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DEVELOPMENTAL ASPECTS OF THE ENDEMIC CICHLID FISH *ETROPLUS SURATENSIS* (BLOCH, 1790) IN CAPTIVITY

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Abstract: *Etroplus suratensis* is an endemic cichlid fish of commercial importance, common in the brackishwater habitats of Kerala, India. It is a substrate spawner having high degree of parental care. This paper describes the developmental stages (embryonic, larval and early juvenile periods) of *E. suratensis*. Fertilised eggs hatched out in 72 to 80 h; the hatchling possess a mean size of 4.48±0.103mm. The yolk was absorbed completely in six days. The characteristic vertical bands appeared when they attain 15mm and the parental care lasts for about two months.

Key words*: Etroplus*, breeding, fertilization, embryonic development, larvae, yolk sac, hatchling

INTRODUCTION

The Pearlspot, *Etroplus suratensis* is a tropical brackishwater fish belonging to the order Perciformes and family Cichlidae. They are one of the most popular attractions in the food fish trade of Kerala, India. Due to good flesh quality, high market price and culinary delicacy *E. suratensis*, the State fish of Kerala, is suitable for diversification of fresh and brackishwater fish farming in India. Even though captive seed production and larval rearing of this species have been accomplished, their production is not presently done on a commercial scale. In India most of the seeds for the culture systems collected from wild. The existing stocks seem to be threatened by factors like habitat degradation and pollution. The expansion of water tourism and dredging of the lake bottom for lime shell are catastrophic to natural breeding of pearlspot. Since wild seeds are scarce, captive production is essential and through this good quality seeds can supply throughout the year.

The species is listed as 'Least Concern' in the IUCN Red List of Threatened Species in view of its wide distribution, presumed large overall population, even though it has a relatively declining trend in the wild, in Kerala (Abraham, 2011). Previous research in India focused mainly on its biology (Jayaprakas and Nair, 1981; Jayaprakas and Padmanabhan, 1985; Jayaprakas *et al.,* 1990; Padmakumar *et al.,* 2004a; Bindu and Padmakumar, 2008, 2014), breeding (Padmakumar *et al.,* 2009a, 2012; Biswas *et al*., 2014) and culture (Padmakumar *et al.,* 2004b, 2009b; PramodKiran *et al.,* 2014).

With more than 1350 described species, cichlid fish form one of the most diverse and successful groups of teleosts (Nelson, 2006). However, there have been only a few studies on cichlid fishes so far, most of which deal with the development of African species such as *Oreochromis niloticus, O. mossambicus, Labeotropheus fuelleborni* and *L. trewavasae* (Kratochwil *et al.,* 2015). The description of eggs, embryonic development, larval rearing and juveniles production are available for relatively few cichlid fishes and such reports are lacking in Indian waters. Fish spawning, egg hatching and larval rearing are the major factors in successful hatchery management. This paper provides basic information on its different stages of development to enhance the captive production of *E. suratensis*.

MATERIALS AND METHODS

Live brooders of *Etroplus suratensis* were collected from the riverine stretches of Vembanad lake (09°31' and 09°41'N and 76°21' and 76°26'E), India and stocked in earthen ponds (250m^2) and cement tanks (70m^2) at Regional Agricultural Research Station, Kumarakom, Kerala. In the ponds eggs were attached on different type of natural substances (Fig. 1a, b). The tank was provided with different types of substrate to promote spawning behavior and egg deposition (Fig. 1c) and 25% of the water was exchanged once in a week for ensuring the clarity of water.

Eggs were collected from the breeding tank (Fig. 1d), at the time of fertilization itself and kept in glass tanks (123x49x47 cm) containing filtered freshwater (10-15 cm depth) with vigorous aeration. From this 10 to 12 eggs were removed carefully and observed under a trinocular microscope (CETI, Belgium) at 10x magnification. They were continuously monitored for studying the daily changes in embryonic development. Time of fertilization was denoted as 0:0 h. All the developmental stages were documented using computer aided 'Magnus Imaging System' supported by *Pixel View* software connected with microscope. The hatching tanks were provided with well-aerated water. Temperature (25.5–28 ^{0}C), dissolved oxygen (5.0–5.6 mg.l^{-1}) and pH (6.5–7.5) of the water were monitored using digital water quality analyzer (Eutech, Singapore).

Early developmental stages of fishes were grouped into embryonic, yolk-sac larvae period, post yolk-sac larvae period and juvenile period (Liang *et al.* 2003). Rema *et al.* (2012) divided the embryonic period into three phases: cleavage , embryonic and elutheroembryonic (yolk-sac larvae) stages. In the present study, developmental stages are summarized as embryonic (cleavage, embryonic), larval and juvenile period. The embryonic and post embryonic stages were recorded as hours post fertilization (HPF) and days post hatching (DPH) respectively. Length of larvae (LL) from hatching to 6 DPH were measured by taking the mean total length of 10 individuals on each day and expressed as Mean ± Standard Deviation. After one week they were shifted to cement tanks (5x3x1.2 m) containing filtered lake water. During this period, the larvae were fed twice a day with powdered commercial pellets (Crude Protein 20%), containing fish meal as the major ingredient.

Fig. 1. Eggs collected from pond (a, b); Attached eggs on the substrate placed in the tank (c, d)

RESULTS AND DISCUSSION

Cichlids are typically categorized into substrate brooders and mouth brooders (Keenleyside, 1991). *E. suratensis* is a substrate spawner with an asynchronous ovarian development (Bindu, 2006). Like other substrate brooding cichlids, the eggs remain cemented to the nesting object by a stalk with out any overlap. Spawning fecundity of pearlspot is low, 382 to 1966 eggs (835 ± 499) were laid per brood (Padmakumar *et al.,* 2009a). The absolute fecundity is however higher (average 2748) compared to *E. maculatus* (Bindu and Padmakumar, 2014).

1. Embryonic Development

Eggs of *E. suratensis* have an ovoid shape, with the longitudinal axis longer (2.23±0.191mm) than the transverse axis (1.03 ± 0.11 mm) and the animal pole narrower than the vegetal pole. They were pale yellow in colour and after fertilization the color changes slowly and they became brownish just prior to hatching. The egg is surrounded by the chorion, a translucent envelope that sticks closely to the egg. This persists throughout later developmental stages. The embryonic period began with fertilization and ended with hatching out from the chorion and was characterized by the utilization of exclusively endogenous nutrition from the yolk.

1a. *Cleavage Phase*

Cell division in *E. suratensis* eggs were found to be slow, probably due to the high amount of yolk (Fig.2a-2l). By 1 HPF cytoplasm accumulated as a blastodisc at the animal pole (fig 2a) similar to other fish species and is termed as one uncleaved cell blastomere stage (Fujimura and Okada, 2007; Rema *et al.*, 2012). The cleavage is restricted to the animal pole region. The egg is telolecithal and cleavage mode is meroblastic and discoidal. The meroblastic divisions keep a connection between yolk and blastodisc during the cleavage period.

The first cleavage was meridional and initiated at 1.30 HPF resulting in the formation of two blastomeres of equal size. The second cleavage plane is at right angle to the first and resulted in four equal blastomeres at 2.0 HPF, and the third division occurs at two plane parallel to the first cleavage plane, resulted in 8 blastomeres of equal size followed by the fourth division leading to 16-cell stage, four rows of four blastomeres, at 4 HPF(Fig. 2d). Further division resulted in 32-cell stage at 4.30 HPF (Fig. 2e) and from this time onwards there were no regular pattern in the cell division. At this stage, the blastomeres appeared irregular in size and curved around the yolk.

The sixth cleavage resulted in the formation of 64-cell stage and the cells appeared more or less crowded and occupy the whole space in two layers (Fig. 2f). Cleavage continued and no clear cleavage planes can be identified. Consequently, by 7.30 HPF, cells became smaller and this stage is referred to as blastula stage (Fig. 2g). The 7th, 8th and 9th cleavage results in 128, 256 and 512 blastomeres respectively. By 12 HPF, the blastomeres appeared as a mass of cells above the yolk mass. Gradually the blastodisc flattened and cover the top of the yolk, bulges towards the animal pole in an dome-like shape. It gradually transforms into a uniformly thick layer called blastoderm or germ ring. The epiboly depends on the spreading of the blastoderm margin over the yolk (Kratochwil *et al.*, 2015). In 14 to 15 HPF early epiboly occurs, the blastoderm spread over 10-20% of the yolk. As epiboly progresses, the blastoderm at the animal pole thins out and extend on to either side of the yolk. By 17 HPF, 30% of epiboly reached(Fig. 2h). The gastrulation commences at about 18 HPF by the involution of cells as described in Midas cichlid by Kratochwil *et al.* (2015). The blastoderm become differentiated at this point of time and by 21 HPF, epiboly continues and leads to neurulation. A thickening appears at one position of the blastoderm (Fig.2i) The extension of the germinal ring is gradual and slow owing to the quantity of yolk present.

1b. *Embryonic Phase*

At 24.30 HPF, epiboly came to close and future embryonic axis conspicuous. A thin layer of cells were found to envelop the yolk (Fig. 2j). By 30 HPF, the assemblage of cells was most prominent in the anterior region of the embryo forming the brain rudiment (Fig. 2k) and the neural plate, appear as a layer dorsally at the axial end. A thickened median ridge also grew downwards along one side enveloping the yolk. By 33.30 HPF,

the anterior end further thickened as a head fold and the embryo extends as an elongated tube (Fig. 2l). Formation of optic bud started with the evagination of two lateral bulges. At this time onwards somites gradually become recognizable on either side of the embryonic axis. Heart was visible as a tube at the ventral part of the head at 38 HPF and the heart began to beat by 40 HPF. There were 12 pairs of somites in the 41 HPF embryo and it form the embryonic axis (Fig. 3a). At 41.30 HPF, the brain primordium, differentiation of the brain vesicles started, hollow otic vesicles are observed on the posterior part of the head. At 42 HPF, the two lobed heart, beats @ 86 min^{-1}. The optic primordia, which evaginates from the future diencephalon part of the brain primordium became visible. By 43 HPF, the cerebral region became more differentiated and otic vesicles were conspicuous and heart beated more rapidly(Fig. 3b). At this stage 17 pairs of somites were clearly visible. Head and tail were more distinct, projecting distinctly and the embryo covers almost 50 % of the yolk. Blood circulation to the caudal region was clearly visible and the muscles contracted at irregular intervals at 48 HPF. At 49.30 HPF, length of the embryo increased and eye lens was clearly visible (Fig. 3c).

By 52 HPF, the tail bud protruded away from the yolk. At 54 HPF, the optic primordium with lens placodes becomes enlarged and prominent, the otic vesicle are also discernible (Fig. 3d). The body of the embryo elongated and encircled almost 60% of the yolk and became 'C' shaped. The embryo also exhibit wriggling movements at times within the egg case. By 60 HPF, the embryo covers almost 80 % of the yolk and the anterio-posterior circulation of blood become conspicuous. By 64 HPF, embryo encircled 90 % of the yolk. Head region more prominent and a pair of otolith was observed in the ear vesicle. Heart beats became rapid and regular, counted as 128 min^{-1}(Fig. 3e). The embryo completely encircled the yolk by 66 HPF. The eye and lens have expanded in size and the head thickens due to brain growth. The forebrain, midbrain, and hindbrain regions became structurally differentiated. During 68 HPF, the yolk sac became transparent and stellate chromatophores and the oil globules became visible (Fig. 3f). By 70 HPF the muscles contracted more frequently and the egg case became thinner and ready to rupture dorsally at the cephalic end (Fig. 3g).

Hatching occurred at 73 HPF and the tail emerges out first (Fig. 3h,i). The embryo remain quite for sometime, within the broken egg shell and with the lashing and wriggling of embryonic movements, the opening get widened. At 78 HPF, the heart beated @ 138 min^{-1} and became more rhythmic and rapid. Slowly, the membrane near the head also gets separated and the hatchling becomes free from the shell membrane by 75-80 HPF (Fig. 4a,b). This process of hatching is protracted *i.e.* time interval between the rupture of egg membrane and emergence of hatchling is quite long. The tail is still curled and the head is bent around the yolk. Hatching of eggs in one brood is also protracted and is completed only in 24-26 hrs. In *E. maculatus* hatching takes place in 48 to 60 h (Bindu and Padmakumar, 2012) and 70 to 90 h in *O. niloticus* (Fujimura and Okada, 2007). The developmental stages of the pearlspot from fertilization to hatching were summarised as follows (table 1).

2. Larval Development

Larval development started after the hatching period and end by the yolk absorption (Fujimura and Okada, 2007). We compared the development of *E. suratensis* with other substrate-brooding cichlid like *E. maculatus* and mouth brooder cichlid *O. niloticus*. Early larval period was 4 to 5 days in *O. niloticus* (Fujimura and Okada, 2007) and 3 to 4 days in *E. maculatus* (Bindu and Padmakumar, 2012)

2a. *1 DPH* - Newly hatched larva (*LL* = 4.48 ± 0.103 mm)

The yolk was voluminous with large oil globules and the larvae being heavy sinks to the bottom. Yolk sac, ovoid and enormous, broader at the proximal end and narrow towards the distal end and the yolk extended almost upto the anal opening. The hatchling is characterized by large pigmented eyes and olfactory pits. Mouth and jaws were not fully developed at this stage (Fig.5a). Anal opening was seen located 4-5 somites below the level of yolk sac. Dorsal and

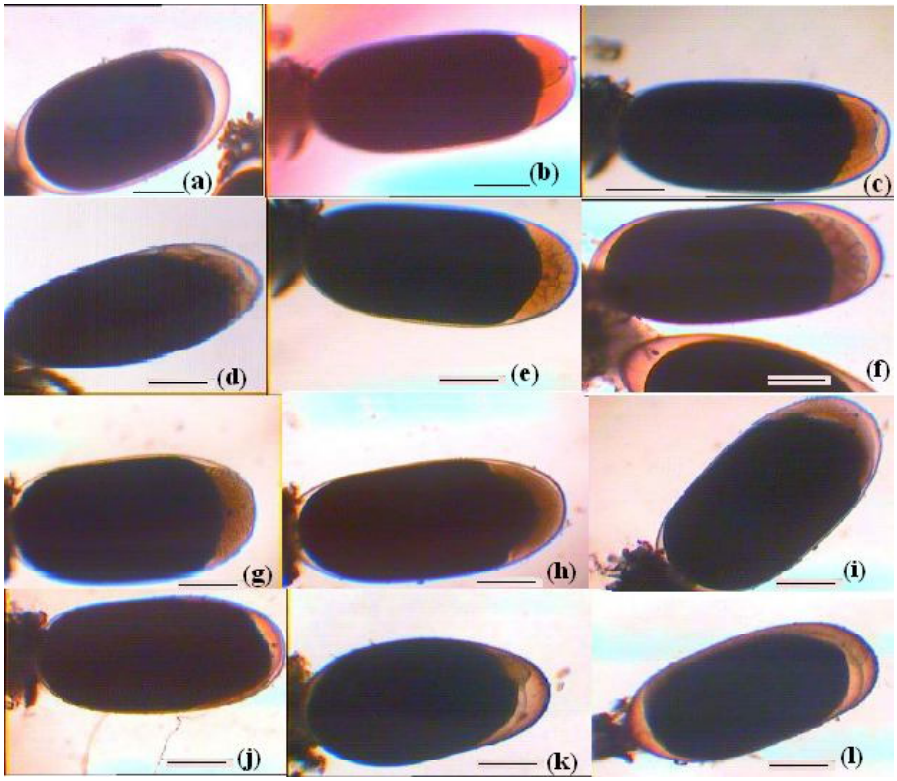

Fig. 2. Early development of egg (2a- l); 1 HPF (2a); 1.30 HPF (2b); 2 HPF (2c); 4 HPF (2d); 4.30 HPF (2e); 7 HPF (2f); 7.30 HPF (2g); 17 HPF (2h); 21 HPF (2i); 24.30 HPF (2j); 30.30 HPF (2k); 33.30 HPF (2l). (scale bar = 1mm)

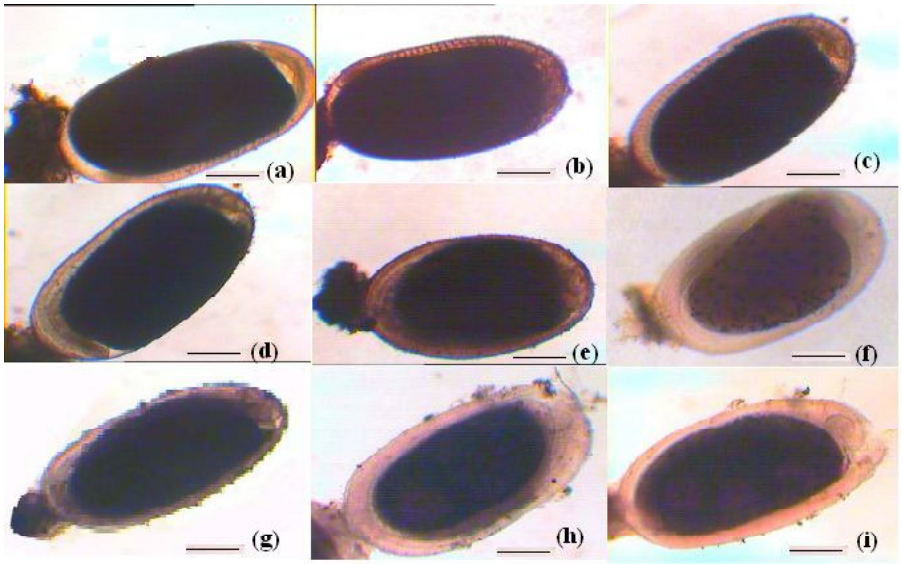

Fig. 3. Development upto hatching (3a-i); 41.30 HPF (3a); 43 HPF (3b); 49.30 HPF (3c); 54 HPF (3d); 64 HPF (3e); 68 HPF (3f); 70 HPF (3g); 73 HPF (3h,i) (scale bar = 1mm)

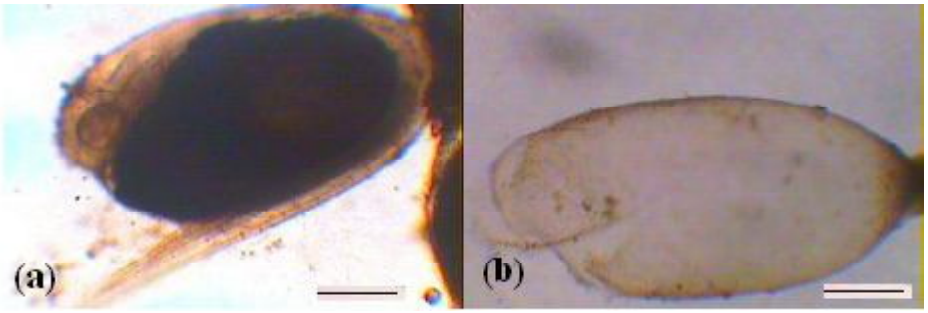

Fig. 4. Hatching (a); egg shell after hatching (b)

Table 1. Embryonic development in *Etroplus suratensis*

HPF* (h:min.)	Characteristics
0.00	Fertilisation
1.00	Blastodisc formation
1.30	Two-cell
2.00	Four-cell
4.00	Sixteen-cell
4.30	Thirty two-cell
7.00	Morula
7.30	Blastula
10.00	Late blastula
15.00	20 % epiboly
17.00	Early gastrula; 30 % epiboly
18.00	Gastrula
21.00	Neural plate formation
24.30	Head and tail bud formation
30.30	Slight enlargement of head region
33.30	Appearance of somites
34.00	Optic rudiment visible
38.00	Appearance of heart
40.00	Heart beat started
42.00	Head differentiation more conspicuous
43.00	Appearance of otic capsule
46.00	Ventricles of the brain was visible
48.00	Muscle contraction started; Blood circulation visible
49.30	Formation of eye lens
52.00	Tail became free
54.00	Olfactory capsule visible
64.00	Appearance of Otolith
68.00	Scattered chromatophores on the yolk
70.00	Egg membrane softened
73.00	Egg membrane breaks and hatching started
80.00	Hatching completed

*Hours of post fertilization

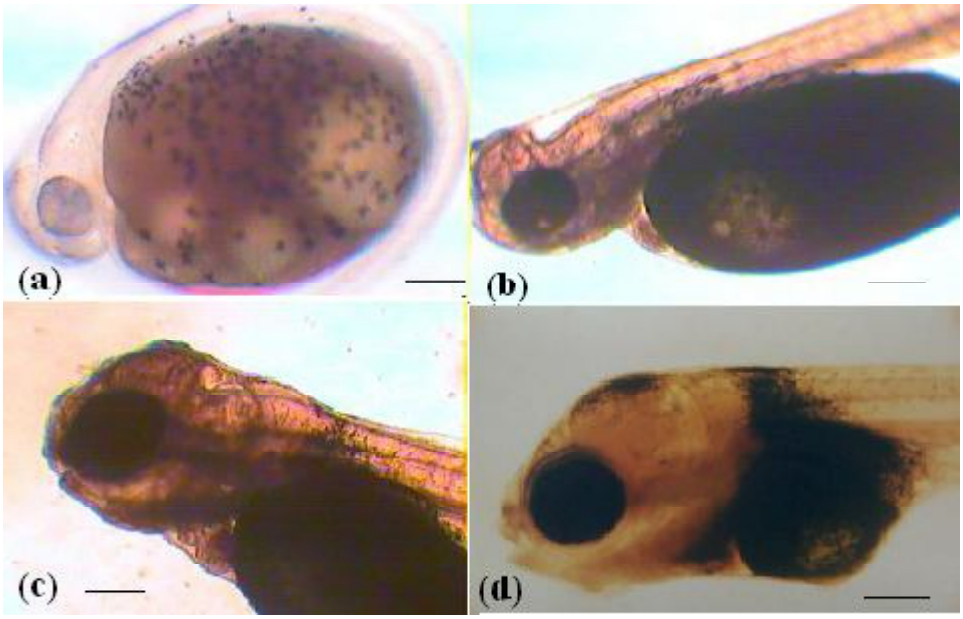

Fig. 5. Hatchling (a); 2-DPH (b); 3-DPH (c); 5-DPH (d) (scale bar = 1mm)

anal fin fold continuous. Pulsating, tubular heart located between the head and yolk sac was more prominent. The movement of red blood corpuscles through the circulatory system was better visualized. Caudal circulation through dorsal and ventral blood vessels and interconnecting canals were conspicuous. Notochord and myotomes were clear, with little ossification in the skeleton. A full complement of fins was also absent. The newly hatched larvae congregated in pits and bottom corners of the larval rearing tank, head down and tail up with lashing movements and apparently exhibited positive geotaxis and a negative phototaxis. By 16-18 h of hatching the head started to lift slightly from the yolk.

2b. *2 DPH* (*LL* = 4.98 ± 0.132 mm)

Yolk sac that remain attached to the head region of the larvae became separated and the head appear free from the yolk mass (Fig.5b). The yolk sac became gradually reduced and large eyes appeared deeply pigmented. Otolith was clearly visible within the transparent body. The two chambered heart located ventrally, anterior to the globular yolk, is observed to pulsate vigorously at 99 beats $min.^{-1}$. Mouth cleft appears during this period, although not with any marked mobility. At this stage, blood circulation was rapid and continuous, discernable through the transparent body. Dorsal fin fold also appeared continuous through the caudal end.

2c. *3 DPH* (*LL* = 5.84 ± 0.196 mm)

Head region was well developed with the formation of a characteristic spout like mouth(Fig.5c). Lower jaw began to exhibit rapid movements. Yolk sac became reduced to half its size and heart appeared spherical with rapid pulsation. The transparent body becomes pigmented gradually.

2d. *4 DPH* (*LL* = 6.12 ± 0.132 mm)

Gradually yolk mass decreases in size. There were more melanophores on the head, beneath the pharynx and trunk region. Alimentary canal became visible through the body, marked by intermittent pulsative movements. The larvae started gliding on the bottom and began to swim up with yolk sac down. Inflation of swim bladder started, but the larvae cannot float to the surface completely because of the yolk. Gills were almost completely covered by the operculam. In this stage the larvae began to move its jaws and

the young ones were found to congregate inside the artificial pits. Since the fins are not functional, larvae creep around the tank bottom and form gregarious patches (Fig. 6a).

2e. *5 DPH* (*LL* = 6.45 ± 0.085 mm)

Yolk absorption continued and the size of the yolk was smaller than the previous stage. Fin rays in the caudal region became conspicuous and the fin fold appeared continuous. More melanophores are concentrated on the top of the head and beneath the pharynx than earlier(Fig.5d). Pectoral fin buds were present beneath the operculam.

2f. *6 DPH* (*LL* = 6.66 ± 0.097 mm)

As more than 90% of the yolk was absorbed, the larva started foraging for food. Pectoral fin rays became conspicuous throughout the length of the fin fold. Operculum also became prominent. There were numerous melanophores in the trunk and tail region also. When kept in glass tank, inside the laboratory, the young ones were found to congregate near the aeration points and move in swarms.

As the yolk gets absorbed and the pectoral fins became active in 5-6 days the fry became free swimming in natural condition also. The yolk was fully absorbed by this time and the hatchlings attained an average size of 7.31± 0.31mm. The craniofacial morphologies were functionalized in the late larval and early juvenile period.

3. Nursing of juveniles

Juvenile development begins with yolk absorption and last until the first maturation of gametes (Fujimura and Okada, 2007). Here only the early periods of development were described. This period was characterized by the onset of exogenous feeding. The fin rays were more differentiated and fins became more functional during this period. The characteristic vertical bands on the hatchling appeared on the body when the hatchlings reached 15mm. In the fry nursing tanks, the young ones attains a size of about 20.01±0.62mm in one month and freely feed on zooplankton and powdered pellet feed. The larvae attained 30- 35mm, in two months and ready for stocking in the rearing ponds. The juveniles during this stage is characterized by the appearance of a black spot on the dorsal fin, which gradually disappeared as they attain a size of 45 to 50mm (Fig.6b). A similar 'tilapia mark' was observed in the juveniles of *T.niloticus* (Fujimura and Okada, 2007). In natural environment the food contain mainly filamentous algae. They were also fed with ground nut oil cake, rice bran and feed pellets. Growth performance of the larvae from hatching to juvenile stage is shown in figure 7. Differentiation of paired and unpaired fins, inflation of swim bladder, expansion of melanophores and maturation of intestine are considered as 'larval to juvenile' transition in cichlids (Fujimura and Okada, 2007).

4. Parental Care

E. suratensis exhibits a prolonged parental care till the young ones attained a size up to 40mm. In both tank and pond breeding, the parents plays a major role in determining the survival rate of larvae (Padmakumar *et al.,* 2012). During incubation, the major role in parental care was played by the male, which mainly involves fanning and mouthing the eggs. Similar to other substrate brooders such as *E. maculatus*, *Herotilapia multispinosa etc.* (Baylis, 1974; Smith-Grayton and Keenleyside, 1978; Bindu and Padmakumar, 2012), both active and passive fanning were observed. After fertilization, the parents aerate the eggs by fanning with their pectoral fins, which created a cooling effect to the clutch that reduced the damage of eggs. The unfertilized, dead eggs and dust particles were removed by the process of mouthing. The eggs hatched in 70-72 h, parents took the hatchlings in mouth and transferred to the breeding pit which was already prepared on the shallow pond bottom. Seven to fifteen pits of 3 to 10cm diameter and 2 to 7 cm deep were made by the parents after egg laying. In *Tilapia rendalli,* 5 to 24 nesting pits with a maximum depth of 6cm were observed (Ribbink *et al.,* 1981). During pit guarding also fanning and mouthing of the larvae continued. This mouthing helps the sticky larvae to remove the adhering substances and were shifted from one pit to another. In the pits the young ones remain attached by mucous threads of head glands, a characteristic feature of substrate brooders (Keenleyside, 1991; Bindu and Padmakumar, 2012).

Fig. 6. Hatchlings congregated in the tank bottom (a) young ones reared in captivity (b)

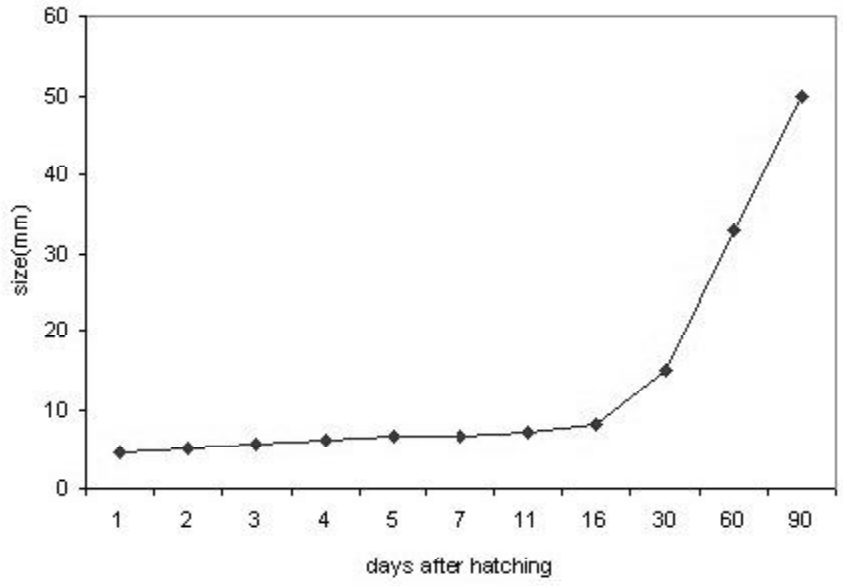

Fig. 7. Size attained by the young ones of *E. suratensis* in the experimental conditions

When the larvae became free swimming, the parents lead them out to the open waters. They move in shoals guided by the parents, swimming mostly underneath the parents. The youngones began to feed on food particles especially filamentous algae on or near the substrate. The turbulations on the tank/pond bottom created by the fin digging activity of parent fishes help in making available suspended particles as food for the young ones. The youngones also ingest the mucous from the parent body by regular biting called micronipping. This behavior is common in *E. suratensis* and *E .maculatus* even in the presence of other foods (Ward and Wymann, 1977). Protein, the major component of this parent mucous assist in the nutritional management of larvae (Khen and Chien, 2006). The high fry yield under protracted parental care is apparently linked to increased availability of particulate feed for the young.

CONCLUSION

The diversity of this species is at risk in the wild due to over exploitation and habitat disturbances like dredging and unethical fishing practices. Their stock can be improved through habitat improvement or restoration to encourage spawning and development of young by imparting protected areas to promote spawning and recruitment. Since the staging series is a useful standardization tool that provides accuracy in developmental studies, more researches are needed in the case of this valuable food fish.

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