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Study of Embryonic and Larval Development of *Rasbora daniconius*

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ABSTRACT: *Rasbora daniconius* commonly known as slender Rasbora is a lower risk threatened species in Assam. Though the population is not at risk presently but the natural population is showing a declining trend due to habitat loss as well as anthropogenic stress. The species is a potential ornamental fish. Therefore the present studies elucidates the embryonic and the larval development of *Rasbora daniconius* from fertilization to juvenile stage. The most important criteria for breeding programs are–in-vitro sexual dimorphism selection of brooders, male and female ratio for breeding. In males colouration is bright and the lateral band is prominent whereas the females are fainter. The ratio of male to female is 1:1 or 2:1. The fish is bred by striping and artificially fertilized. The fertilized eggs are spherical, semi adhesive and the diameter of egg proper ranges between 0.8–1.0 mm × 0.96 mm. First sign of cleavage is observed at 20m from hatching, the appearance of germ plug commences the start of gastrula at 7h and organogenesis starts at 10h 30m after fertilization. The period of incubation is about 32h at a room temperature. The hatching percentage varied from 75% to 80%. The hatched larva is c 3.0 mm to 3.2mm in length. The yolk absorption is completed in five days from the day of hatching. The larva metamorphosed into juvenile within 25 days of hatching. The study will help aquaculturist and researchers to generate knowledge about the different developmental stages of the test species. It will also help to formulate feeding schedule for fries by aquarists.

Keywords: Embryonic, larval, *Rasbora daniconius*, in-vitro sexual dimorphism, organogenesis.

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INTRODUCTION

Slender Rasbora or *Rasbora daniconius* is a slim fish. Its body is compressed and oblong. The fish is silver in colour and the undertones are olive coloured. The belly of the males are white whereas in the females it is yellow to orange. A bluish black coloured line outlined with golden colour runs through the entire length of the body upto the tail. In Assam the fish inhabits streams, rivers, beels, ponds and fields. *Rasbora daniconius* is a lower risk threatened species in N.E. India and its population is dwindling in natural environment due to anthropogenic stress. Although some studies have been made in India namely by Basavaraja *et al*, 1988; Belsesware and Naik, 2001a; Nair, 2001; Mukhopadhya, 2001; Sinha *et al.*, 2001; Pandian *et.al*., 2001 and Swain and Das, 2001, Borcato *et al.*,(2014), Sutradhar *et al.*, (2016) and Saha *et al.*, (2017) on the food, nutrition, embryonic, larval and rearing of some freshwater ornamental fishes. In Assam, Dey and Sarmah 2000; Sarmah and Dey (2003, 2004a and 2004b); and Paul and Sarmah (2011) and Mahapatra and Krishna (2016), DiMaggio *et al.*, (2017), Dil Afroza *et al.*, (2018), Shadrin and Emel'yanova, (2019) who made empirical studies on the breeding of some native ornamental fish species of N.E. India hitherto remain unattended. In teleost, fertilization is external followed by absorption of water by the egg and hardening of the chorion. Hatchling is the tiny free swimming larva. The larval development begins with the external feeding of fry till the formation of juvenile. In the Northeastern region, aquarist of ornamental fish species mainly depends on natural catch for supply of indigenous ornamental fish species. This is because of lack of scientific knowledge of breeding of fish species. Therefore, the present paper depicts the developmental stages of the test species. In the present treatise the entire development process has been grouped into three phases-embryonic phase, hatchling phase and larval phase. Further the study will help in understanding the variations in developmental stages of other related species.

MATERIALS AND METHODS

The body of the males of the test species is short, slender and narrow, ventral profile is straight, mouth is blunt, fin edges are smooth and semi circular whereas in females the body is long comparatively deep, dorsal and ventral profile convex, mouth pointed and fin edges uneven.

In males colouration is bright and the lateral band is prominent whereas the females are fainter. For breeding brood stock with good finage and disease free are selected. Total length of 56.0-60.0 mm and 61.0-65.0mm for males and females respectively is suitable for breeding with weight ranging between 4.17-5.01gm for males and for females 8.5-9.2 gm. Two ratios have been found suitable for breeding. The ratio of male to female is 1:1 or 2:1.

The eggs and milt are stripped from the spawners and artificially fertilized by the standard wet method. During incubation, the aeration and temperature of aquaria are kept at optimum level for desired effect.

The egg samples are taken every 10 minute in the first 2 hours to determine the first cleavage and then at 2-4 hours intervals. Microphotographs of the different stages of development of each species are taken as far as practicable.

Free embryo is reared in the aquaria with a fix temperature and one third of the water is changed daily. Three to five days after hatching live food (mainly infusoria) are added into the aquaria. Sampling is made to record the daily events in free embryo and larva are examined under binocular microscope. Length of the individual is measured with micrometer and photographs are taken. The critical progressive developmental stages of the larva are recorded in aquaria and under microscope to define phase after Balon (1975a), Dujakovic *et al.,* (1995) and Unal *et al.,* (2000). The water of the aquaria was maintained at a temperature in between 22-29°C.

III. RESULTS AND DISCUSSION

1. Embryonic Phase

Table 1.

Hrs	Min	Description
		The fertilized eggs are spherical, semi- adhesive and pale grayish in colour. The size of the eggs are 1.7- 2.0 mm (× 1.76 mm), while diameter of egg proper is 0.8- 1.0 (× 0.96 mm)
00	15	A cap like blastodisc was observed.
00	20- 30	Cleavage starts about 20 mins after fertilization, 2- celled stage is attained.
00	45	4 celled stage
1	15	8 celled stage
1	45	16 celled stage
2	00	32 celled stage is reached after fertilization
2	30	Morula stage and extends upto 2h 45 mins after fertilization
4	00	Early blastula is marked by the spreading of blastoderm over the yolk
7	00	Beginning of gastrula stage by appearance of germ ring
7	15	Yolk plug
10	30	Organogenesis process starts, antero-posterior axis is discernable
12		6-somite , formation of optic cup, buccal invagination was prominent
14		Notochord antecedent is followable with clear optic cup.
20		The tail becomes free. Lens, pericardium and faint pulsating mechanism are noticeable
22		Fin folding is observed
30		Switching movement of the embryo gradually became vigorous. The neural chord over the trunk and caudal region is visible. Eyes were fully developed, pectoral fin formation was indicated
32		The embryo ruptures the vitelline membrane and the larva comes out from the egg capsule with tail first

2. Hatchling Phase

(a) **Free embryo stage:**

(i) The prolarva is laterally compressed, transparent with rounded yolk sac and measuring 3.0-3.2 mm (3.05 mm). The hatchling showed uncoordinated movement and remains at the bottom A circulatory path of blood corpuscles could be traced clearly up to the caudal region.

(ii) At 12 hrs, buccal invagination is prominent. Heart beating was recorded @ 50-55 beat per minute. Formation of alimentary canal starts. The hatchling settles on the side of breeding tank.

Cling stage: This state is observed for a short period at 18 hrs after hatching. The cling stage persists up to 22 hrs

(c) **Mouth formation stage**: This stage is attained at 24 hrs after hatching: The size of prolarva is 4.0-4.2 mm (4.1 mm) 34 somite stage. Upper jaw and lower jaw were formed. Vent is also formed. The larva exhibited a tendency to swim freely.

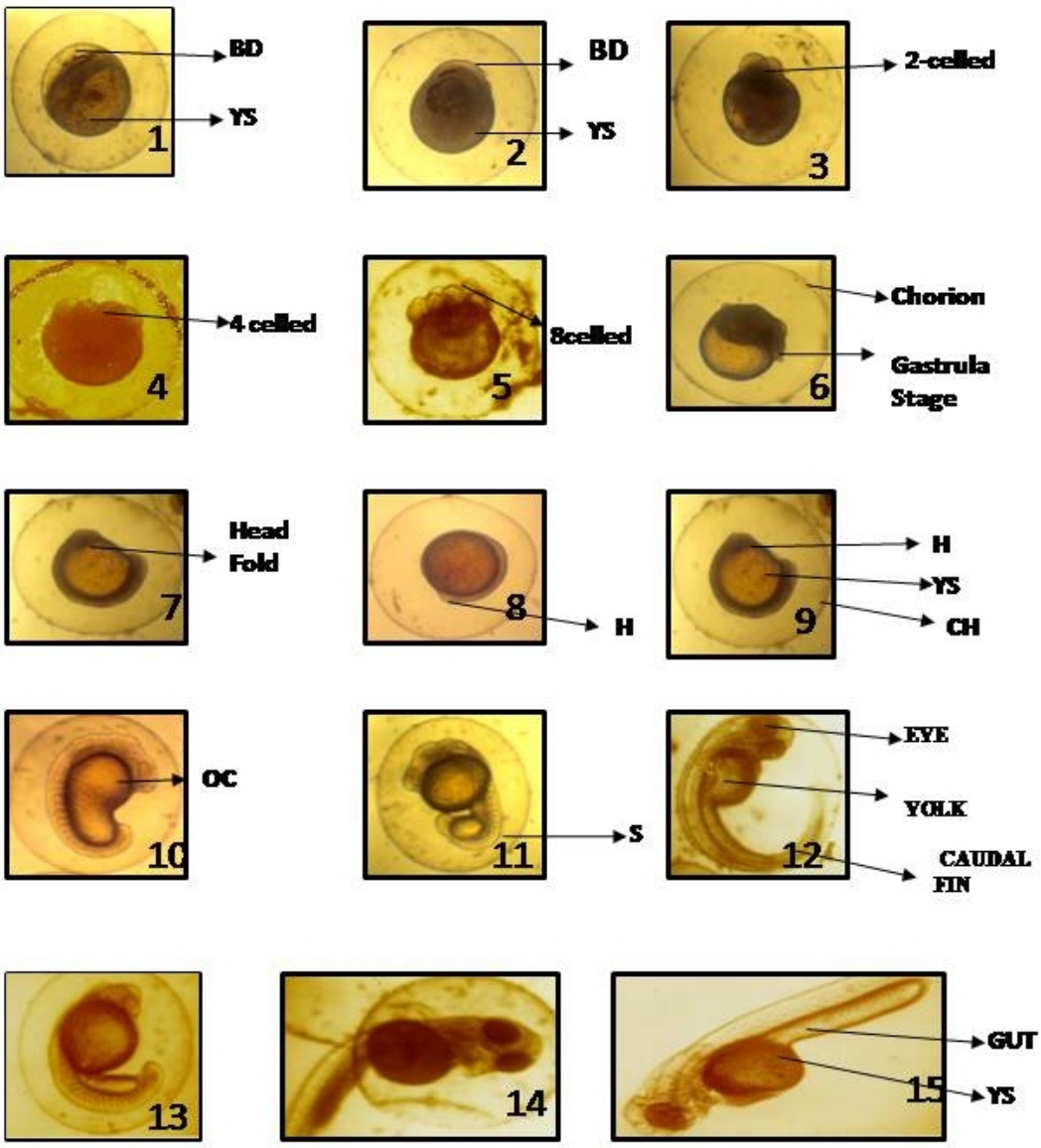
3. Larval Phase:

(a) **Free swimming stage:**

The larva attains the free swimming stage at 48h after hatching. The length of the larva is observed to be 4.5-4.8 mm.

(b) **Fin formation stage**:

(i) *Dorsal fin*: The first indication of dorsal fin formation is noticed at 5th day after hatching with thickening and elevation of embryonic fin fold. The rudimentary rays are noticed at 10th day after hatching.



Note: BD: Blastodisc; CH: Chorion; E: eye; H: Head; OC: Optic Cup; S : Somite; and YS: Yolk Sac.

Fig. 1. Different stages of Embryonic and Larval stages of *Rasbora daniconius.*

Dorsal fin development almost completes with distinct fin rays, although not free from embryonic fin fold at 20th day after hatching.

(ii) *Anal Fin*: Anal fin bud became prominent from 3rd day. An indication of fin rays formation is visible from 10th day after hatching and completed by 20th day.

(iii) *Pectoral fin*: The first sign of pectoral fin formation was noticed at 30h. At 6h. after hatching pectoral fin appears but rudimentary. The full grown fins appears with fin rays at 15 day after hatching.

(iv) *Pelvic fin*: From 5th day after hatching the pelvic fin formation starts, and completes at 20th day after hatching.

(v) *Caudal fin*: Formation of caudal fin is indicated at 24 hrs after hatching with some streaks. The rudimentary rays are noticeable at 5th day after hatching. Caudal fin is forked with distinct fin rays are noticed at 10th day after hatching, and complete fin is formed at 15th day after hatching.

(c) **Complete fin articulation stage:** All fins of the larva are fully developed and become active after 25th day of hatching.

(d) **Colour formation stage:** The first colour pigment are distributed over the mid dorsal side and caudal region at 30h after fertilization. Some melanophores are also concentrated in the post opercular region at 6 hrs after hatching. Black melanophore are observed on the post orbital dorsal side. At 4th day after hatching black melanophores are distributed along the embryonic dorsal fin fold. On 5th day, black melanophores redistributed on both dorsal and ventral side forming a band like appearance. At 10th day after hatching black melanophores are concentrated in the dorsal side of head, and caudal region. Colour patterns started from 20th day after hatching. A bluish black-cum-golden pigmentation are concentrated in the post opercular region to mid lateral side and a faint black-blue stripe are also noticeable at this stage. The complete colouration of the larva became clear on 25th day after hatching.

4. Growth Length of Larva: The growth length of larva are observed from 3rd day to 30th day after hatching and the results are presented below:

Table 2.

Day	3	5	10	15	20	25	30
Range (average)	4.8-5.0	5.0-5.2	5.5-5.8	6.0-6.5	6.6-6.8	7.0-7.2	7.4-7.5
Length (mm)	(4.9)	(5.08)	(5.56)	(6.24)	(6.7)	(7.1)	(7.42)

Other major features of the study-Notochord turns upward direction from 2nd to 4th day after hatching, yolk sac is fully absorbed on 5th day, operculum movement became prominent on 15th day, transparency of larva gradually decreased on 15th day after hatching. Scale appearance is noticed after 25th day of hatching.

As reported in many fish species (Blaxter, 1969; Suzuki & Hibriya, 1984 & Unal *et al.*, 2000) the newly hatched free larva of present test OFS have chromatophores in the body. Presence of some cement organs for attachment to substratum as reported in some cyprinids, like carp & bream (Nikolsky, 1963), Blaxter, 1969; Balon, 1975) is evident in the present test OFS through a cling stage covering *c* 24 hrs duration. Significantly, pre hatching latency was not observed in case of *R. daniconius* which may be an indication of lentic habitat of the species.

CONCLUSION

R. daniconius can easily be reared, matured and bred in aquarium condition. The study reveals precisely the time taken for the development of key stages of the species. It will further help in comparing the variation in organogenesis of related species of Assam. The larval and fry development will help in the replenishment of this species in natural environment and also help to improve the captive culture of the test species.

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