

Induced spawning and embryonic development of striped murrel *Channa striata* (Bloch, 1793) under indoor conditions using salmon gonadotropin releasing hormone analogue (sGnRHa)

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ABSTRACT

Ten successful trials on induced breeding of the striped murrel *Channa striata* (Bloch, 1793) under indoor conditions were carried out during July-August 2019. Brooders reared in cement tanks (males weighing in the range of 290-700 g and females 220-600 g), were used in a set of 2:2 male to female ratio for induced breeding with salmon gonadotropin releasing hormone anologue (sGnRHa) and dopamine antagonist commercial formulation. Each brooder set was kept in an FRP tank (13'x3'x14'') along with submerged macrophyte *Hydrilla verticillata*, which was sparsely spread all around the tank. Maturity of brooders was screened on the basis of secondary sexual traits. The brooders were given hormonal injection after 6 h of acclimatisation under indoor conditions @ 1 ml kg⁻¹ body weight (containing sGnRHa 20 µg ml⁻¹ and domperidone 10 µg ml⁻¹) to both the sexes. Natural spawning occurred in all ten sets within 21.5 ± 3.2 h (17-26 h) of injection. In all the tanks, two separate egg masses were observed with 30-95% fertilisation. Approximately 0.14 million eggs were scooped out from the tanks and reared in round plastic tanks (70 l) for hatching, where hatching occurred within 17-26 h post-fertilisation (hpf) with hatching rates of 50.76 to 93.18% at $28\pm2^{\circ}$ C. The embryonic development was recorded under a stereo-zoom microscope under controlled temperature conditions and described with digital images. The study revealed that induced breeding of *C. striata* can be undertaken in FRP tanks under indoor conditions for mass scale seed production, which will provide a platform for diversification of this high valued fish species, for large scale farming practices in ponds as well a new species for culture in recirculatory aquaculture system (RAS) and biofloc technology (BFT).

Keywords: Channa striata, Embryonic development, Induced breeding, sGnRH, Striped murrel

Introduction

Striped murrel Channa striata (Bloch, 1793) (family Channidae) is one of the preferred freshwater food fish in the Indian sub-continent and south-east Asia. Out of the total production of 92,523 t during 2016 (FAO, 2016), capture fisheries dominated the major market share (70,802 t) and only small share of 21,721 t is contributed from aquaculture mainly by Thailand, Malaysia, Cambodia, Vietnam, India and Pakistan (Wee, 1982; Haniffa et al., 2001). The major problems with culture of striped murrel are non-availability of hatchery seed, commercial feed and inherent problem of cannibalism (War and Altaf, 2014; Yadav et al., 2016a; Hien et al., 2017). The species breeds in ponds and rivers a little prior to or with the onset of monsoons (Bhattacharya, 1946; Alikunhi, 1953; Ravindranath, 1988) and their spawning season extends from March to September in north India though it has been reported to breed during October-December in southern India (Bilal et al., 2012; Dayal et al., 2013). It is a batch spawner and breeds two to three times in a year,

laying floating egg mass having a small number of eggs at a time, in weed infested marginal water areas of ponds (Haniffa et al., 1996). Both parents guard the eggs and fry (Bhattacharya, 1946). Captive breeding of striped murrel has been attempted in India, Bangladesh, Cambodia, Malaysia and Vietnam (Yaakob and Ali, 1992; Hossain et al., 2008; Bilal et al., 2012; Dayal et al., 2013; Rahman and Sadigul, 2016; Nen et al., 2018) using natural and synthetic hormones in various systems such as hapa; open cement tank and FRP tanks. Carp pituitary gland has been used to spawn striped murrel with varying success (Haniffa et al., 2000; Hossain et al., 2008; Roy et al., 2016). Marimuthu et al. (2007) successfully carried out spawning of striped murrel in a net enclosure fixed in pond and in cement tanks using Ovatide (sGnRHa) at a dose of 0.4-0.6 ml kg⁻¹ body weight to both female and male fishes. Pati et al. (2004) reported breeding in small fibre reinforced plastic (FRP) tanks (1.2 x 0.6 x 0.6 m), but they did not mention whether the breeding was undertaken in indoor or outdoor conditions and how

many pairs were used for spawning in a tank. The major problems with captive breeding of striped murrel is difficulty in the identification of maturity so as to judge ready to spawn brooders particularly male fish; lack of optimised hormonal doses for both female and male fish and suitable indoor hatchery conditions for mass seed production. Therefore, the aim of the present study was to define full maturity stage in male and female brooders and to standardise the hormonal requirement of sGnRH commercial formulations and also to attempt breeding the fish under indoor conditions with more than one pair in a system.

Materials and methods

Brood stock rearing

Ninety juveniles of striped murrel were procured from wild sources located near Lucknow or adjacent areas during March-May, 2019 and stocked in an open concrete tank of 5 x 7 m at the farm facility of ICAR-National Bureau of Fish Genetic Resources (ICAR-NBFGR), Lucknow, India. Brooders were fed small sized wild fish @2-3% of body weight per day during morning and evening.

Maturity assessment

The brooders were assessed for maturity by morphological examination. The features considered for maturity identification were shape of the belly; shape and colour of gonadal orifice as well as distance between gonadal orifice and origin of anal fin. In case of male fish, prominence of genital papilla was considered as an important sign of full maturity. Based on morphological examination, selected brooders were also dissected to confirm whether these traits match with the maturity status of the testis and the ovary.

Preparation of spawning tanks and induced breeding

Two identical FRP tanks of size 13'x3'x14" (LxWxD) were used for spawning under indoor conditions. The tanks were filled with borewell water upto 10-11" height. The entire water surface was sparsely covered with freshwater macrophyte Hydrilla verticillata. The brooders were collected from the tank and identified for sex and maturity status by morphological examination and sets of fully mature brooders were grouped in to sets at a ratio of 2:2 (male to female). Generally, two sets were simultaneously arranged on a day in two FRP tanks. The brooders were acclimatised for about 6 h and given single intra-peritonial injection of the commercial hormone, GonoproFH (M/s APC Nutrients, Secunderabad, India), containing salmon gonadotropin releasing hormone anologue (sGnRHa, (20 µg ml⁻¹) and dopamine antagonist, domperidone (10 mg ml^{-1}) . The hormone was given (a) 1.0 ml kg^{-1} to both female (Pati *et al.*, 2004) and male brooders. As maturity in male striped snakehead is a critical issue, higher dose of 1.0 ml kg⁻¹ of hormone was given as suggested by Yadav (2016) with sGnRHa and So Nam *et al.* (2012) with HCG application. The tanks were provided aeration from a portable air blower and covered properly with greenhouse netting to avoid fish escape.

Collection of eggs, hatchling and spawn rearing

The fish laid eggs in the form of a floating egg mass in between the macrophytes that looks like a nest. The eggs were floating, non-adhesive and pale yellow in colour having diameter of 1.5+0.5 mm. They were easily scooped out with the help of small plastic sieve in a bucket. The eggs were counted (Pati el al., 2004) and shifted to round plastic tanks (801), holding approximately 501 water. The tanks were provided with mild aeration from a portable air blower. The eggs float and settle mostly on the margin of the tank. Around 40% tank water was changed after every 8 h. The density of eggs in each tank was kept around 5000 (100 larvae l⁻¹). The rate of fertilisation was assessed 4-5 h after collection of eggs. Randomly, 20 eggs were observed under the microscope for the assessment of fertilisation rate as no difference was seen between the fertilised and unfertilised eggs by visual comparison in this species for at least 12 h. The eggs were allowed to hatch in the same tanks and reared till yolk-sac was completely absorbed. The rate of fertilisation, hatching and duration of hatching were recorded.

Embryonic and larval development

Approximately 200 eggs were taken in a tray in an air conditioned room maintained at 26 ± 1.0 °C. The fertilised eggs were examined under a stereozoom microscope (Leica model S8APO) for the embryonic and larval developments up to yolk-sac absorption stage and digital images were recorded whenever, change in the internal structures were observed.

Water quality

The water quality parameters of the spawning and larval rearing tanks were analysed for temperature, pH, electrical conductivity (EC), total dissolved salts (TDS), total hardness and dissolved oxygen using multiparameter recorder (Thermo) and were found to be within the normal ranges: Water temperature - $28\pm2^{\circ}$ C, pH - 7.4 ± 0.2 , EC - $660\pm16 \ \mu$ S cm⁻¹, TDS - $225\pm18 \ mg \ l^{-1}$, Total hardness - $330\pm8 \ mg \ l^{-1}$ and Dissolved oxygen - $6.0.\pm0.5 \ mg \ l^{-1}$.

Results and discussion

The sexes looked alike morphologically when they were immature. However on attaining maturity, a good fecund mature female could be easily distinguished having bulged belly nearing the sides of pectoral fins. The gonadal orifice of females was found round, little fleshier (button shaped), pinkish-red and with no genital papilla even when fully mature (Fig. 1a). Males on the contrary have slender body, no bulging abdomen, elongated genital orifice and having slightly fleshy and pointed genital papilla at the time of complete maturity. Males have distinct and much larger space between genital orifice and anal fin in comparison to females (Fig. 1b). Besides, fully mature males also had darker body colour in comparison to females. Parmeswaran and Murugesan (1976) and Devraj (1973) have also reported more or less similar findings except that they did not take into consideration the distance between the gonadal orifice and origin of anal fin for differentiating sexes, which is more reliable character for differentiating sexes in this species even during nonbreeding season. The males did not exude milky-white milt on applying pressure over the abdomen as in case of carps and catfishes due to low GSI (0.214 in June) of testes even during peak spawning season (Yadav et al., 2016b). Anatomy of the abdomen implies that unlike carps and catfishes, whose testes are large and the major part of the testes body remain embedded within the alimentary canal; the testes in case of striped murrel remains attached up to long distance with the ventral coelomic wall and then finally gets embedded in the alimentary canal with left lobe larger than the right (Fig. 1c). The testes in fully mature fish were very small in size, translucent and reddish white in colour (So Nam et al., 2012; Irmawati et al., 2019) and filled with transparent milt (Yadav, 2016) (Fig. 1d). As the size of testis is very small and the abdominal wall very thick due to thick musculature even in case of mature male specimens, the milt did not come out on applying

pressure over the abdomen as in the case of carps and catfishes and hence chances of extracting milt by stripping is very difficult. The success of breeding in this species also depends on assessment of the maturity stage of fish particularly male fish in comparison to female as latter can be much accurately identified in mature condition based on bulging abdomen and the shape of gonadal orifice. Therefore, the authors are of the opinion that maturity status of the male fish could be better judged by observing body colour, which becomes darker with the advancement of maturity and of genital papilla, which becomes prominent with slightly swollen pinkish body and reddish pointed tip in fully mature males (Fig.1b). Hossain et al. (2008) have also expressed similar views and hence it can be concluded that assessment of male maturity in murrels is one of the biggest problems in captive breeding (Irmawati et al., 2019). According to Bolaji (2011) and Irmawati et al. (2019) and also based on personal observation of the authors, poor sex ratio of males in nature is another problem affecting captive breeding of this species.

All 20 females in 10 sets spawned naturally laying distinct floating egg masses between *Hydrilla* twigs (Fig. 1e, f) that contained 4050-9500 eggs in 21.5 ± 3.2 h (Table 1). Generally, one egg mass was found close to one end of the tank (west direction), whereas second one more or less somewhere in mid of the tank. Therefore unlike in the wild conditions, where egg masses are generally observed at the margins of the water bodies, the fish did not choose margins of tank for spawning, probably due to shallow and uniform water depth all along the tanks in the present case. Therefore, shallow tanks or shallow

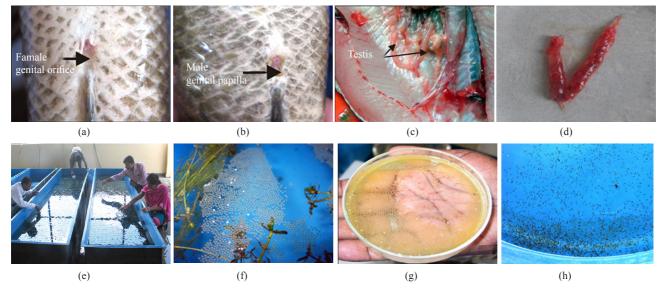


Fig. 1. Various events during induced breeding of *C. striatus*. (a) Gonadal orifice of female, (b) Gonadal orifice and genital papilla of male, (c) Mature testis *in situ*, (d). Fully mature testis after extraction, (e) Collection of eggs from spawning tanks, (f) A single egg mass in the FRP tank, (g) Fertilised eggs in petridish (white eggs are unfertilised), (h) Swarm of spawn in the FRP tank

water depths may be considered ideal for the spawning of this species as shallow tanks provide more or less similar natural breeding habitat of this species (Amilhat, 2005). This also accounts for the successful natural spawning of striped murrel in indoor conditions using FRP tanks with cent percent success in which two pairs were successfully bred in single tank repeatedly.

As the egg masses were almost intact and approachable, they could be easily scooped out from the tanks and transferred to the hatching tanks. Out of 20 females, fertilised eggs were obtained from 18, whereas one female in each set of VII and IX laid all unfertilised eggs Though the eggs of these females had close resemblance to fertilised eggs in appearance, they perished after >12 h, which is an indication that the female did not get chance to cohabit with male probably due to selection of immature second male in these sets.

Induced breeding of striped murrel was demonstrated with pituitary gland extract in the past (Alikunhi, 1953; Parameswaran and Murugesan, 1976). Hossain *et al.* (2008) reported successful induced spawning in this species using carp pituitary at very high doses in Bangladesh. Haniffa *et al.* (2000, 2001); Marimuthu *et al.* (2000, 2007) and Dayal *et al.* (2013), induced bred this species with sGnRHa hormonal formulations in pond condition by putting them directly in the open pond/cement tank or

in hapa in earthen pond. Marx and Stephen (2004) also succeeded in spawning this species in pond condition using pituitary extract but they did not get successful response both with Ovaprim (M/s Syndel, USA) and Ovatide (M/s Hemmo Pharma, Mumbai, India). Pati et al. (2004) first reported successful spawning in FRP tanks using Wova FH (sGnRHa; Biostadt India Ltd., Mumbai, India) but they did not mention whether it was under indoor or outdoor conditions and number of pairs tried and sex ratio used. Dayal et al. (2013) attempted spawning in indoor conditions with sGnRHa but could not succeed in spawning this species. In most of the breeding trials, the successful spawning rate has not been defined and different opinions have been expressed about the suitability of inducing agents and the breeding environment. Based on the present success and the views of the earlier workers, the authors suggest three criteria for successful induced spawning. Firstly, proper assessment of maturity stage of brooders as suggested previously in this paper, which needs to be carefully examined before selection of the brooders more particularly that of male fish. Secondly, the hormone dose to be applied to brooders for both the sexes. Females have been reported to breed even with low (0.5 ml kg bw⁻¹) and similar doses of sGnRH as used in this study (Haniffa et al., 2000; Marimuttu et al., 2000; Dayal et al., 2013; Rahman et al., 2016; Roy et al., 2016; Yulintine et al., 2017). Successful spawning of all 20 females with

Table 1. Induced breeding details of C. striata spawned naturally in FRP tanks under indoor conditions

Set No.	Weight of female (g)	Hormone dose for female (ml kg bw ¹)	Weight of male (g)	Hormone dose for male (ml kg bw ⁻¹)	No. of eggs	Fertilisation (%)	Incubation period (hpf)	No. of hatchlings	% Hatching
Ι	520	1.0	470	1.0	7900	95	17	6500	86.60
	390	1.0	340	1.0	5200	90	17	4100	87.60
II	260	1.0	290	1.0	4500	90	19	3550	87.65
	220	1.0	250	1.0	4050	80	19	2800	86.41
III	425	1.0	525	1.0	8200	85	17	5525	79.26
	475	1.0	450	1.0	7400	75	17	4500	81.08
IV	400	1.0	450	1.0	7000	95	20	6600	89.57
	450	1.0	550	1.0	8000	93	20	6600	88.70
V	500	1.0	400	1.0	9500	85	22	7525	93.18
	450	1.0	450	1.0	8500	70	22	5050	84.87
VI	500	1.0	700	1.0	7800	75	21	5150	88.03
	500	1.0	500	1.0	7500	65	21	4300	88.20
VII	500	1.0	600	1.0	8500	75	24	4050	63.52
	450	1.0	450	1.0	7000	nil	-	-	-
VIII	550	1.0	600	1.0	7500	70	25	3400	64.76
	500	1.0	500	1.0	7000	60	25	3200	76.20
IX	600	1.0	700	1.0	8000	40	24	2400	75.00
	500	1.0	500	1.0	7000	nil	-	-	-
Х	500	1.0	400	1.0	6000	40	26	1575	65.62
	600	1.0	500	1.0	6500	30	26	990	50.76
Total					143050			77815	

dose of 1.0 ml kg bw⁻¹ revealed that this rate is suitable for female fish. In the present study, in case of males too, a dose of 1.0 ml kg bw⁻¹ gave excellent results in spawning. As in most of the cases, failure in induced breeding is generally due to poor response from male, a dose of 1.0 ml kg bw⁻¹ might trigger final maturity in males better than low dose usually applied by other researchers. So Nam *et al.* (2012) have also recommended higher dose of HCG for male than to female striped snakehead, which confirm the authors' assertion. Thirdly, the spawning tank should have shallow water depth and provided with submerged sparsely spread aquatic macro-vegetation for supporting formation of nest like structure by the brooders.

It has been observed by the authors (from our unpublished previous work) that majority of the females mature more or less at the same time during the peak breeding season in a pond/tank, but lesser number of males are found in fully mature stage. This is the reason that although this species being a pond breeder, only very few spawning occurs in a pond at a time even during peak spawning season, where large population of striped murrels are available. This phenomenon has shown some sort of anti-cohort linkage attached with poor availability of mature male brooders in a pond/tank. This needs to be verified in future research, though several studies have suggested significantly poor female to male ratio in natural population in murrels (Olurin and Savage, 2011; Koundal *et al.*, 2014).

Striped murrel is a low fecund as well batch spawner species having ova at different maturity stages at a time in the ovary and hence does not release all ova in a single spawning. The ovarian fecundity of this species has been reported to vary from 29000-55000 per kg body weight (Alikunhi, 1953) and 22000-34000 per kg body weight (Chen, 1990). Considering the above ovarian fecundity, the number of fully mature ova/eggs available from floating egg masses both in the wild and captive spawning is generally very low, which in the present case were only 4050-9500 for fish in the weight range of 260-600 g during spawning, which were more or less similar to the finding of Hossain *et al.* (2008). This implies that the species is a batch spawner and release only those eggs which are fully mature at the time of spawning.

A total of approximately 143050 eggs were collected from 20 sets (18 females). The fertilisation percentage ranged from 30-95%. The rate of fertilisation was higher (highest was 95%) in the beginning of our trials *i.e.* during third week of July to first week of August, thereafter it declined (30%) in the end of August. This implies that the peak spawning season lasts upto first week of August and there is slump in spawning thereafter. The eggs hatched out in 24.15 ± 01.00 h post-fertilisation (hpf) at $26\pm1^{\circ}$ C and yolk-sac was completely absorbed in 101.30 ± 0.30 hpf with success rates of 50.76-93.18%. The present success rate was found contrary to the observations of Marimuthu *et al.* (2001), who reported that eggs kept for hatching in aquarium in the absence of brooder parents suffer fungal and parasitic infections leading to poor survival.

Embryonic development

The fertilised eggs were round, translucent, pale yellow, floating and non-adhesive, measuring 1.50 ± 0.05 mm in dia (Fig. 1g). They were unique in having single large round oil globule of 1.1 ± 0.1 mm dia, which occupies the greater part of the ovum and immersed in the golden yellow yolk having 1.25 ± 0.1 mm dia and both were surrounded within a space bounded by the vitelline membrane (Fig. 2a). Willey (1909) has also described similar structure in *C. striata* egg. The blastoderm was found situated on the lower side of the yolk as a thin layer under the microscope and usually not visible up to morula stage due to high buoyancy of the oil globule which always keeps egg upside down.

The first cleavage took place with equal division of blastoderm (2-cell stage) within 20+04 min postfertilisation (mpf). It was followed with second and third cleavages with the formation of 4-cell and 8-cell stages (Fig. 2a) respectively, within 30+05 mpf. The fourth cleavage with formation of 16 cells was observed at 45±05 mpf (Fig. 2b). Further cleavages resulting in formation of 64 cells were completed within 60+10 mpf. The blastoderm attained morula stage in 1.45+0.15 hpf (Fig. 2c) and blastula stage in 03.50±0.15 hpf (Fig. 2d). The embryo developed to gastrula (Fig. 2e) and early neurula stages (Fig. 2f) in 05.45+0.15 hpf and 06.30+0.30 hpf respectively. At this stage, head and tail ends became distinct and formation of germ ring was seen. Cephalic region became wider with formation of primitive brain, extension of tail region and rudiments of heart could be seen at 10 myotome stage (Fig. 2g). The size of the embryo expanded further with increase in the number of myotomes and development of internal organs, which have been summarised in Table 2 and depicted in Fig. 2 (h-n). The embryo hatched in 24.15±01.00 hpf (26±1°C). The pattern of embryonic development of C. striata observed during the present study were found to be more or less similar to the findings of Marimuthu and Haniffa (2007).

Larval development

Details of larval development up to yolk absorption stage are presented in Table 3 and Fig. 3. Newly hatched larva was translucent, dull brown in colour with well defined yolk. The head and yolk together looked like a

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Stage

8	duration	1
Fertilised egg	-	Eggs are round, transluscent, non-adhesive, floating and pale yellow in colour having 1.50 ± 0.05 mm dia. Diameter of oil globule and yolk-sac was 1.1 ± 0.1 mm and 1.25 ± 0.1 mm respectively
2-cell stage	20:00 <u>+</u> 04.00 min	First cleavage
8-cell stage	30.00 <u>+</u> 05.00 min	Second cleavage (Fig. 2a)
16-cell stage	45.00 <u>+</u> 05.00 min	Third cleavage (Fig. 2b)
64-cell stage	60.00 <u>+</u> 10.00 min	Fourth cleavage
Morula stage	01.45 <u>+</u> 00.15 h	Blastoderm cell walls become undifferentiated (Fig. 2c)
Blastula stage	03.50 <u>+</u> 00.15 h	Embryonic shield formed and invaded around half of the yolk (Fig. 2d).
Gastrula stage	05.45 <u>+</u> 00.15 h	Gastrulation converts the blastoderm into a two-layered structure, with an outer epiblast and inne hypoblast. Head and tail region not differentiated (Fig. 2e)
Early neurula	06.30 <u>+</u> 00.30 h	Cephalic region distinct with fore brain and narrow tail region. Formation of germ ring is seen (Fig. 2f).
10 myotome	11.30 <u>+</u> 00.30 h	Cephalic region further broadened and primitive brain visible, 10 myotomes visible, tail extends upto half of the yolk periphery, heart rudimentary (Fig. 2g).
15 myotome	12.15 <u>+</u> 00.15 h	Length of embryo further extends, around 15 myotomes visible, heart formed, eye lens formed in the rudimentary eyes Kupfer's vesicle (Fig. 2h).
22 myotome	13.45 <u>+</u> 00.30 h	Length of embryo further extends, around 22 myotomes visible, heart well developed and commenced blood circulation, eyes rudimentary (Fig. 2i)
25 myotome	14.15 <u>+</u> 00.15 h	Length of embryo further extends and cover around 80% yolk surface, heart well developed and commenced blood circulation but pulse rate very slow, around 25 myotomes visible, melanophores visible over the yolk-sac, tail narrow (Fig. 2j)
27 myotome	18.30 <u>+</u> 00.15 h	Length of embryo further extends and cover around 85% yolk surface, cephalic and tail regions further widened, heart beat rhythmic and fast, melanophores increased in number (Fig. 2k)
35 myotomes	19.00 <u>+</u> 00.15 h	Embryo covers entire yolk, cephalic region further enlarged, tail started detaching from yolk, melanophores increased further in number, around 35 myotomes visible (Fig. 21)
Pre-hatched embryo	22.15 <u>+</u> 00.30 h	Tail detached from posterior side and moves at intervals, myotomes more than 35, heart showed flow of red blood, eye precursor visible, auditory vesicle formed, twitching movement initiated, melanophores appeared over the body (Fig. 2m)
Hatching embryo	24.15 <u>+</u> 01.00 h	Embryo coming out of egg shell with more vigorous twitching movement, myotomes more than 35, melanophore denser over the body, eye rudimentary (Fig. 2n)

Table 2. Details of embryonic development of C. striata at 26+1 °C (n=4)

Developmental details

Post-fertilisation

bulb and anal portion has a distinct transparent caudal fin-fold having approximately 38 myotomes. It measured 3.14 mm in total length (1.61 mm thoracic length+1.42 mm anal length) with yolk length and yolk width of 1.20 and 0.80 mm respectively. Marumuthu and Haniffa (2007) however, recorded size of newly hatched larvae as 3.4 mm, which is slightly larger as compared to the present observation. The hatchling moves with jerks up and down but unable to swim (Fig. 3a). The heart was functional but flow of blood was not visible as being transparent at this stage. In subsequent developments, the hatchling increased both in head and anal lengths (Fig. 3b, c). The rudimentary eyes and mouth became prominent at 8.00+0.20 h post-hatch (hph) i.e., 32.15+0.20 hpf (Fig. 3d). At 25.15±0.20 hph (49.30±0.20 hpf), eyes were formed having 0.35 mm dia and distinct mouth appeared (Fig. 3e). The larva started vigorous swimming movement at 52.15+0.30 hph (76.30+0.30 hpf) with well developed mouth and pectoral fins. At this stage, yolk-sac is reduced to a very small size (Fig. 3f). Yolk-sac is totally consumed at 77.15 \pm 0.30 hph (101.30 \pm 0.30 hpf) when hatchling attained a total length of 6.20 mm and started to move freely and consume live feed voraciously (Fig. 3g). These developments were found to be more or less similar as reported by Marimuthu and Haniffa (2007) for *C. striata* except that the length of yolk-sac absorbed larvae recorded was lesser (5.8 mm) as compared to the present observation.

It is concluded that induced spawning of *C. striata* in FRP tanks can be undertaken under indoor conditions using synthetic commercial sGnRHa formulations. Three main considerations suggested for indoor spawning of striped murrel based on the results are: correct maturity assessment of brooders particularly of male ; a higher dose of sGnRHa @ 1.0 ml kg⁻¹ body weight to both the sexes and long tanks with shallow water depth provided with submerged macro-vegetation. The latter also provides scope for breeding more than one pair in a tank at a time. With proper identification of mature brooders, a sex ratio

Induced breeding of Channa striata under indoor conditions

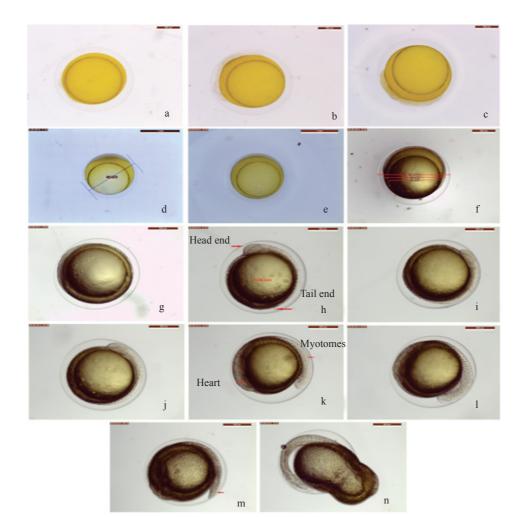
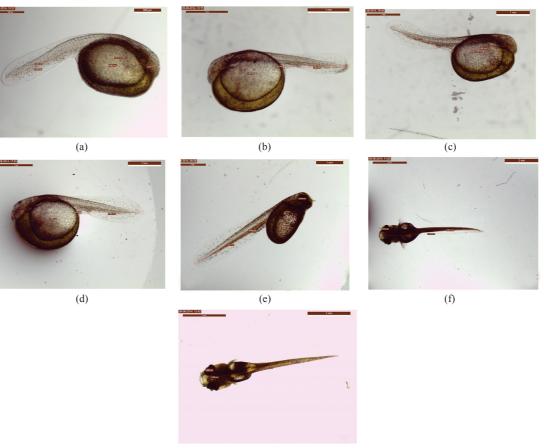


Fig. 2. Embryonic development in C. striatus under controlled temperature of 26±1°C

Table 3. Larval development of C. striata	<i>i</i> up to spawn stage at 26 ± 1 ^o C (n=4)
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hph	hpf	Developmental details
0.0	24.15 <u>+</u> 0.30	Hatchling dull brown in colour, yolk-sac well defined, caudal finfold transparent, myotomes around 38, average total length 3.14 mm, anal length 1.42 mm, yolk length 1.20 mm, yolk width 0.80 mm, length of eye optic 0.34 mm, eye optic distinct but eyes are not developed, mouth not formed. The larvae move up and down with jerks but could not swim (Fig. 3a).
5.15 <u>+</u> 0.30	29.30 <u>+</u> 0.30	Average total length 3.40 mm, anal length 1.38 mm, yolk length 1.44 mm, yolk width 1.20 mm, melanophores increased in number (Fig. 3b),
8.00 <u>+</u> 0.30	32.15 <u>+</u> 0.30	Average total length 4.08 mm, anal length 2.06 mm, thorax length 2.02 mm, yolk length 1.42 mm, yolk width 1.08 mm, eyes rudimentary (Fig. 3c)
8.45 <u>+</u> 0.20	33.00 <u>+</u> 0.20	Rudimentary eyes and mouth prominent, average total length 4.08 mm, anal length 1.85 mm, yolk length 1.47 mm, yolk width 0.80 mm (Fig. 3d)
25.15 <u>+</u> 0.20	49.30 <u>+</u> 0.20	Eyes and mouth formed, eye dia 0.35 mm, average total length 4.03 mm, anal length 1.95 mm, thorax length 1.78 mm, yolk length 1.43 mm, yolk width 0.79 mm (Fig. 3e)
52.15 <u>+</u> 0.30	76.30 <u>+</u> 0.30	Average total length 4.96 mm, anal length 2.99 mm, thorax length 2.0 mm, eye dia 0.35 mm, head width 1.0 mm, yolk-sac reduced 1.29 mm, pectoral fins developed and larvae move vigorously for swimming, mouth gape well formed (Fig. 3f)
77.15 <u>+</u> 0.30	101.30 <u>+</u> 0.30	Average total length 6.20 mm, anal length 3.99 mm, thorax length 2.59 mm; eyes increased in size with eye dia 0.42 mm and eye width 0.40 mm; head width 1.29 mm, mouth cleft 0.53 mm, vestigial yolk-sac present, melanophores dense, pectoral and caudal fins developed (Fig. 3g)

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(g)

Fig. 3. Larval development from hatchling to spawn stage of C. striatus under controlled temperature of 26±1°C

of 1:1 (female:male) is ideal. The spawning success achieved under indoor conditions in the present study could pave the way for mass-scale seed production of this species.

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