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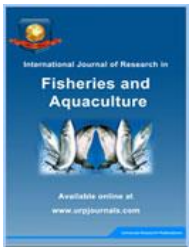

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Original Article

Breeding and early development of the nest building gourami *Trichogaster trichopterus* (Family Osphronimidae)

Bindu L.*, Amina. S, Linsha. R, Sreelekshmi. G, Archana. P. L

Post Graduate Department of Zoology, M.S.M.College, Kayamkulam, Kerala, India – 690502.
(* Correspondence Email, bindukylm@gmail.com)

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Abstract

The three spot gourami, *Trichogaster trichopterus* is a high valued ornamental fish remarkable for its nest building behaviour. This study was designed to evaluate the breeding and early development of blue and gold varieties of *T.trichopterus*. Since they build bubble nest for egg deposition, plantain leaf was provided as substrate for nest building. The average fertilization and hatching rates were observed 99.6% and 95% respectively. The hatching period was observed to be 24h at water temperature 26-27^{0}C. The diameter of fertilized egg varied between 1.37 and 1.45 mm. Hatchling size varied between 2.5 and 2.75. Yolk was completely absorbed by the 4th day and they were fed with pellet feed. In backyard rearing tanks, they attained sexual maturity in ten weeks.

Key words: *Trichogaster trichopterus*, nest builder, ornamental fish, breeding, hatching, larval rearing.

1. Introduction

Gouramis commonly known as labyrinth fishes belong to the suborder *Anabantoidei*. They are remarkable for air-breathing behavior and over one hundred *Anabantoids* are currently traded in the tropical fish industry. *Trichogaster trichopterus* is the naturally occurring colour morph of three spot gourami and it is considered as a good community fish (1). They fetch high price even in the local market itself due to their brilliant coloration. The species is omnivorous and feeds mainly on zooplankton, macroinvertebrates and occasionally on terrestrial macrophytes and detritus (2,3). Since mosquito larvae is the favoured food of most of these fishes, we can effectively use them for biocontrol of mosquito. In addition to this, very active and compatible nature make them a suitable candidate for our aquariums.

They are well known for their fascinating reproductive behaviour, including bubble nesting and parental care. Blue and gold varieties of *T.trichopterus* have high demand in the ornamental market. So this species is selected for studying the breeding behavior and production potential of nest building fishes. Earlier studies on this species focused on its growth and reproduction (4,5,6). But there is only limited information on the breeding and early development of this species from India. The proposed study aims to evaluate the breeding protocols and early development of this species under laboratory condition.

2. Materials and methods

We can identify the male and female fishes of gourami from a very young age. The dorsal fin of male is elongated, pointed and reaching beyond the caudal peduncle while in female it is short and rounded. Since they are monogamous in nature, one female with conspicuously swollen belly and one healthy male forms a breeding pair. Blue (n=12) and gold (n=12) varieties of *T.trichopterus* were maintained in separate tanks (male and female separately) during the study period. All fishes, ranged from 3.0 to 9.5 cm, were of breeding or near-breeding age when paired. During the study period (2013-2014) three breeding trials were conducted for blue gourami (BT_1, BT_2, BT_3) and two for gold variety (GT_1, GT_2). In the present study male and female fishes were placed together in separate glass tanks (60 x 30 x 30 cm) filled with freshwater at a depth of 23cm. As the fish is categorized as a nest builder with unique habit of parental care, their breeding behaviour was monitored in these tank. The fish was allowed to breed naturally in the tanks. Plantain leaf (20cm×18cm) was provided in each tank for facilitating the characteristic bubble nest building. Bubble nest formation, breeding behavior, egg laying, hatching and hatchling development were closely monitored. No aeration was provided in these

Table 1. Details of Blue and Gold colourmorphs of *Trichogaster trichopterus* selected for breeding trials

	Male				Female			
	Total Length (cm)		Total weight (g)		Total Length (cm)		Total weight (g)	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Blue gourami	6.5-7.5	6.95 ± 0.438	2.51-4.304	3.437± 0.8231	5.5-6.5	5.92± 0.421	2.181-3.391	2.912± 0.519
Gold gourami	5.2 -9.5	6.84± 1.624	2.181-9.9	4.304± 3.0554	5.0-6.5	5.83± 0.623	1.99-3.391	2.719± 0.550

Table 2. Breeding of *Trichogaster trichopterus* in laboratory conditions

Parameters	**Blue Gourami**			**Gold Gourami**	
	BT_1	**BT_2**	**BT_3**	**GT_1**	**GT_2**
Length of fish (cm) Female Male	 6.5 7.5	 7.8 10	 7.0 6.5	 5.5 9.5	 6.5 9.5
Weight before breeding (g) Female Male	 3.391 4.304	 6.24 5.8	 5.0 4.3	 3.065 9.9	 3.281 9.7
Weight after breeding (g) Female Male	 3.002 4.101	 5.82 5.3	 4.24 3.82	 2.923 9.3	 3.003 9.2
No. of eggs	1050	1300	1100	800	900
Size of eggs (mm)	1.3	1.4	1.3	1.4	1.4
Fertilization rate (%)	100	98.8	99	100	100
Hatching rate (%)	98	98.5	98	89	91
Incubation period	24	22	22	24	23

Table 3. Water quality parameters (mean ± SD) in *Trichogaster trichopterus* breeding

Parameters		**Blue Gourami**		**Gold Gourami**	
		Breeding tank	**Hatching tank**	**Breeding tank**	**Hatching tank**
p^H		7.33±0.288	7.33±0.288	7.16±0.288	7.166±0.288
Temp. (^{0}C)	(air) (water)	28.33±0.577 25.33±0.577	29±1.0 26.33±0.577	29±1.0 25.66±1.154	29±1.0 26.33±0.577
Salinity (ppt)		0.197±0.036	0.204±0.083	0.155±0.063	0.197±0.036
DO		3.46±0.462	4.53±0.46	4±0.8	4.53±0.462

tanks. After breeding the female fish was removed first and after two days male also transferred from the spawning tank. Among the trials BT_3 was conducted by utilising the 10 week old young ones of BT_1. Fertilization and hatching rates were calculated according to Lagler (7). Development of the fertilized egg and larvae were monitored under a trinocular microscope (Almicro) at 10x magnification. Egg diameter was determined to the nearest 0.01mm by averaging the measurements of at least 10 eggs. Developments of the hatchlings were documented using a camera (Sony, Japan) connected to the microscope. One week after hatching, larvae were transferred into separate tanks for further rearing. They were fed with commercial pellets containing 25% protein (*Meenoot*, Kerala Agricultural University). After ten weeks their growth and maturity were assessed. The water quality of the breeding and rearing tanks were monitored using standard methodology (8).

3. Results

3.1. Breeding

Mature males having an average size of 6.84±1.624cm (4.304±3.055g) and females having 5.83±0.623cm (2.719±0.550g) were observed to be suitable for breeding (Table 1). Male started to build a bubble nest on the surface below the plantain leaf in the first three to four hours. This was followed by natural spawning and fertilization. Usually spawning occurred after a latency period of 18 to 20 hours and in two trials on the third day of stocking. The fertilised eggs were spherical, yolked and golden yellow in colour. The diameter of mature eggs varied between 0.8 and 1.0 mm. It is difficult to distinguish the eggs from the nest. No aeration was provided in the tank. The male became very aggressive after spawning and the female fish always stayed away from the nest and this help to protect herself from damages. At this time, the female was carefully removed from the tank.

Table 4. Larval development of *Trichogaster tricopterus*

Days after hatching	Size (mm)	Morphological Description
Newly hatched larva	2.5	Transparent embryo with dark eyes, mouth not visible, pulsating heart is seen on the anterior side of the yolk ventral to head region, auditory vesicles visible, stellate chromatophores scattered throughout the body.
1	2.9	Size of yolk reduced, caudal fin visible, mouth visible, a three lobed pattern of yolk, oil granules visible, chromatophores arranged in the body in a linear pattern
2	3.4	Size of larva increased, yolk reduced , mouth and opercular movement faster, pectoral fin movement visible
3	3.7	Mouth stronger and jaw movement faster, rapid pectoral fin movement, caudal fin rays are visible, size of yolk sac reduced, size of oil globules also highly reduced.
4	3.9	Yolk completely absorbed and the larva started to feed external food
5	4.0	Size of the larvae increased, chromatophores concentrated on the dorsal body surface.
6	4.1	No yolk, auditory chamber visible, jaw bones stronger, intestine well developed.

The spawning fecundity, fertilization and hatching rate of the two varieties were provided in table 2. Spawning fecundity of blue gourami was higher than that of gold gourami. In BT_3 the fishes utilized were ten weeks old and were produced in the laboratory itself by breeding the BT_1 fishes. Hence spawning fecundity, fertilization and hatching rate were lower than BT_1 and BT_2. Temperature, pH, salinity and dissolved oxygen (DO) of the breeding and rearing tanks were provided in Table 3.

3.2. Breeding behaviour

After introducing mature fishes into the tank the male *T. trichopterus* built up bubble nests under the floating plantain leaf (Fig.1). It was the first indication of readiness displayed by the male. The male gulped air and expelled it as a bubble coated with mucus towards the water surface, often below the floating plantain leaf. This process was repeated several times and all the bubbles adhered together to form floating bubble nest. When nest building finished, fish was ready to mate. Intensification of coloration in males also indicated its readiness and it started to chase the female. The male initially stroked on the ventral side of the female with his dorsal fin and then wrapped the female with his body to exert pressure on the belly to expel her eggs. Spawning occurred simultaneously and male fertilized the eggs. The eggs were lighter and the male picked up those eggs that sink and those that have floated outside the nest in its mouth and placed into the nest. Eggs were adhered to the nest up to hatching. During this time the male fish was highly aggressive towards the recently-spawned female. So the female fish was removed from the tank in order to avoid the attack of male. After the young fish leave the nest, the male ceases care.

Figure 1. Male *T.trichopterus* building bubble nest

3.3. Early development

Diameter of fertilized egg varied between 1.37 and 1.45mm (1.405±0.031mm). Heart beat and twitching movement of the embryo started 3 to 4 h and 2 to 3h respectively before hatching (Fig.2). The eggs were hatched in about 22 to 24 hours and the hatchlings remain in the nest itself. Size of the hatchling varied between 2.5 to 2.75 mm (2.573±0.085 mm), retained the remnant yolk (Fig.3 & 4). The hatchlings were slender and transparent with unpigmented eyes and without a distinct mouth (Table 4). They appeared swimming in the water column with their attached spherical yolk sac on the ventral side. Pigmentation appear on the dorsal portion of the head from the second day onwards. As the hatchling development proceeds, the yolksac gets fully reabsorbed by the fourth day.

After the absorption of yolk, the young fry were fed with freshly prepared egg custard during the first 4 days and then with pellet feed. One week old gourami was reared separately in outdoor tanks. They attained a size of 4.37±1.265cm (1.097±0.733g) when fed with commercial pellets @10% of body weight. In the present study ten weeks old young ones attained first maturity at ten weeks (Fig.5), when reared in outdoor silpolin tanks.

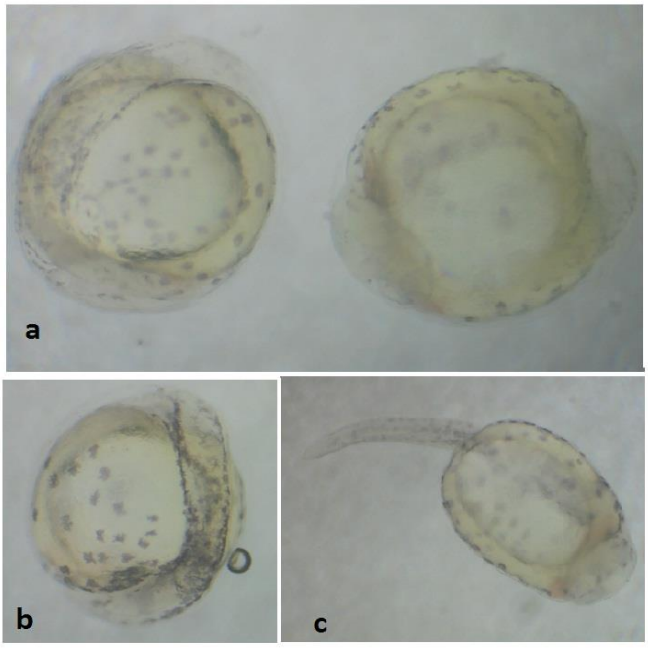

Figure 2. Developing eggs (a&b) and hatching © in *T.trichopterus*

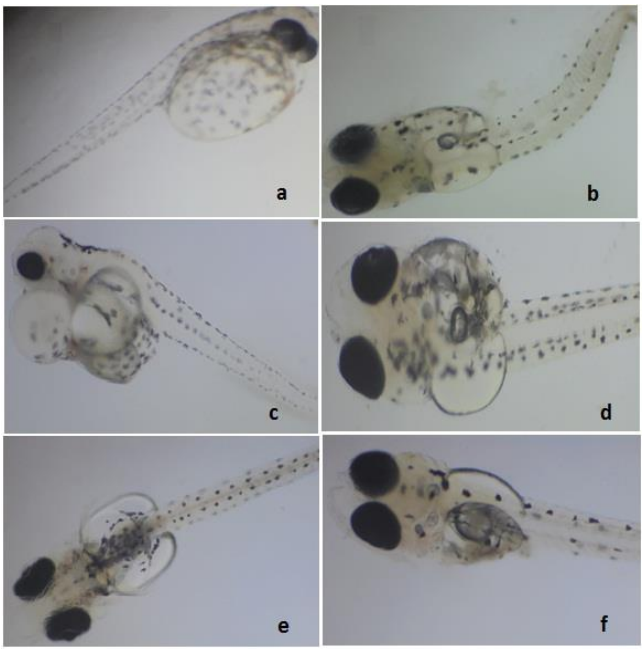

Figure 3. Larval development in *T.trichopterus* (a) hatchling (b)one day old (c) 2 day (d) 3 day (e) 4 day and (f) 5 day old larvae

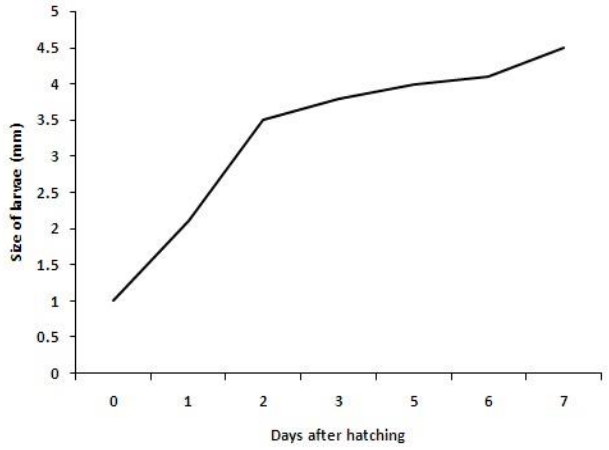

Figure 4. Post embryonic development of *T.trichopterus* in the first week of hatching

Figure 5. Ten weeks old *T.trichopterus*

4. Discussion

Most of the anabantids are exported from India as very good ornamental fishes (9). Breeding behaviour, spawning, embryonic development, hatching and larval development of *T.trichopterus* resembles other members of anabantidae with slight variations. Observation on the mating and spawning behaviour of this species shows that the fish was a broadcast spawner and the scattered eggs were collected and deposited in the bubble nest by the male. It is capable of year round spawning in its natural habitat depending on prevailing conditions, with temperature and day length being the two main reproductive cues (10). *T.trichopterus* was reported to attain maturity at 12-14 weeks of age and a maximum length of 15-20 cm (11). The species breed at temperatures between 18 and 29°C (12) with spawning enhanced in acidic water with a pH range between 5.5 and 6.5 (13) where as in the present study pH was slightly above this range. Male *T. trichopterus* are territorial, bubble nest builders and exhibit complex behaviours, associated with establishment and defense of reproductive territories (14,15). Similar to this other anabantids like the paradise fish, *Pseudosphromenus cupanus* (16) usually make their nest among floating or emergent vegetation. Since aeration will impede construction of the bubble nest, the containers are without any type of aeration.

Degani (17) reported that nest building and reproductive behaviour are at its peak under low light conditions. In the present study all the experiments were conducted in glass tanks and in day light. The nest is the focus of the territory and serves as a protective, oxygen-rich environment for fertilised eggs after spawning. The aggressive behaviour of male was evident with the intensification of colouration, erected median fins and vigorous body movements and tail beating. Male and female of equal or near-equal size were paired in order to prevent excessive aggression and lessen the danger of infection and mortality. The male guide a female into the territory under the nest with slow movements around the female and frequent parallel alignment, usually with depressed fins (18). The nest advertise the reproductive fitness of the male and the female lays her eggs according to the size of the nest (19). The water in which male builds the nest contain steroid glucuronidis which, promote maturation in females. In female ovarian development was asynchronic and the final oocyte maturation will occur only in the presence of the male (20). The male then tends the brood for several days and retrieves eggs and fry that drift from the nest (21, 22,18). During the post spawning and nursing phase, the male becomes highly aggressive towards other fishes including the recently-spawned female. Usually the male continues to maintain the nest and during this period will court and spawn with other ripe females (22, 23). The specialised nesting and parental behaviour enhances early fry survival and recruitment.

Spawning fecundity in *T.trichopterus* is reported up to 1000 (24), and others up to 4000 (25). The fecundity observed in the present study comes under this range or slightly above and more number of eggs were produced by larger females. Combined with multiple spawnings, this

enables rapid population growth. In the present study hatching time varied between 22 and 24 similar to that of *T.pectoralis* (26). The newly hatched larvae of *T.pectoralis* was 2.73±0.02mm similar to that of *T.trichopterus* in the present study. Using feed with 25% protein they attained better size and maturity earlier than the previous reports (8). *Trichogaster* spp. fed with formulated diets (6, 27) showed better growth and survival. By providing high protein feed we can improve the growth and breeding potential of this economically important ornamental fish for the commercial market. There was not much variation in the behaviour, spawning and larval rearing of blue and gold populations of *T. trichopterus.* Since they are easy to breed this technology can be adopted by the women entrepreneurs as an income generating activity and offer immense scope for the upliftment of rural women.

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