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Breeding of an ornamental fish - black skirt tetra (*Gymnocorymbus ternetzi*, Boulenger, 1895) and study of its embryonic development

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Abstract
The aim of the study was to breed the Black Skirt Tetra under controlled conditions and also to study its embryonic development. The experiment was successfully conducted during the months of July - December, 2023. It was found that the fish bred during the dawn and courtship behaviour lasted for 3 hours, which lead to the release of eggs. The fertilized eggs were slightly adhesive, demersal and spherical in shape. The egg capsule was transparent while the yolk was brownish yellow. The eggs were incubated at a temperature of 24±1.0°C and embryonic development was recorded at every interval and was categorized into seven main stages-zygote, blastula, gastrula, segmentation, pharyngula, pre-hatching and hatching. The eggs finally hatched at 21:00 hours. The results of this study can give a better understanding of the breeding behaviour and the embryonic development of other similar commercially important ornamental fishes as well.

Keywords: *Gymnocorymbus ternetzi*, breeding, embryonic development, hatching

1. Introduction
Ornamental fishes really are nature's wonderful creation. Ornamental fish keeping is the second most preferred hobby in the world and the number of hobbyists for ornamental fish keeping is rising day by day because it provides great opportunity for entrepreneurship development and income generation [16]. Ornamental fishes, due to their different and brilliant colours, shapes and behaviour, are often referred to as living jewels and are kept in aquaria or garden pools for their beauty as well as entertainment [8]. The rearing of Ornamental fishes is an activity which provides not only aesthetic pleasure but also financial openings. This has resulted in a steady increase in its trade globally. Global trade in ornamental fish has become a multi-billion-dollar industry, with the entire value of the industry inclusive of retail sales, associated materials, wages and non-exported product being estimated at around US $15-30 billion per year. Some 55% of the global market supplies originate from Asia, where the sector contributes significantly to the national economies of the countries involved [6].
Characidae is a family of freshwater subtropical and tropical fish, found in Southwestern Texas, Mexico, Central and South America and it is a large family that comprises about 152 genera and 776 species [12]. The true Characins, family Characidae, are found in Africa and more abundantly in South and Central America [7]. The *Gymnocorymbus ternetzi* (Boulenger, 1895), commonly known as Black Skirt Tetra, is one of the fishes in the group of tetras.
Fishes exhibit the most diverse reproductive and developmental strategies. Breeding of *Gymnocorymbus ternetzi* can usually be done naturally by stimulating natural environmental conditions and can easily breed in aquarium. They are egg scatterers and the adults may eat their own eggs. The most important environmental factor that affects spawning and egg hatching is temperature. In the commercial development of freshwater ornamental fish culture, provision of appropriate temperature for egg incubation and further development of the fish at various stages is an important aspect in successful attainment of seed production [9]. The embryonic phase refers to different stages in eggs which include fertilized egg, cleavage, morula, blastula, gastrula, embryonic body formation, optic vesicle and auditory vesicle

formation, blastopore closing, tail formation and hatching stages. The period between fertilization of egg and outlet of organism is called incubation period [11]. Information on embryonic development of fish is a fundamental key which enables a closer approach to their biology and taxonomy [14]. It is essential to study the embryonic development as such studies can provide important information relevant to developmental and hatching success [3].

Little research has been done on the breeding and embryology of *Gymnocorymbus ternetzi*. An early report has been given by Çelik, *et al.*, (2012) [2]. This study can provide more information about the breeding techniques, behaviour and embryonic development of *G. ternetzi*.

2. Materials and Methods

2.1 Study Area

The study was conducted in the Meghalaya State Fisheries Research and Training Institute, Mawpun, Ri Bhoi District, Meghalaya, India, during the months of July - December, 2023.

2.2 Collection of brooders

The brooders were collected from Satsoma Aquarium shop, Chandmari, Guwahati. A total of 30 brood fishes were collected. The brood fishes were transported from the aquarium shop to the Institute by keeping them in plastic bags filled with water and oxygen, and were tied securely. On reaching the Institute, the brood fishes were treated with KMnO4 for 2-5 minutes so as to control their stress or any kind of infection.

2.3 Broodstock maintenance

Water Quality: During the experimental period, different physical and chemical parameters such as Temperature, pH, Dissolved Oxygen (DO), Hardness and Alkalinity were analysed weekly as per the APHA standard methods [15].

Feeding: The brood fishes were given a high-quality diet rich in proteins. They were fed twice daily in the morning and evening with Tetra Bits Complete (Crude Protein 47.5%, Crude Oil 6.5%, Crude Fiber 2.0%) a highly nutritious aquarium fish food along with Tubifex worms.

2.4 Setting up of breeding tank

An aquarium of dimensions 36×18×18 inches was used for the purpose of breeding. The aquarium was filled with water and a thermostat was placed inside the aquarium to regulate the temperature of about 24±1.0 °C. Aerators connected with air stones were placed inside the aquarium to provide dissolved oxygen. A net (0.5 cm mesh size) was placed on the aquarium surface and was submerged halfway with the help of a bamboo frame so that the eggs could fall to the bottom of the aquarium and this will prevent the brooders from eating their own eggs as they are carnivorous.

2.5 Releasing of brooders into breeding tank

A total of 10 pairs were released in the tank for breeding at a ratio of 1:1 (Male: Female). These fishes were left without any disturbance in order for them to start their courtship behaviour. After spawning was done, the brooders were taken out immediately and kept in another tank.

2.6 Calculation of Fertilization Rate, Hatching Rate and Survival Rate

The following formulae were used for the calculation of fertilization, hatching and survival rates:

$$\text{Fertilization rate (\%)} = \frac{\text{Number of fertilized eggs}}{\text{Total number of eggs}} \times 100$$

$$\text{Hatching rate (\%)} = \frac{\text{Number of hatchlings}}{\text{Total number of fertilized eggs}} \times 100$$

$$\text{Survival rate (\%)} = \frac{\text{Number of survived fry}}{\text{Total number of hatchlings}} \times 100$$

2.7 Study of Embryonic development

Some of the fertilized eggs were taken out in order to study the embryonic development. The egg was placed on a slide using a dropper and was observed under a trinocular compound microscope. All the stages were studied from spawning to hatching. Photographs were taken each time the stages of the egg changes.

3. Results and Discussion

3.1 Water quality parameters

The water quality parameters are very important for both broodstock management and breeding of the fishes. The recorded water quality parameters are given in Table 1.

Table 1: Water quality parameters of broodstock management and breeding of *G. ternetzi*

Parameters	Values
Temperature (°C)	24±1.0
DO (ppm)	4.0±1.3
pH	6.0±1.0
Hardness (ppm)	100±12.0
Alkalinity (ppm)	110±8.0

Table 1 displayed mean water quality parameters. Good water quality is very important for successful breeding of fishes and for their growth and development. During the study period, a thermostat was used to maintain the water temperature. The temperature range was 24±1.0°C, which is the optimum temperature for the growth of *G. ternetzi* as stated by Çelik, *et al.*, (2012) [2] and also by Kadtan & Shillerwar, (2022) [9] for *Paracheirodon innesi*. Also, the pH range was 6±1.0, hardness 100±12.0 ppm, Alkalinity 110±8.0 ppm and Dissolved Oxygen (DO) 4.0±1.3 ppm, which showed good quality of the water for the purpose of rearing and breeding this fish.

3.2 Fertilization Rate, Hatching Rate and Survival Rate

The percentage of fertilization rate, hatching rate and survival rate is given in Table 2 and the chart showing these percentages is given in Figure 1.

Table 2: Percentage of Fertilization Rate, Hatching Rate and Survival Rate

Species	Fertilization Rate (%)	Hatching Rate (%)	Survival Rate (%)
Gymnocorymbus ternetzi	75	60	50

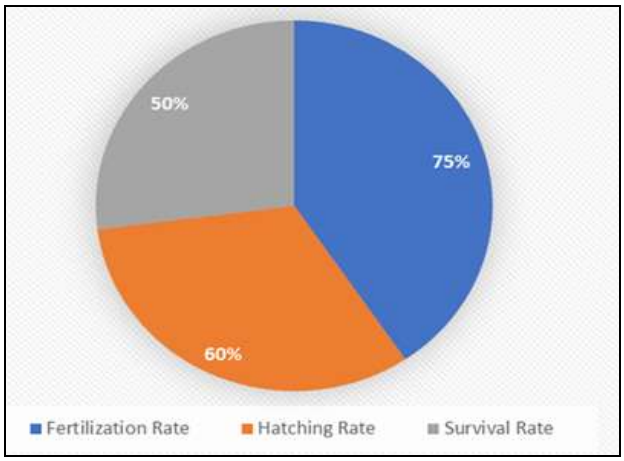

Fig 1: Chart showing the percentage of Fertilization Rate, Hatching Rate and Survival Rate of *G. ternetzi*

In the present study, the fertilization rate was 75%, hatching rate 60%, with a survival rate of 50%. The results obtained were slightly lesser as compared to the results obtained by the findings of Kadtan & Shillewar, (2023) [10] for P. innesi which recorded the fertilization rate to be 90% and also the report given by Çelik, *et al*., (2012) [2] for *G. ternetzi* which recorded the hatching rate to be 85-90% at 24±0.5°C for *G. ternetzi*. This may be due to some slight fluctuations in temperature during spawning and incubation, as temperature is the main deciding factor for development of fishes.

3.3 Stages of Embryonic Development

It was observed that the egg capsule was transparent, while the yolk was brownish in colour. The embryonic development was categorized into seven main stages – zygote, blastula, gastrula, segmentation, pharyngula, pre-hatching and hatching, along with many sub-stages.

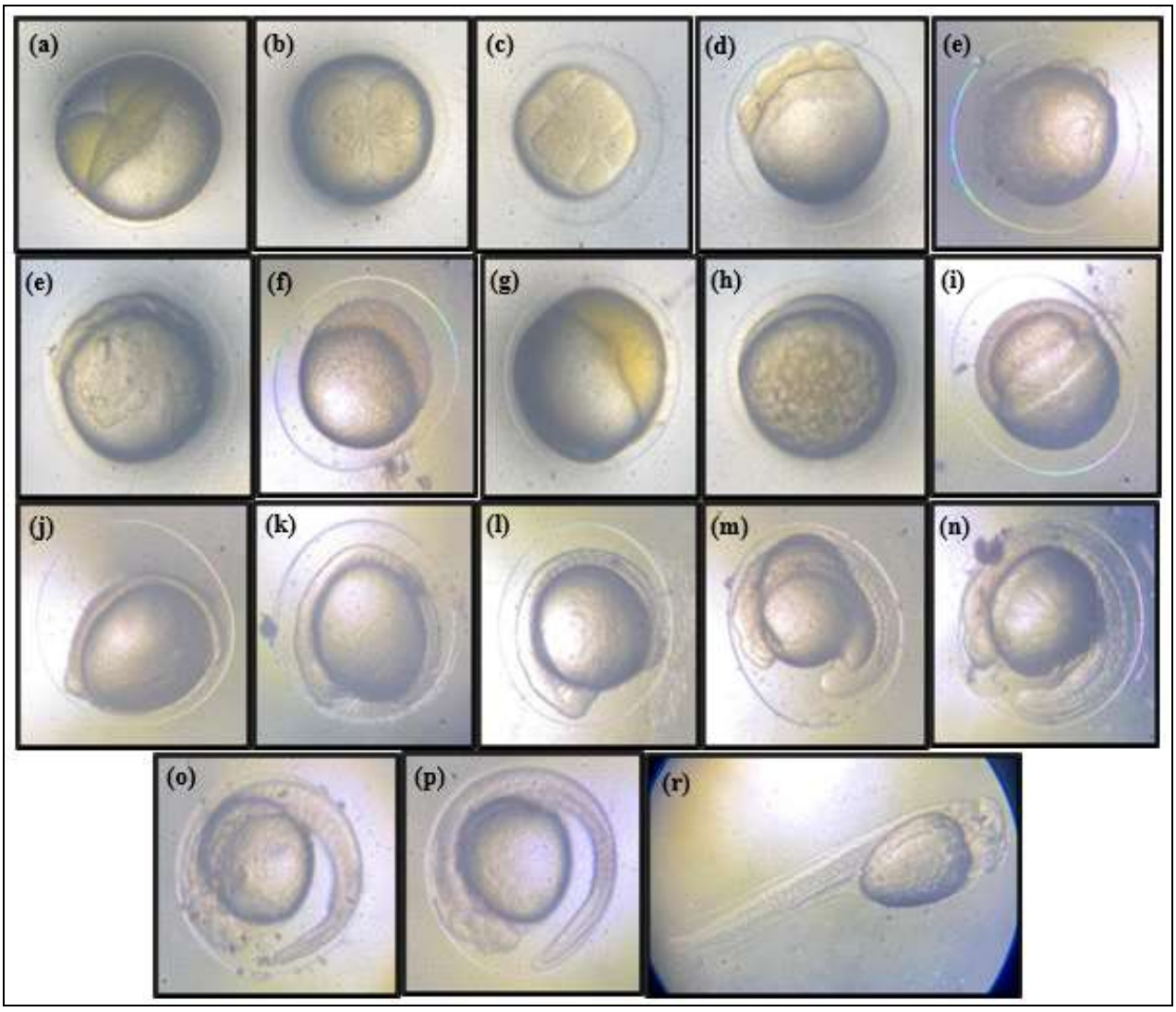

Fig 2: The stages of embryonic development of *Gymnocorymbus ternetzi*: (a) 2-blastomere stage (b) 4-blastomere stage (c) 8-blastomere stage (d) 16-blastomere stage (e) 32-blastomere stage (f) Early blastula stage (g) Late blastula stage (h) Early gastrula stage (i) 30% epiboly (j) 50% epiboly (k) 75% epiboly (l) 8-somite stage (m) 11-somite stage (n) 13-somite stage (o) Otic capsule (p) Muscular effect (q) Otolith appearance (r) Hatchling

Table 3: Embryonic developmental stages of *G. ternetzi* at 24±1.0 °C

Main Stages	Sub-stages	Time (Hour: Min)	Figure
Zygote	2 cells	00:25	2(a)
	4 cells	00:38	2(b)
	8 cells	00:48	2(c)
	16 cells	01:01	2(d)
	32 cells	01:08	2(e)
Blastula	Early blastula	01:55	2(f)
	Late blastula	02:20	2(g)
Gastrula	Early gastrula	03:00	2(h)
	30% epiboly	03:15	2(i)
	50% epiboly	03:55	2(j)
	75% epiboly	04:48	2(k)
Segmentation	8 somite	06:58	2(l)
	11 somite	07:30	2(m)
	13 somite	08:33	2(n)
Pharyngula	Otic capsule	10:30	2(o)
	Muscular effect	12:35	2(p)
Pre-hatching	Otolith appearance	15:05	2(q)
Hatching		21:00	2(r)

3.3.1 Zygote Stage: The cleavage of the egg was meroblastic. The first cleavage (2 cells) occurred at 00:25 hours. The blastodisc was being divided by meridional cleavage to form two equal cells (Figure 2a). The second cleavage (4 cells) occurred at 00:38 hours and the blastodisc was being divided into 4 blastomeres to form four equal cells (Figure 2b). The third cleavage (8 cells) occurred at 00:48 hours. The blastodisc was being divided horizontally, dividing the blastodisc into 8 blastomeres, forming eight equal cells (Figure 2c). The fourth cleavage (16 cells) occurred at 01:01 hours in two separate planes and resulted in 4 × 4 array. 16 blastomeres can be seen (Fig: 2d). The fifth cleavage (32 cells) occurred at 01:08 hours. The cells were smaller and were not uniform. 32 blastomeres can be seen (Figure 2e).

3.3.2 Blastula Stage: The blastula stage is the period where the blastodisc looks like a ball until the onset of gastrulation. Early Blastula occurred at the vegetal pole at 01:55 hours. The blastomeres continued to divide and expand over the yolk, but were asynchronous (Fig: 2f). Late Blastula occurred at 02:20 hours and consists of multicellular blastomeres. There was an increase in the epibolic cells (Figure 2g).

3.3.3 Gastrula Stage: The primary germ layers began to form during this stage. Early Gastrula started at 03:00 hours. The blastoderm cells were found to spread over the yolk (Fig: 2h). The embryo reached 30% epiboly at 03:15 hours and the blastoderm covered 30% of yolk sac (Figure 2i). 50% epiboly occurred at 03:55 hours. The blastoderm covered 50% of yolk sac (Figure 2j). 75% epiboly occurred at 04:48 hours and the blastoderm covered 75% of the yolk sac (Figure 2k).

3.3.4 Segmentation Stage: Formation of somites in an anterior to posterior sequence occurred during this stage. The tail bud became more prominent. The 8 pairs of somites were formed and was observed at the central part of the embryo at 06:58 hours. (Fig: 2l). 11 pairs of somites were formed at the central part of the embryo and was observed at 07:30 hours. (Fig: 2m). 13 pairs of somites were formed and this was observed at 08:33 hours (Figure 2n).

3.3.5 Pharyngula Stage: During this period, the embryo was developing well and twitching movement was observed. The Otic Capsule formation started at 10:30 hours after spawning (Figure 2o). Muscular Effect was observed at 12:35 hours when the embryo started moving and began to spin. The eyes and brain were also developed (Figure 2p).

3.3.6 Pre-hatching Stage: The otolith began to from at 15:05 hours. The embryo also showed conspicuous muscular contractions. The tail was well extended and was free from the yolk sac. (Figure 2q).

3.3.7 Hatching Stage: The embryo developed completely and hatched at 21:00 hours. The embryo is transparent and there was no pigmentation during this period. The larva showed locomotory movements. (Fig: 2r).

The eggs obtained were slightly adhesive, demersal and spherical in shape. The embryonic development lasted for 21 hours at 24±1.0°C, which was the same as the report given by Çelik, *et al.*, (2012) [2], and was the first to study and describe the embryonic and larval development of this fish. The difference from the previous findings is the timings of changes in the stages of embryonic development before hatching. The cleavage of eggs was meroblastic and the first, second, third, fourth and fifth cleavage occurred at 00:25, 00:38, 00:48, 01:01 and 01:08 hours respectively, whereas Çelik, *et al.*, (2012) [2] found the timings of these stages to be 00:30, 00:43, 00:50, 01:04 and 01:10 hours respectively. The early blastula and late blastula occurred at 01:55 and 02:20 hours respectively, which was different from that of the findings of Çelik, *et al.*, (2012) [2] i.e., 02:04 and 02:26 hours respectively. The blastomeres of *G. ternetzi* are regular in size and shape and this property is similar to black neon tetra (*Hyphessobrycon herbertaxelrodi*) [3] and serpae tetra (*Hyphessobrycon eques*) [4]. Gastrulation started at 03:00 hours and the embryo reached 30%, 50% and 75% epiboly stages at 03:00, 03:15 and 03:55 hours respectively, different from the findings of Çelik, *et al.*, (2012) [2] at 03:20, 03:34 and 04:10 hours respectively. The segmentation stages were characterized by formation of somites. The 8- somite, 11-somite and 13-somite occurred at 06:58, 07:30 and 08:33 hours respectively. The pharyngula stage started at 10:30 hours and is sub-divided into two sub-stages the otic capsule and muscular effect. The formation of the otic capsule started at 10:30 hours and the embryo began to spin in the muscular effect at 12:35 hours. The eyes also started developing. In contrary to this, the findings of Çelik, *et al.*, (2012) [2] showed

that the otic capsule formed at 13:15 hours and the muscular effect started at 15:30 hours. Hatching of the eggs occurred at 21:00 hours, which is the same as that of the first findings by Çelik, *et al.*, (2012) [2]. The reason for varying timings in between the stages before hatching may be due to slight fluctuation of temperature during the developmental period of the eggs, despite the presence of a thermostat. The hatching periods in some Characidae species are similar to those in each other [13]. However, egg hatching time was different from that of others. The developmental time of *G. ternetzi* also differs in several respects from that of *Puntius conchonius* as this species takes around 26 hours for their eggs to hatch as compared to *G. ternetzi* which could take around 22 hours to hatch [1]. Also, the hatching period of *Rasbora lateristriata* was around 24 hours after fertilization [17] and that of *Devario aequipinnatus* was found to be around 35-36 hours [5].

4. Conclusion

One of the most common problems in aquaculture to be solved is obtaining high quality gametes which applies not only to fishes reared for consumption but also aquarium fish species. For the successful breeding of ornamental fishes, proper knowledge of maintaining good water quality, proper broodstock management and understanding of habits and biology of the fish is required. It is also necessary for the conservation of the natural stock. This study can be helpful for the commercial production of not only the Black Skirt Tetra but also many other similar ornamental fishes having similar breeding patterns, behaviour and also embryonic development stages.

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