 ± 0.10
Average	41.61±1.3	1.441±0.17	1178±136	24.4	63±.50	0.64±0.08

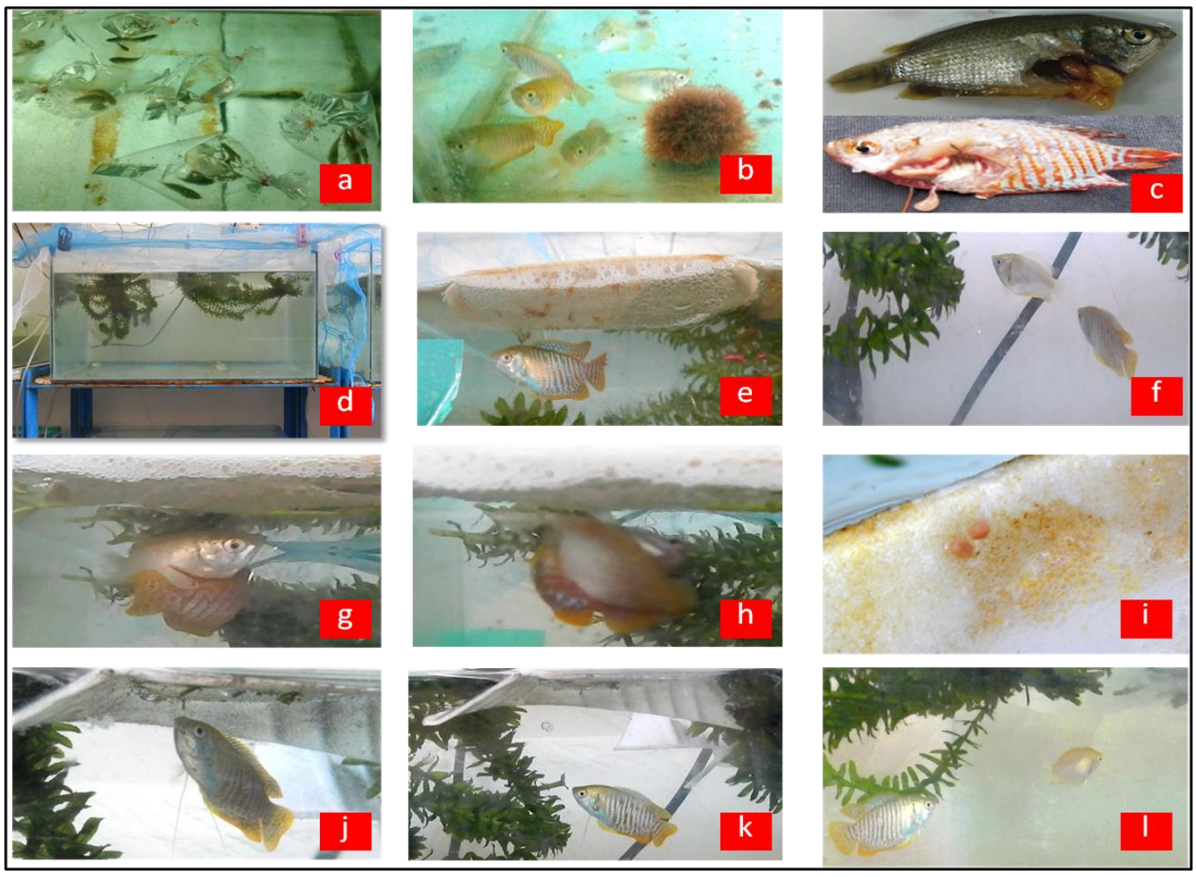

Fig 1: Pictorial presentation of *T. lalius* breeding: a. Acclimatisation of brooders, b. Live *Tubifex* feeding to the brooders, c. Matured male and female brooders, d. Maintained male and female ratio 1:1 in the tank, e. Male making bubble nest, f. Male chasing towards female, g. Mating of male and female, h. Female releasing eggs, i. Fertilized eggs of *T. lalius.*, J. Male placed eggs into the nest, k. Male guarding eggs, l. Male chasing away the female after breeding.

3.4 Hatching

The eggs were hatched within 24-26 hours after spawning. The newly hatched larvae attached to the bubble nest for next three days, until the yolk sac was totally absorbed and then the hatchlings became black, coma shaped fry swim horizontally after the 3rd and 4th day of breeding.

3.5 Embryonic Development

The embryonic development of *Trichogaster lalius* was divided into several stages namely zygote, cleavage, bastula, gastrula, segmentation, pharygula andsegmentation, pharygula and hatching [10]. The recorded embryonic development is described as follows:

Zygote stage: The fertilized eggs were not adhesive, golden in colour and optically transparent. The cytoplasm movement was activated after fertilization. The diameter of the egg was between 0.64±0.08. The first visible cell appeared about 10 minutes after fertilization. After 10 minutes it became flattened (Fig. 2 a).

Cleavage stage: The first cleavage occurred 15 minutes after

fertilization. The two blastomeres rounded just after first cleavage. After 1 hour the second cleavage occurred and it was continued up to 2 hours (Fig. 2 b, c and d).

Blastula stage: Blastula period began with further cell division and ended with the beginning of gastrulating. From the 3 hours to 6 hours the germinal disc flattened and grows around the yolk. The cells extended around the yolk at 7 hours. By the 8 hours the germinal disc had fully encircle the yolk (Fig. 2 e and f).

Gastrula stage: In the gastrula period, excessive cell movements were observed, including involution, convergence and extension, producing germ layers. Gastrula period was observed to be 7-11 hours (Fig. 2 g, h and i).

Segmentation stage: The segmentation period was characterized by further development of organs; tail bud becomes more prominent, first body movement occurs; neural cord and the notochord continue formation. It lasted just prior to hatchling. Segmentation period was observed to be 12-19 hours (Fig. 2 j, k, l and m).

Pharyngula stage: In this period, the embryo was well developed. Heart beat and twitching movement of the embryo was started. It was extended upto 20-23 hours (Fig. 2 n and o).

Hatching stage: During this period frequent twitching movement by lashing the tail against the egg capsule was observed. After few seconds, it was become violent and breaks out from the embryo with tail first (Fig. 2 p and q).

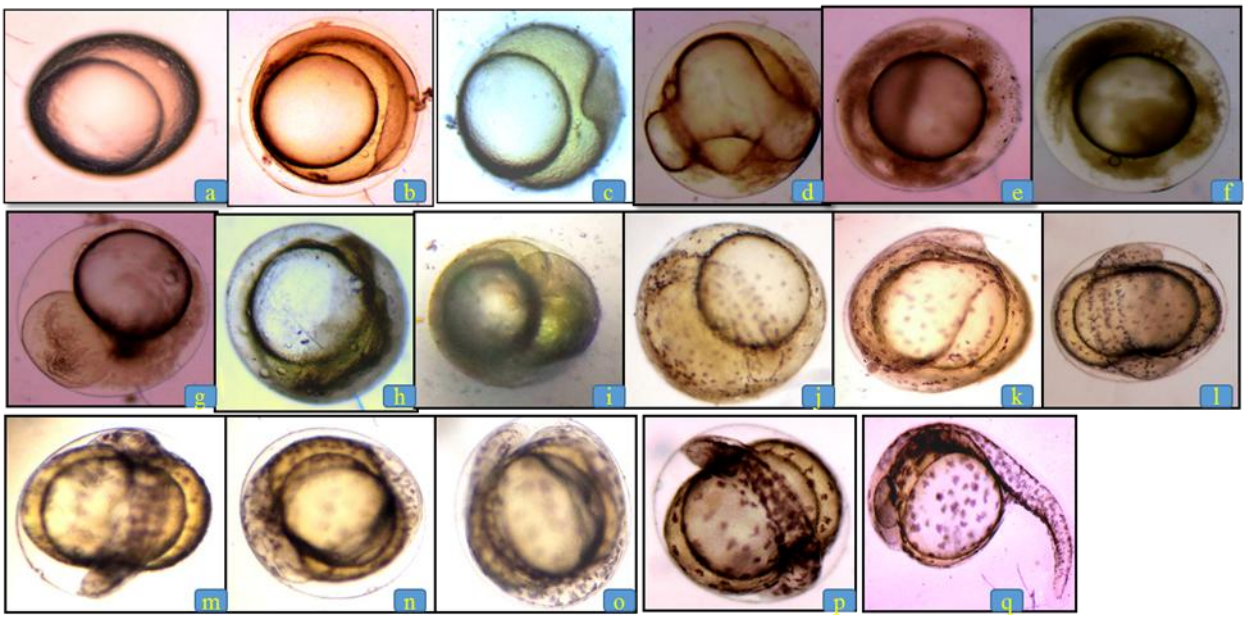

Fig 2: Different stages of embryonic development in *T. lalius.*

3.6 Fecundity and fertilization rate

For fecundity estimation eggs were collected randomly from different parts of the gonad. The absolute fecundity was ranged from 1000 to 1350. The fertilization rate was found to be 63±0.50% and incubation period was recorded 23 to 26 hours.

5. Discussion

5.1 Water quality parameters

The water quality parameters during the study period were found to be optimum for breeding and embryonic development, except hardness. In the present study, mean DO value was found to be 6.10 ± 0.13 mg l^{-1} which was found to be suitable for breeding. In case of carp [11], found that DO level range between 4.5 to 6.2 mg l^{-1} was good for growth and reproduction of carps. The temperature ranged between 27-28 ºC was very much effective for captive breeding of honey gourami, *Colisa sota* and was reported by [12]. [13] reported that in case of *Trichogaster trichopterus* temperature range between 28-29 ºC is suitable for breeding. [13] recommended that the ideal range of total alkalinity should be 60-300 mg l^{-1} which was within the recommended limit. Hardness of water between 150-160 mg l^{-1} is considered best for breeding and larval development in case of Honey Gourami [12]. In the present study hardness was observed little higher (461-471 mg l^{-1}), which reduced the fertilization rate.

5. 2 Breeding and Embryonic development

Drawf gourami *Trichogaster lalius* is a potential candidate species in eastern India for the ornamental fish traders. Breeding of this species in wild gradually decreasing due to habitat alternation, environmental degradation etc. which reduced the natural seed collection. Management and breeding in control condition gaining importance to conserve and culture of this species.

The incubation period was recorded 23-26 hours which was found to be little less from the study of [12] in horney gourami, *Colisa sota* and reported to be 28-30 hours. According to [13] the hatching period of *Trichogaster trichopterus* was reported to be 24 hours at water temperature of 26-27 ^{O}C which was similar to our work. According to [14] the incubation period was varied from 24-30 hours for *Colisa faciatus*. Comparatively less hatching period was observed (15-16 hours) in *Puntius sarana* at water temperature of 26.5-28.5 °C by [15] which was due to administration of inducing agent for breeding. [16] found that, incubation period of thick-lipped Gourami, *Trichogaster labiosua* was 20-30 hours.

The fertilization rate was recorded 63±0.50%. [17] observed an average fertilization rate of 82.09% in *Botia dario* (Hamilton) by using synthetic hormone. A very high fertilization rate (99.6%) of *Trichogaster trichopterus* was reported by [10] which were achieved due to maintaining high water quality

parameters.

The first cleavage occurred 15 minutes after fertilization in the present study of *T. lalius*. [17] reported that in *Devario aequipinnatus* first cleavage was occurred after 45 minutes, in *Puntius sarana* it was observed after 30 minutes [15] and in *Danio rerio* it was after 40 minutes [18].

The fertilized eggs were not adhesive, golden in colour and optically transparent. The size of fertilised eggs was recorded from 0.60 ± 0.05 to 0.69 ± 0.08 mm. [12] reported the size of eggs in *Colisa sota* was 0.5 to 0.68 mm in length. [13] mentioned diameter of fertilized eggs varied between 1.37 to 1.45 mm in *Trichogaster trichopterus*. [13] observed that in *Trichogaster trochopterus* the size of fertilized eggs was 0.70 to 0.91 mm.

The absolute fecundity was ranged from 1000 to 1350. According to [1] the average pre-spawning fecundity of *T. lalius* was reported 1024 to 1351 numbers which was found within the ranged of present findings. The present study on breeding, embryonic development, fecundity and fertilization rate on *T. lalius* was resemblance with others studies by [12] in *Colisa sota* (Ham.-Buch.), [13] in *T. trichopterus* [19], in *T. lalius*, [10] in *Botia dario* (Hamilton) and in [15] *Puntius sarana*.

6. Conclusion

Trichogaster lalius can be easily reared, matured and bred in confined environment both naturally and artificial propagation. Brood stock management and hatcheries should be established for recruitment of the species in natural condition. By disseminating the breeding techniques generated through the study to the fish breeders, natural recruitment might be regain side by side new employment may create which substantial increase the foreign earnings. The success of this study will be useful for the ornamental fish breeders, aquarium keepers and further study on genetic characterization and larval and fry development will conserve this species in natural environment.

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