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Breeding behaviour and embryonic development in the Orange chromide, *Etroplus maculatus* (Cichlidae, Bloch 1795)

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Original Article

Abstract

Etroplus maculatus, commonly known as Orange chromide, is an indigenous cichlid of the Peninsular India and Sri Lanka. They are asynchronous, substrate spawners showing biparental care. Breeding behaviour of this species including pair formation, nesting, parental care and spawning intensity were continuously observed in the laboratory conditions. *E. maculatus* lay 140 to 231 eggs per spawning and the mean size of fertilized egg was 1.6 mm. Hatching was recorded in 48 hr. of incubation (27^{0}C) and hatchling has a size of 3.9 mm. Yolk absorption was completed in three days and after that the larvae accept external food. Spawning interval was estimated based on observing isolated pair continuously for a period of three months in aquarium tanks. Hatching of the eggs was facilitated with/without parental care in the laboratory conditions. Embryonic and larval developmental stages were continuously monitored. By reducing the spawning interval, maximum number of seeds can be produced and utilized in the ornamental fish markets. This helps to overcome the difficulties during natural collections and also conserve the valuable natural populations.

Keywords: *Orange chromide, substrate brooder, biparental care, spawning interval, fanning, mouthing*

Introduction

The family Cichlidae comprises over 700 species, occurs in both fresh and brackish waters. Among the cichlid group, *Etroplus* is the only genus endemic to India and three species have been reported from Indian backwaters, viz., *E. maculatus, E. suratensis* and *E. canarensis*. *E.maculatus* and *E.suratensis* have potential for both food and ornamental markets. Popularly known as Orange chromide, *E. maculatus*, an omnivorous species, is widely distributed in almost all rivers and backwaters of Peninsular India and Sri Lanka (Jayaram, 1999). Its small size, bright orange colour and black spots on the body, calm nature etc., make them attractive candidates for the tropical aquariums.

In the backwaters of Kerala, *Etroplus maculatus* breeds throughout the year (Jayaprakas *et al*., 1979). It is a substrate spawner exhibiting high degree of parental care on eggs and larvae. Biparental guarding is considered as a primitive pattern of parental care as compared to mouth brooding seen among Tilapias (Balshine-Earn, 1997). There are many reports regarding breeding and parental care of this species in Sri Lankan waters (Ward and Wyman, 1977; Ward and Samarakoon, 1981; Samarakoon, 1981, 1983, 1985). Despite these reports, there is no account on the embryonic development of *E. maculatus* in Indian waters. Overexploitation of the wild stock due to its commercial value and pollution by chemical wastes from industries and agricultural paddy fields has threatened the very existence of this endemic species. The upstream Vembanad lake has also been attributed to persistent exposure to high concentration of agricultural pollutants, often higher than maximum allowable toxicant concentration (Sulekha, 2001).

This study aims to investigate the breeding behaviour, embryonic and larval development of *E. maculatus*. Understanding these aspects will largely benefit captive breeding programme, which are useful for mass production of seeds. The development and growth pattern can be used to assist in restoration of natural population and commercial exploitation using aquaculture practices and employment generation.

Material and methods

Brooders of *E. maculatus* were collected from the farm ponds of the Regional Agricultural Research Station, Kumarakom (9°37' N Lat; 76°25' E Long.) by cast netting. Captive breeding experiments were conducted in the laboratory during the period 2006-07. Fishes were stocked (approx. 12 each) in two large glass tanks (123 x 49 x 47cm) containing freshwater at a depth of 35cm. As the fish is categorized as nest builder with unique habit of parental care, breeding behaviour of Orange chromide was monitored in these tanks. Sand bottom was provided in the tank for facilitating the characteristic pit caring of the hatchlings. Among the stocked fishes, male and female with specific attributes gets 'attached' and a spawning pair is formed. The most prominent indication of the premating pair formation was the conspicuous intensification and darkening of color pattern in males. In the case of females, black spots and blotches appear on the ventral side between pelvic and anal fins, during spawning period. Replicated trials on breeding indicated that pairing occurred among 3-4 sets in each tank and pair selection was conspicuous.

From the fishes that formed breeding pairs, two pairs were transferred to two spawning tanks (60 x 30 x 30 cm) where breeding trials were done. Water was transferred twice a week and the brooders were fed two times daily with commercial pellets (*Higashi Fresh*) having 20% protein. Environmental manipulation was resorted by simulating natural conditions in the experimental tanks through periodic water exchange and providing nesting materials. Pebbles of size 5-10 cm were provided for egg attachment. Spawning frequency was observed under two conditions: 1) reducing the parental care period by periodic removal of eggs and fry from the caring parents 2) allowing parental care by keeping the young ones along with the parents itself. Breeding behaviour and development of the fertilized egg and larvae was monitored continuously using computer aided Magnus Imaging System. Spawning fecundity, period of incubation and hatching, fertilization and hatching rate, critical environmental factors such as pH and temperature were also monitored.

Results and discussion

Captive Breeding: When transferred to the spawning tanks, the paired fishes were found to spontaneously searching for nesting substrates. They cleared the surface of the nesting substrate using their spout like snout. The pair exhibited a characteristic territorial behaviour and in the culmination of courting process the female attaches the eggs one by one on to the substrate and the male fish spontaneously fertilize the eggs by sprinkling milt over them. The fish utilize even the glass surfaces of the tanks for egg fixing, approximately at a height of 15-17 cm above the tank bottom.

Even though mean fecundity in *E. maculatus* was reported to be 1,378 (Jayaprakas *et al*., 1979), in this study spawning fecundity varied between 140 to 231 per female. This is because of the 'asynchronous' development of oocytes in the ovary of *E. maculatus* and multiple batches of eggs were spawned successively within a spawning season as reported by Wallace and Selman (1981) and Nagahama (1983). The eggs of *E. maculatus* were pale yellow coloured, ellipsoidal in shape, with an average size of 1.6 mm, attached to the nest surface by a stalk. After spawning, both parents alternately guard over nest while the other leaves the territory to forage; their roles were reversed in every few minutes and this keep the pairs in good health (Perrone and Zaret, 1979). This time allocation for guarding nest is only half as compared to *E. suratensis* which spend 68-77 % of the time budget for guarding nest (Ward and Samarakoon, 1981). Incubation of eggs with parental care, provided high hatching up to 99.5% whereas in rearing tanks without parental patronage, hatching was reduced to 21.5% (Table 1). Survival rate of hatchlings in tank conditions were also rather poor presumably due to lack in parental care. This indicates that breeding of *E. maculatus* in laboratory conditions with parental patronage is an appropriate technology for their mass seed production.

Rapid removal of eggs and young ones from the spawning tanks and rearing them in separate systems possibly have reduced parental care period. In the present study it was clear that this time interval between two successive spawning can be minimized by periodic removal of the eggs and hatchlings from the caring parents, and within three months nine spawning were observed from an isolated pair (Table 2). The time interval has reduced about 2 weeks, when the hatchlings removed while the same was further reduced to one week in the case where eggs were removed. Lee (1979) reported that parental care suppresses expression of full reproductive potential and fry removal shortens the time interval between spawning. The spawning frequency and fry production can be increased by the frequent removal of incubating eggs

Table 1. Breeding of *Etroplus maculatus* in the glass aquarium tanks

Breeding variables	Range	Mean
Total No. of eggs per nest	140 - 231	183
Fertilization (%)	90.0 - 100	95.0
Hatching % (with parental care)	97.0 - 99.5	98.0
Hatching % (without parental care)	21.5 - 50.0	33.8
Water temperature (^{0}C)	26.0 – 29.0	27.0
Water pH	07.0 - 07.5	07.3
Incubation period (hr)	47.00 -48.30	48.00

and freshly spawned hatchlings (Peters, 1983; Verdegem and McGinty, 1987). Legendre and Trebaol (1996) observed a similar condition in mouth brooding cichlid *Sarotheradon melanotheron*. Whereas in larger tanks, in which the larvae were allowed to live with the parents, spawning occurred after 5-6 months. In natural conditions, the parental care continued until they are ready to breed again. Even after the brood reaches free swimming stage, parental protection and constant vigilance prevailed as in the natural environment (Ward and Wyman, 1975, 1977), whereas in *E. suratensis*, this spawning interval is comparatively short (Bindu, 2006). Since gonad development is inhibited during parental care,

Table 2. Spawning intensity in two isolated pairs of *E. maculatus* by periodic removal of egg and larvae

No. of Spawning	Trial I		Trial II	
	Spawning interval (days)	Stage*	Spawning interval (days)	Stage*
1st	00	+	00	+
2nd	13	+	16	+
3rd	15	+	14	√
4th	16	+	09	√
5th	11	√	10	√
6th	09	√	09	√
7th	07	√	14	+
8th	08	+	16	+
9th	18	--	16	--

*Stages removed : Eggs (√) and Larvae(+)

the spawning interval is considerably prolonged. The observations in this study support the hypothesis that removal of eggs or fry from the caring parent helps to reduce the spawning interval. In contrast to the polygamous fishes, like centrarchids, male-female association in monogamous pairs does not break after spawning, it continues during the entire parental care period (DeWoody and Avise, 2001). It again confirms that in fishes like *E. maculatus*, the same pair bond is maintained throughout the sequential breeding attempts and this helps to avoid the costs associated with searching for a new mate. This technique of breeding of Orange chromide is thus of immense importance in the context of high demand in the ornamental fish trade.

In *E. maculatus* the embryos kept without parents hatch later (Zoran and Ward, 1983) and also heavy infestation with fungal mat resulted in large scale mortality of eggs and poor hatching (Takahashi *et al.*, 2004). Whereas a more synchronized hatching was apparent when facilitated with parental patronage. In the experimental conditions fungal infections can be avoided by providing continuous aeration and increasing the salinity of water up to 5 ppt.

Breeding behaviour: Breeding behaviour of *E. maculatus* is unique and involved a series of events such as pairing, nest making and parental care. The most prominent indication of pre-mating pair formation was darkening of colour patterns. As a monogamous species sex differentiation is possible only during the breeding period. Just prior to spawning, the males become deeply coloured and the dark blotches are present along sides of the body become strongly marked. Spots are very much clear in both the fishes during spawning period. The black belly signal in *E. maculatus* has a visual communicative and synchronizing function and that directs its mate during spawning (Zoran and Ward, 1983). Male coloration intensifies during courtship and these colour patterns are very important channel for communication in cichlids (Nelissen, 1991) and are attributed as an important component of the specific mate recognition system in cichlids (Ribbink, 1990).

The breeding pair then started searching for a suitable substrate for nest making, which is then cleared off by the pair. Both partners are actively engaged in nest preparation. During ovulation, the female attached sticky eggs, one by one by pressing on to the substratum (Fig.1a). The male fish fertilizes them instantly by releasing a spray of milt and this process is continued several times. The small pebbles in the tank and also the tank wall was selected for fixing the eggs (Fig. 1b and 1e). The process of spawning is completed in about 30 minutes. Ovulation is accompanied by the production of a sticky material, mucopolysaccharides, by which eggs are attached to the substrate (Nicholls and Maple, 1972; Baroiller and Jalabert, 1989) and is characteristic to substrate spawners. In the large experimental tanks, after egg fixation, mainly the female parent fish, excavated small cup like pits on the sand bottom, just below the selected nesting substrate, using her mouth. After spawning both parents alternately cared the eggs by fanning and mouthing (Fig.1c and 1d), and also engaged in protecting the territory.

Fanning helps to remove metabolic waste from the egg surface and also increase the oxygen level near the eggs. The fanning

Fig.1a. Egg fixing on the wall of the glass tank

Fig.1b. Eggs on aquarium wall

Fig.1c & d eggs are alternatively cared by the parents. Fig.1c. Female

Fig.1d. Male

Fig.1e. Eggs attached on substratum

Fig. 1(a – e). Breeding of *Etroplus maculatus*

parent holds position close to the clutch and moves the water with large amplitude beats of pectoral fins. *E. maculatus* exhibit both active and passive fanning. In the former both pectoral fin and caudal fin beats vigorously to oxygenate the eggs, whereas in passive fanning pectoral fin only beats slowly, compensatory caudal fin movements are absent (Zoran and Ward, 1983). The guarding female occasionally places its mouth gently against the eggs and sucks away the adhering particles. This 'mouthing' helps to clean the eggs. Dead and fungus ridden eggs are removed by a more vigorous mouth contact (Keenleyside, 1991). This process continues till hatching and the eggs hatched out generally in 48 hours. However, when the brood was disturbed, the parent fish was found to devour the developing eggs.

Embryonic and larval development: Embryonic development completed in 48 hours after fertilization (Fig. 2a – 2l) and the details are presented in Table 3. Complete hatching occurred in 8-12 h. There is a positive correlation between egg size and parental care duration. In species having larger eggs, larger proportion of the total caring period is invested into pre-hatching care as the egg require more time for development (Gillooly et al., 2002; Kolm *et al.*, 2006).

During hatching, most of the hatchlings remained attached to the substrate itself. They were picked up by the female in mouth and transferred to the nursery pits on the sand bottom of the tank. As the hatchlings were fully transferred,

Table 3. Embryonic development in *Etroplus maculatus*

Time after fertilization(hr)	Developmental stages
0.00	Fertilization
0.15	Formation of two blastomeres ; egg size 1.6 mm
0.45	Four blastomeres
1.15	Eight blastomeres
1.35	Formation of morula
1.50	Multilayered blastula appears ; egg size 1.65 mm
2.15	Cell division continues; become irregular and indistinct mass
6.30	Gastrula stage; The germ ring migrated equidistantly around the yolk and covers half of it
21.00	Yolkplug projects; rudiment of the head lift
24.45	Embryo covers 50% of the yolk mass. Ten somites are visible
26.45	Heart is visible
28.45	Optic rudiment very clear, cerebral vesicles more prominent; egg size 1.7mm
29.30	Two chambers of the heart is clearly visible; tail rudiment become more prominent; 17 pairs of somites are visible
30.00	Heart beat starts
30.30	Heart beats *@ 75 min^{-1}.
32.40	Embryo covers 60% of the yolk and become 'C' shaped. 19 to 20 pairs of somites are visible. Tail region become free. Heart beats @ 96 min-1.
33.55	Blood streaming through the ventral region to the caudal region is visible
35.15	Tail region become much elongated. Movement of the embryo started
36.45	Cerebral region become well developed, number of somites increased.
37.45	'C' shaped embryo covers 70% of the yolksac
39.15	Vigorous tail lashing. Embryo encircles almost 80% of the yolk
39.35	Heart beat @ 122 min^{-1}.
41.25	Embryo covers 85% of the yolk
43.00	Embryo encircles 90% of the yolk
43.40	Heart beats @ 133 min^{-1}.
48.00	Hatching started and whole eggs hatched out in 8-12h

*@ = at the rate of

the female actively engaged in 'pit guarding' and closely care the deposited young ones in the pits. In tanks without sand bottom, the hatchlings sinks to the bottom and congregated

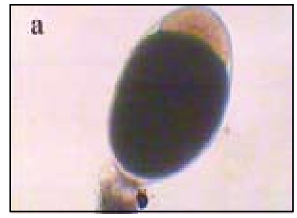

Fig. 2a 3hr old

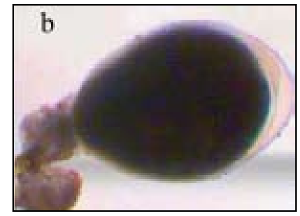

Fig. 2b 21hr old

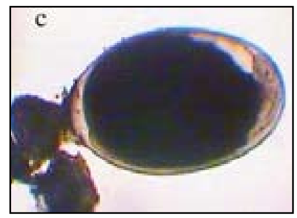

Fig. 2c 26.30hr

Fig. 2d. 30.30hr

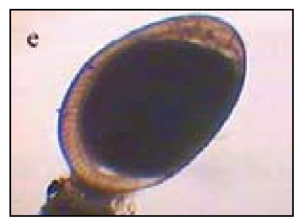

Fig. 2e. 33.45hr

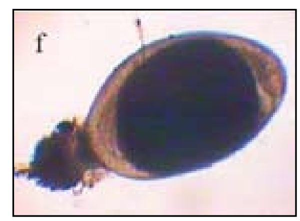

Fig. 2f. 37.30hr

Fig. 2g. 41hr

Fig. 2h. 42.30hr

Fig. 2i. 48hr

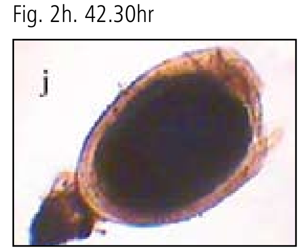

Fig. 2j-l Different stages of hatching

Fig. 2k.

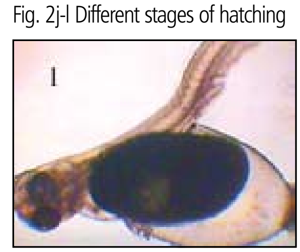

Fig. 2l.

Fig. 2(a – l). Embryonic development in *Etroplus maculatus*

Table 4. Stages of larval development in *Etroplus maculatus*

Stages	Avg.Size (mm)	Morphological description
Newly hatched larva	3.9	The hatchlings appear slender and transparent. The yolk is voluminous with large oil globules and the yolksac is ovoid with a length of 1.25mm The hatchling is characterized by large pigmented eyes and olfactory pits. Mouth and jaws are not fully developed at this stage. Heart is prominent and is seen pulsating, located between the head and yolksac, ventral to the eyes. Caudal circulation through dorsal and ventral blood vessels and interconnecting canals is conspicuous, and is visible through the transparent body (Fig. 3a).
1 day (24 hr.) old Hatchling	4.9	The head region that attached to the yolk sac appeared free from the yolk mass and the size of yolk reduced to 1mm. Mouth cleft appears during this period, although not with any marked mobility. The pulsating, two chambered heart located ventrally anterior to the globular yolk and the streaming larval blood circulation to the caudal fin became perceptible ventrally at this stage. Eyes are large and highly pigmented. Dorsal and caudal fins developed. Continuous fin rays are developed. (Fig. 3b)
2 day (48 hr.) old Hatchling	5.3	Yolk sacs become reduced to half its size and heart appear spherical with rapid pulsation. Melanophores become conspicuous on the body. Gill movements visible, Jaws are movable. Pectoral fins developed and show vigorous movement. (Fig. 3c)
3 day (72 hr.) old Hatchling	6.0	Yolk absorbed completely. Upper and lower jaw becomes well developed with characteristic jaw movements. Pigmentation becomes remarkable on the head and trunk region. Operculum and pectoral fins highly developed and functional. Caudal fin rays are clearly visible. (Fig. 3d)

in corners, head down and tail up with lashing movements. In the larger tanks, they remained in the pits attached by mucous threads from the head glands and were transferred from one pit to another by the parents (Keenleyside, 1991). Occasionally, a few wrigglers were picked up by the parents, rolled out in the mouth and returned to the pits. This process probably helps to cleanse the sticky larvae by removing the adhering particulate matter. During this period also the parent fish continue fanning of pits with their fins and render oxygenation to the hatchlings that were sheltered in the pits.

Details of larval development in *E. maculatus* is summarized in Table 4. The fry became free swimming from the 4th day onwards and move in shoals guided by the parents, swimming mostly underneath the parents whereas the young ones were found congregated near the aeration points as swarms in the

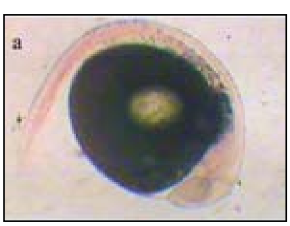

Fig. 3a. Hatching

Fig.3b One day old larva

Fig.3c Two day old larva

Fig.3d Three day old larva

Fig. 3(a-d). Larval development in *Etroplus maculatus*

tanks without parental care. During this period, the movement of larvae was largely limited within the territorial limits and returned to the pit nurseries while disturbed. Individual fry that stray from the brood were orally retrieved by the parent and brought back with the others.

Like other cichlids, micronipping, a regular feature of foraging by the fry through parent contacting, common in *E. maculatus* (Ward and Wyman, 1977). In the experimental tanks where fry and brooders are kept together, young fish regularly bite at the parent's body. By this the fry ingest mucous from the body of the parent even in the presence of other foods and the parent's epidermal mucous production increased during this period (Hildemann,1959) and is demonstrated that taste was also a way of communication in cichlid species. Since the major component of parental mucous is protein, apparently this assists in nutritional management of broods and larviculture (Khen and Chien, 2006). Longrie *et al*. (2008) reported acoustic communication in *Oreochromis niloticus* during the period of extensive parental care. With the vigorous beats of pectoral fins also, the fishes stir up loose materials for feeding the fry. Communication of parents with the young ones through signaling by jolting movements and 'fin flicking' evolved spontaneous response in the young ones.

The information obtained on breeding behaviour in *E. maculatus* from the present study reveals that periodic removal of eggs and fry from the caring parents is helpful to increase the breeding intensity, even though fecundity and actual yield of hatchlings per brood is low. This would help to reduce the spawning interval and produce more seeds within a short period for commercial exploitation in ornamental fish trade and thereby conserve the valuable natural population.

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