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Research Paper

Induced breeding, seed production and larval rearing of striped murrel (*Channa striatus)* in FRP tank systems

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ABSTRACT

Striped murrel is considered to be highly nutritious and are known to have medicinal values. However, the production couldn't meet the demand due to unavailability of suitable breeding technology. Therefore, a study was conducted to investigate the induced breeding and larval rearing techniques of striped murrel (*Channa striatus)* in FRP tank systems. The breeding experiment was carried out in FRP tanks of 1000 litre capacity. The female and male fishes were injected with ovatide hormone at 0.6 ml / Kg of body weight and released into the breeding tank. Spawning was noticed after 36 hours of hormone injection and eggs hatched 20 hours after spawning. The induced fishes spawned 8300 numbers of eggs and the diameter of the egg mass was 6 – 8 cm with 1.2 – 1.3 mm egg size. The fertilization and hatching rates were 60 % and 60 % respectively. The hatchlings were transferred to FRP tank of 300 L capacity for larval rearing. Survival in the larval rearing tank was 60% from spawn to fry. The total length of the newly hatched larvae was 4.0 ± 0.089 mm 24.81 ± 0.832 mm on 30th day when reared in the FRP tank. It can be concluded from the results that breeding and larval rearing of striped murrel can be successfully undertaken in FRP tanks with maximum fertilization, hatching and survival rate.

Key words: Artificial reproduction, cannibalism, hatching, ovatide, spawning

Channa striatus is one of the significant native freshwater fish of tropical Asia and is a member of the Channidae family (Ng and Lim, 1990). Along with other species of the genus *Channa, C. striatus*, commonly known as "Shol," is an economically significant species in Bangladesh (Mollah et al., 2009; Rahman *et al.,* 2012). It is a staple fish in Malaysia, Indo-China, and Thailand. Commercial snakehead cultivation is practiced in Pakistan, India, Thailand, Philippines, Vietnam, and Cambodia (Mollah *et al.*, 2009; Rahman *et al.,* 2012; Wee, 1980). This fish has the most delectable flavour and has firm, white, virtually boneless flesh. It is extensively taken due to its nutritional benefits and usefulness in the healing of wounds (Wee, 1980). Due to their ability to thrive in harsh environments with low levels of dissolved oxygen and high ammonia content, this air-breathing fish have significant advantages for aquaculture (Ng and Lim, 1990; Qin *et al.*, 1997).

In their natural habitat, *C. striatus* often constructs nests prior to the start of the spawning season. The process of building the nest is crucial, and the parents typically remove aquatic vegetation from an area of the water's surface. It typically reproduces in flooded paddy fields, ponds, and ditches at the beginning of the monsoon season (Rahman *et al.*, 2012). The knowledge of how snakeheads breed and nourish their early larval stages is still insufficient (Marimuthu and Haniffa, 2007). The captive breeding and seed production system of the striped murrel in concrete tank has been developed and standardised by the ICAR-Central Institute of Freshwater Aquaculture, Bhubneshwar. Haniffa *et al.* (2000) injected *C. striatus* with luteinizing hormone releasing hormone analogue, human chorionic gonadotropin, pituitary extract, and ovaprim for artificial reproduction. Spawning took place in 24-26 h after *C. striatus* were injected with ovaprim hormone at a dosage of 0.5 ml/kg body weight (Marimuthu and Haniffa, 2007).

Since snakehead larvae are carnivorous, piscivorous, and cannibalistic, rearing them is a monumental undertaking. Although snakehead captive reproduction and larval rearing have been tested, they are not being carried out on a commercial scale (Mollah *et al.*, 2009). Development of suitable feeds and feeding techniques is necessary for successful larviculture in snakehead culture. Numerous studies have discussed using cultivated live food organisms as food. The availability of fry or fingerlings is one of the major challenges to overcome for the growth of any aquaculture operation (Webber and Riordan, 1976).

Studies on induced breeding and larval development offer intriguing information on their own and are crucial to the productive breeding and rearing of larvae for large-scale seed production. Studying larval development is essential for maximising larval growth, survival, and output. However, due to a lack of seed supply and awareness of their feeding and breeding methods, the culture of murrels is still uncommon in India. As a result, the current work was undertaken to develop a straightforward and inexpensive breeding and larval rearing technique for *C. striatus* in FRP tank systems.

MATERIALS AND METHODS

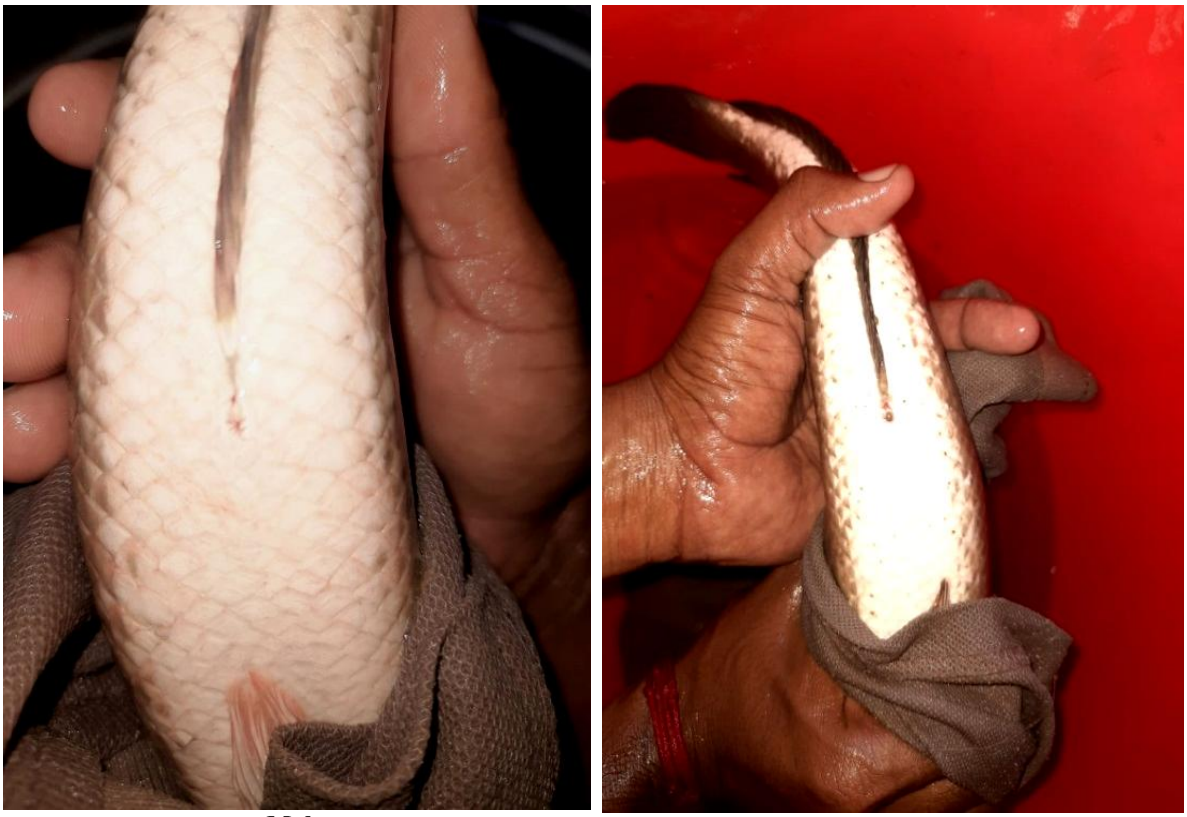
Collection and acclimatization of brooder: The present study was carried out at wet lab, Department of Aquaculture, Fisheries College and Research Institute, Thoothukudi, Tamil Nadu, India. Totally thirty numbers of brooders were procured and were safely transported and transferred to two cement tanks (15 ton capacity- 5m x 2m x 1.5m) for acclimatization. The brooders were acclimatized to laboratory conditions for a month. The cement tank was covered with nylon net to prevent the jumping of fish out of the tank (Fig. 1). Boiled chicken intestine and earth worm were used to feed the animals three times a day at 09:30 am, 01:00 pm and 04:30 pm. The daily feeding rates of fishes were maintained at 3 % of total body weight. Chicken intestine was fed to the fishes during morning and evening hours while, earth worm was fed in the afternoon. Water quality parameter such as dissolved oxygen, temperature, salinity and pH were monitored and recorded on daily basis (Table I).

Fig. 1: Brooder tank covered with net

Table 1: Water quality parameters recorded during the experiment

Stages	Temperature (°c)	pH	Salinity (ppt)	Dissolved Oxygen (ppm)
Brooder tank	28.15 ± 0.547	7.43 ± 0.051	1.5 ± 0.0547	2.93 ± 0.210
During Injection	28.67 ± 0.516	7.5 ± 0.063	0.0 ± 0.000	3.16 ± 0.103
During spawning	29.16 ± 0.752	7.56 ± 0.081	0.0 ± 0.000	3.30 ± 0.089
Larval rearing tank	29.00 ± 0.554	7.65 ± 0.116	0.0 ± 0.000	3.79 ± 0.751

Sexual dimorphism: Based on their exterior morphological characteristics, male and females can be distinguished (Chakrabarthy, 2006). Female fish had a bulged belly. Female vents were rounder and reddish in appearance, whereas male vents were pale and slit-like. Female fish have a dot that is somewhat reddish, while male fish have a prominent characteristic that resembles an anal papilla with a pointy tip (Fig. 2).



Male Female

Fig. 2: Sex differentiation

Brooder selection through cannulation: The brooders were selected based on their maturity. Maturity was assessed using cannulation process wherein a fine polyethylene tubing (3 mm diameter) attached to a plastic syringe was inserted into the gonadal papilla through which oocytes were collected to assess the status of female broodstock for the hormonal induction (Harvey & Carolsfeld, 1993). The collected oocytes were observed for their size, appearance, and diameter homogeneity under a stereomicroscope (Nikon Phase contrast stereomicroscope). The egg diameter was in the range of 0.8 to 1.5 mm (Fig. 3). Based on the results obtained after cannulation, healthy female and male with mean average body weight of 640 ± 0.5 g and 610 ± 1.3 g respectively were selected for induced breeding.

Fig. 3: Cannulated egg sizes under microscope

Experimental setup: The breeding experiment was carried out in FRP tanks of 1000 litre capacity. The tanks were cleaned and sun dried. Washed gravels were added to the bottom of the tank for 5 cm thickness. Once the tank was ready, it was filled with fresh water. Later, aquatic macrophytes like *Eichhornia* and *Pistia* were introduced into the breeding tank for shelter and to mimic the natural environment (Fig. 4).

Fig. 4: Designed FRP tank for breeding

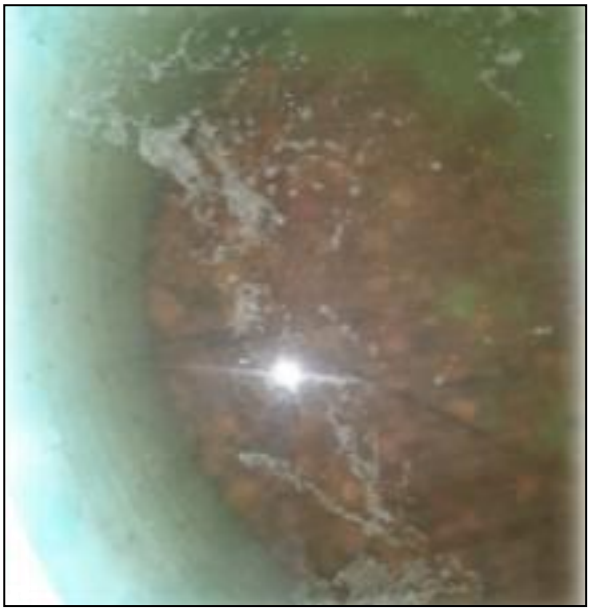


Fig. 5: Egg mass in the FRP tank

Induced breeding : Breeding pair at a ratio of 1:1 was introduced into the breeding tank where the partners moved together. The female and male fishes were injected with ovatide intramuscularly above the dorso-lateral line of the body at 0.6 ml / Kg of body weight. Injected brooders were released into the breeding tank. Spawning was noticed after 36 h of hormone injection. After spawning, parent fishes were removed immediately and transferred to another tank. The water quality parameters in the breeding tank was recorded (Table I).

Table 2: Length of *C. striatus* seeds during the experiment

Particulars	Minimum	Maximum	Mean	Std. Deviation
At hatching	3.90	4.10	4.0000	0.08944
One day old spawn	4.40	4.90	4.6167	0.19408
Two days old spawn	5.30	5.80	5.5833	0.21370
Three days old spawn	6.20	7.40	6.6500	0.51284
Six days old spawn	8.00	9.40	8.7667	0.50859
Ten days old spawn	11.50	12.60	12.1167	0.42622
Fifteen days old spawn	15.20	16.60	16.0167	0.46655
Twenty days old spawn	18.50	20.60	19.7667	0.78655
Thirty days old spawn	23.60	25.90	24.8167	0.83287

Observation of eggs: Size of the egg mass ranged from 6 to 8 cm in diameter (Fig. 5). Number of egg was counted manually after spawning for finding the total number of eggs spawned by the fishes. The number egg was counted using the method of Ross *et al.* (1982) and Harrington and Ikeda (1986). Fertilized and unfertilized eggs were also recorded. Egg samples were collected and observed under the microscope to measure the egg diameter and to study the developmental stages of the egg. The eggs hatched after 20 h of spawning.

Observation of larval stages : After hatching, the larval stages were documented on a daily basis using a binocular microscope (Nikon Phase contrast stereomicroscope). The total lengths of 10 individuals were measured during every sampling (TL is the length from the tip of snout to the end of the caudal fin; Crawford, 1986). The mean total length was also recorded. After a week of hatching, the larvae's total length was physically measured and noted with the aid of a ruler.

The hatchlings were transferred to 300 L capacity of larval rearing FRP tank (Fig. 6) at a stocking density of 200 nos/tank. During first 3 days, yolk sac was absorbed by the larvae. From 4th day onwards, after the formation of mouth, the larvae started exogenous feeding especially on small zooplankton. The larvae were fed with Artemia nauplii (Fig. 7) and zooplankton three times a day at 09:30 am, 01:00 pm and 04:30 pm. To avoid cannibalism, the shoot fry were removed from the tanks after two weeks. Water quality parameter such as dissolved oxygen, temperature, salinity and pH were monitored and recorded on daily basis (Table 1).

Fig. 6: Larval rearing in FRP tank

Fig. 7: Artemia nauplii for larval feeding

RESULTS AND DISCUSSION

Breeding and spawning activities : In the present study, males showed more aggressive and active participation in mating. The active male chased the female and frequently excited its movement which commenced 15 h after the hormone injection. The male was more actively involved in the courtship and spawning. At the time of courtship, the male tilted its body close to the genital papilla of female and the brooders joined together which ultimately resulted in spawning after about 36 h duration followed by external fertilization. According to Paray et al. (2016), the breeding behavior of *C. striatus* was observed 6 h after hormonal injection and continued till spawning up to 22-30 h. These differences may be due to the hormonal variation and environmental conditions.

The fishes spawned during the month of April which falls in between north-east and south-west monsoon. However, Rahman and Awal (2016) reported that *C. striatus* breeds during the onset of monsoon in ditches, ponds and flooded paddy fields. Nevertheless, ovatide hormone given to the fishes have induced the fishes to breed. The attempt of induced breeding in FRP tank was successful and *C. striatus* positively responded to the hormonal stimulation even during off-season. The fishes exhibited external fertilization and produced pelagic eggs. The breeding tank was provided with aquatic vegetation. Parent fishes cleared the area during breeding in the FRP tank. This was in accordance with Rahman and Awal (2016) who reported that parents usually clear an area of the water surface surrounded by aquatic vegetation for breeding. Rahman et al. (2012) reported that the absolute fecundity of the fish varies from 16,330 to 56,467 in the size range of 34.2-51.50 cm. In the present study, the fecundity of *C. striatus* was recorded to be 8300 numbers with the size of 42 cm. As pointed out by Honji (2011), the consistent gonadosomatic index values following hormonal induction of domesticated broodstock, may have contributed to the little amount of eggs released after artificial induction of fish as observed in the present study.

The eggs were noted to be yellow in colour, spherical, non-adhesive which usually floated and adhered to each other forming an egg mass of 6-8 cm in diameter. Fertilized eggs were transparent while the unfertilized eggs were white in colour or opaque. This was similar to the report by Paray et al. (2016) except that the size of the egg mass reported by them was 10 to 15 cm in diameter. This variation in size may be due to the less number of eggs released by the fish during induced breeding. The diameter of the fully swollen fertilized eggs varied from 1.2 to 1.3 mm (Fig. 8) which was in accordance with the report of ICAR-CIFA (2016), who mentioned that the size of the fertilized eggs ranged between 1.1-1.4 mm.

The fertilization and hatching rate was 60% and 60% respectively. This was comparatively lower to the reports of ICAR-CIFA (2016) where the fertilization and hatching rate was 75-98% and 70- 95% respectively in the cement cisterns. This may be due to the various environmental factor particularly temperature variation in the FRP tank. Survival in the larval rearing tank from spawn to fry was 60% and the shoot fry recorded was 2% during its rearing (Fig. 9). Similarly, ICAR-CIFA (2016) also reported 2 -3 % of shoot fry in their study.

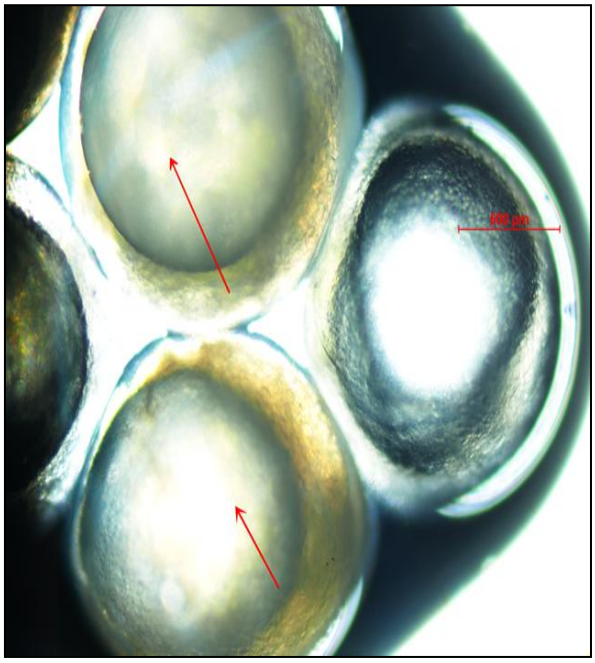


Fig. 8: Fertilized (Transparent) and unfertilized (Opaque) eggs

Fig. 9: Shoot fry

Larval developmental stages : The characteristic features of the larval development along with the body measurements are elaborated in Table 3 and Fig. 10a to 10h. In the present study, hatching occurred at 24 h after fertilization. This was in accordance with Marimuthu and Haniffa (2007) who reported that hatching took place 23-24 h after fertilization in *C. striatus*. The length of the newly hatched larvae was 4.0 ± 0.089 mm. Marimuthu and Haniffa (2007) reported that the newly hatched larva of *C. striatus* was 3.4 mm in length. Parameshwaran and Kamal (1988) mentioned that the length of newly hatched larvae was 2.81-3.22 mm in *C. striatus*. The results of these reports were comparatively lower than the results obtained from the present study. Bagarinao and Chua (1986) stated that there is a positive correlation between egg diameter and length and weight of the larvae while hatching which may be the reason for varied results on the length of the larvae.

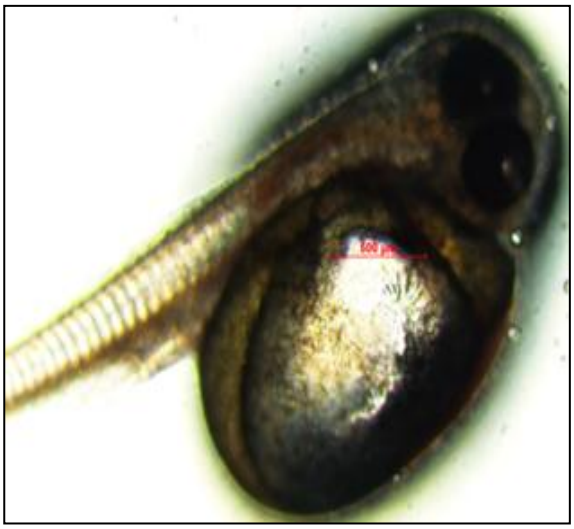
Larvae started feeding after two days at a total body length of 6.65 ± 0.512 mm. The caudal fin and pectoral fin developed after 6th day and started gulping air on 10th day. The length of the larvae was 12.11 ± 0.426 mm on 10th day. From 6th day onwards, larval movement was recorded which may be due to well-developed caudal and pectoral fins and the larvae started hunting feed. These results were in accordance with Marimuthu and Haniffa (2007). They also mentioned that the movement of larvae and capture of prey was improved from 10th day when the larvae measured 12.8 mm. In the present study, the larvae of C. *striatus* grew faster from 10th day of hatching and attained a total length of 24.81 ± 0.832 mm on 30th day when reared in the FRP tank. The main problem with the larviculture of *C. striatus* is the lack of knowledge on larval nutrition.,. It should be mentioned that poor feeding habits have a direct correlation with larval mortality (Appelbaum & Mcgeer, 1998). In the present study, Artemia nauplii was provided to the larvae initially which was weaned to frozen adult artemia from 20th day onwards. However, high rate of cannibalism was recorded during this period.

Cannibalism is more likely to occur when larvae are being reared since there are more of them and there are fewer opportunities for them to escape from predators (Smith & Reay, 1991). Following the advice of Howell et al. (1998), strategies like increasing food availability, maintaining shorter feeding intervals, and lowering stocking density were used to lessen the intensity of cannibalism. Apart from these, the larvae were graded based on the size and distributed from one FRP tank to another FRP tank every week to increase the survival rate.

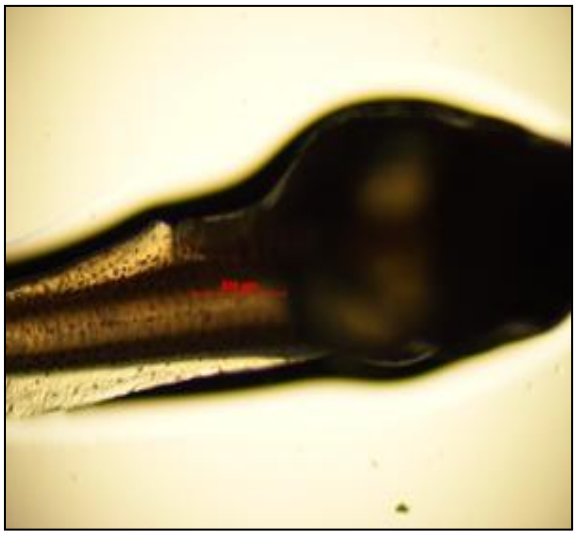
Table: 3. **Larval development of *Channa striatus* during the experiment**

Days	Description
At Hatching	Vigorous muscle twitches of the confined embryo were needed to breach the chorion, which was softened and slimed now. The larval stage started upon total hatching, which happened 24 hours after fertilisation and the average body length was 4.0 ± 0.089 mm (Table 2). Embryo that had just emerged from the egg was translucent and drab brown in colour, lacking a mouth and anus, and able to swim poorly (Fig. 10a).
Day 1	The larvae were still translucent and had a dark eyespot on the front of the head after hatching (AH). Following growth (23 h AH), a minor diminution in the yolk sac was seen (Fig.10b), Pectoral fin buds formed, and the mouth had not yet fully developed (not functional). The average total body length was 4.69 ± 0.194 mm (Table 2).
Day 2	The yolk sac was found to be smaller, and the pectoral fin was found to have a paddle shape. Gill rudiments was developed, movable jaws were seen during the formation of the gill (Fig. 10c). In the FRP tank used for larval rearing, horizontal larvae movement was seen. The average total body length was 5.58 ± 0.213 mm (Table 2).
Day 3	By this time, exogenous feeding had begun; therefore there had been a period of simultaneous endogenous and external food supply. The larva's head is prominent, its mouth, eyes, and pectoral fin are well-developed, and its yolk sac has been absorbed (Fig. 10d). The larva also began swimming vigorously. The average total body length was 6.65 ± 0.512 mm (Table 2).
Day 6	Under a microscope, the rays of the pectoral and caudal fins could be seen to be quite well developed. The body was a brownish golden colour. Large and distinct eyeballs (Fig. 10e). Larval group started settle at the bottom of the larval rearing FRP tank. The average total body length was 8.76 ± 0.508 mm (Table 2).
Day 10	The spawn began gulping air at the surface of the water in the larval rearing FRP tank, and the body colour is yellow with distinct bands (Fig. 10f). Dorsal and anal fins were clearly seen. The average total body length was 12.11 ± 0.426 mm (Table 2).
Day 15	It was easy to see lateral stripes all across the body. Body colour is a yellowish red, and there are more caudal fin rays (Fig. 10g). The average total body length was 16.01 ± 0.466 mm (Table 2).
Day 20	Reddish yellow body hue with distinct dorsal, caudal, anal, and pectoral fins (Fig. 10h). Larvae continuously hunt for feeding. The larvae moved actively in shoals. The average total body length was 19.76 ± 0.786 mm (Table 2).At this time, shoot frys were visible, and their total body length ranged from 28 to 30 mm (Fig. 9).
Day 30	Rays are strongly developed in the dorsal, caudal, anal, and pectoral fins, and the body has a dark yellowish black colour (Fig. 10i). Larvae are always looking for food. The body has distinct stripes running from dorsal to ventral sides.The average total body length was 24.81 ± 0.832 mm (Table II).

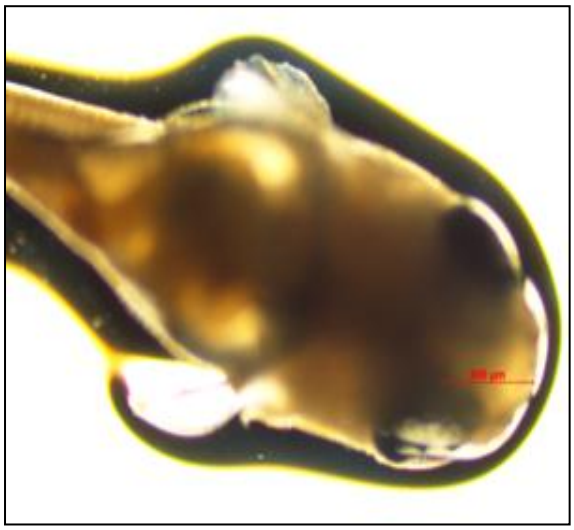
a) Hatchlings



b) One day old

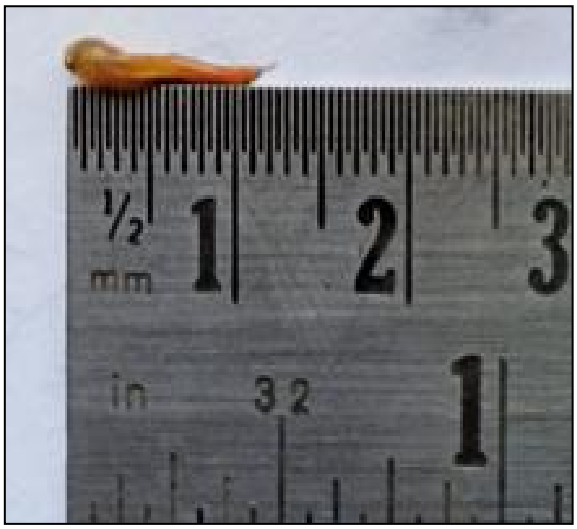


c) Two days old

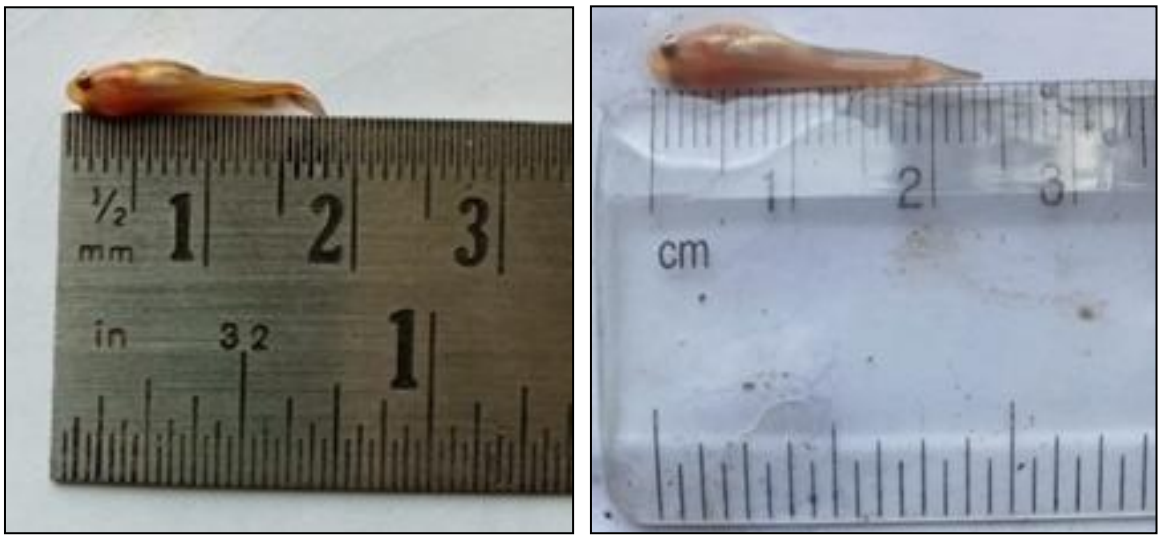


d) Three days old

e) Six days old



f) Ten days old



g) Twenty days old h) Thirty days old

Fig. 10: Larval developmental stages

CONCLUSION

This is the first study to report successful induced breeding of *C. striatus* in FRP tanks with maximum fertilization, hatching and survival rate. There are no reports available for rearing the larvae of *C. striatus*. The present observations on the feeding behavior of the fish larvae enable the development of successful strategies for *C. striatus* larval rearing in FRP tanks. For instance, feeding live food (artemia nauplii) in the beginning of their growth and gradually switching to a frozen adult artemia diet will boost their chances of surviving. Since *C. striatus* seeds are only obtained through natural collection, this knowledge is essential to the success of artificial reproduction, seed production and rearing methods which will enhance the development of *C. striatus* hatcheries to provide consistent seed supplies to aquaculture producers.

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