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Embryonic and larval development of *Rasbora daniconius* (Hamilton): A potential indigenous ornamental fish of north-east India

BK Mahapatra and Gopal Krishna

Abstract
The aim of this paper is to describe the development stages of the embryo and of larval period Indian ornamental fish, *Rasbora daniconius*. Thus, has been characterized seven broad periods of embryogenesis - the zygote, cleavage, blastula, gastrula, segmentation, *pharyngula* and hatching, respectively. These divisions highlight the changing spectrum of major developmental processes that occur since fertilization to hatching. Stages subdivide the periods and their names are based on morphological features, generally readily identified by examination in microscopy of the live embryo. The embryonic development lasted 23-24 hours in water temperature around 26-28 ºC. During larval period, the main aspects were following: yolk resorption, body pigmentation and completed organogenesis, filled with gas of the swim bladder, fins differentiation, the appearance of active body movements and start of the exogenous feeding.

Keywords: *Rasbora daniconius*, morphogenesis, embryogenesis, larval development

1. Introduction
During the past few years the natural population of the freshwater fishes has been rapidly declining due to various man-made and natural causes. Moreover, the fish are also under threat due to drying up of the low lying areas and indiscriminate use of fertilizers and pesticides. It is necessary to undertake proper study to characterize its various stages of embryonic and larval development to understand the biological clock and cultural techniques of these species [1]. Life starts with the unification of male and female gametes. As soon as the egg is fertilized by a sperm, the zygote is formed and embryonic development starts and ends up at hatching. The hatchlings further undergo organogenesis and appear as like as their parents, thus end the larval stages. Egg development in the ovary is maternally derived and is predetermined in the ovary but its genetics complex is determined at the very instant of fertilization [1]. The life stages of fish are formed of 5 stages [2] i.e. Embryonic Phase, Larval Phase, Fry Phase, Ripe Phase and Senescent Phase. The embryonic phase refer 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. Larval phases starts after embryonic phases. After coming out from the egg, fish larvae go through some stages which are named differently by different scientists. The most common among them is one which Hubbs proposed [3]. There are 2 phases of fish i.e. Pre-larval phase and Post-larval phase. Pre-larval phase is the period which starts from coming out from egg to the end of absorption of yolk sac. The most important characteristic of Pre-larval stage is the existence of yolk sac and the Post-larval stage starts after absorption finished to end of the metamorphosis.
The black-line Rasbora, *Rasbora daniconius* (Hamilton) is a popular indigenous ornamental fish of India [4, 5]. It is also a popular food fish for economically weak communities because of low price [6]. Although some work has been done on the fecundity of *Rasbora daniconius* by some workers [7, 8] but there is no information on the early development of this ornamental fish. Very less work has been done on indigenous ornamental fish [9, 10]. So, it is necessary to undertake proper study to characterise its various stages of embryonic and larval developments to understand the biological clock of this species. Hence, in this paper, embryonic and larval developments of Indian ornamental fish, *Rasbora daniconius* were examined.

Material and Methods

The research was carried in the Ornamental Fish Centre of CIFE Kolkata Centre. Mature and healthy Rasbora breeders (6.5–8.0 g), one female and two males, were selected according to sexual dimorphism for the breeding experiment. Fifteen days before being subjected to reproduction, the breeders were separated by sex, in the glass aquarium. The females were usually easier to identify, as the belly of a mature female is generally larger, whereas the male remains streamlined and more torpedo shaped. The incubation took place in a glass aquarium. The diameter of the egg was measured using a calibrated ocular micrometre. Egg samples were taken with regular interval after fertilization. The description of the developing stages was made by examining live specimens under a microscope in 10X and taking microphotographs (developmental stages of eggs and larvae). Eggs that has missed fertilization appeared with a coating of fungus non- productive and hence removed from the aquarium to avoid any contamination to the young ones.

Results and Discussion

The embryonic development of eggs of *Rasbora daniconius* fish from a spawning on July 25, 2013 was described in Table 1. The elapsed time from fertilization was calculated assuming that the eggs were spawned at 04:05pm although it took about half hour to complete spawning in the glass aquarium. The diameter of the fertilized egg varies from 820.00µm to 1000µm.The embryonic development of the Rasbora was divided into six periods: zygote, cleavage, blastula, gastrula, segmentation, pharyngula and hatching period. Details of developmental features are presented in Table 1 and Fig.1-12.

Table 1: Embryonic and larval development of *Rasbora daniconius*

Hrs.	Min.	Water temp.	Fig. No.	Description
00	05	28 ^{0}C	Fig.1	Zygote stage immediately after fertilization, having one uncleaved cell. The eggs were round shaped, nearly transparent and demersal in nature with dimension varies from 820.00µm to 1000µm.
00	30	28 ^{0}C	Fig.2	1st cleavage (cl_1) occurred. The cleavage was meroblastic and meridional in nature. There was comparatively little yolk mass with great deal of cytoplasm in the animal pole and much yolk mass and little cytoplasm in the vegetal pole of the egg. Vitelline membrane with well-defined perivitelline space.
00	35	28 ^{0}C	Fig.3	Just starting of 2nd cleavage (cl_2).
00	40	28.4 ^{0}C	Fig.4	2nd cleavage (cl_2) which occurred meridionally at right angle to first the cleavage resulting in four germinal cells.
00	50	28.6 ^{0}C	Fig.5	3rd cleavage
00	55	28 ^{0}C	Fig.6	Successive stages
18	00	28 ^{0}C	Fig.7	Germ ring was observed
18	10-50	28 ^{0}C	Fig.8A-D	Stages of pre-hatching larvae. The eggs were just ready to hatch. Total length of embryo was 2012.2µm to 2218.6µm.
48	00	28.4 ^{0}C	Fig.9	Total length of the larvae was 4566.9µm. Head length was 572µm. Eye diameter was 234.35µm. Tail was 1115.6µm. Yolk sac length was 1699.1µmAlimentary canal formation was observed.
67	00	28^0-29 ^{0}C	Fig.10	Total length was4681.6µm. Head was 714.5µm.Eye diameter was 267.7 µm. Tail was 1323µm. Yolk sac was 1683.2µm. Pectoral fin was 503.3µm.Formation of the air bladder initiated, which was very small in size and blackish in colour.
119	00	28^0-29 ^{0}C	Fig.11	Total length was4864.8 µm. Head was 746.1µm. Eye diameter 342.5 µm. Tail was 1333µm.Yolk sac was 1583µm. Starting the pectoral fin formation. Formation of pigmentation on their head. Formed airbladder, this was yellowish in colour.
264	00	28^0-28.6 ^{0}C	Fig.12	Total length was 4907 µm. Head length was 904.3 µm. Eye diameters344µm. Mouth opening was 129.1µm. Fully absorbed yolk sac. Alimentary canal and anus observed. Air bladder becomes larger in size. Artemia, artificial feed and mixed zooplankton accepted as food.

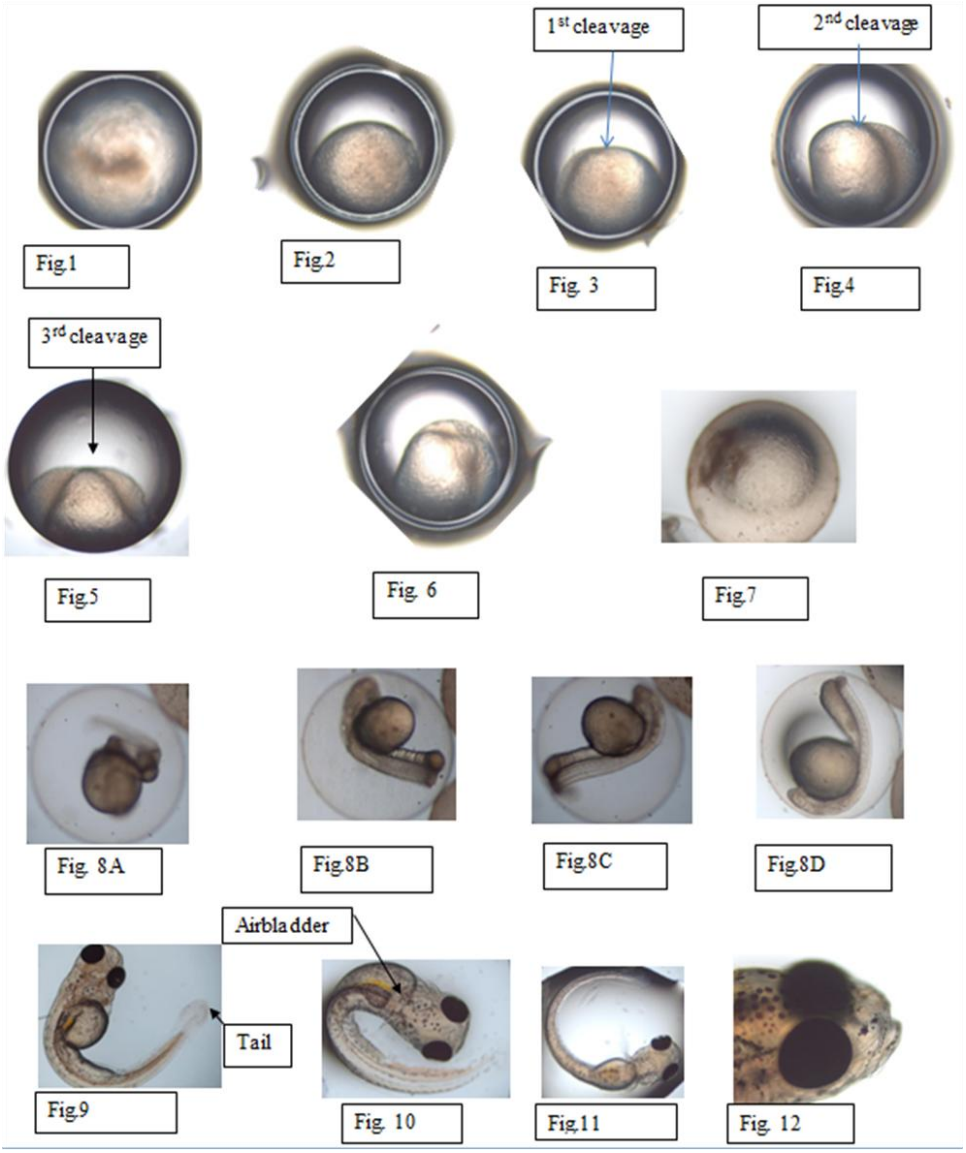

Fig 1-12: Embryonic and larval development of *Rasbora daniconius*

A) Embryonic stage

As soon as the egg was fertilized by a sperm, it swells, the zygote was formed and Perivitelline space was also formed. At this stage, an accumulation of cytoplasm in the animal pole was observed. The blastodisc was easily distinguishable from the yolk mass. The cleavage was typically meroblastic and the first division (2-celledstage) occurred 30 minutes after fertilization, followed by the second cleavage, completed 40minutes after fertilization. Second cleavage occurred meridionally at right angle to first the cleavage resulting in four germinal cells. The 16-celled stage was reached an hour and 30 minutes after fertilization. Yolk invasion started 6-7hours after fertilization and completed 10hrs and 25 minutes after fertilization. Oil globules are absent in fish body. The head and the tail of the embryo became distinguishable at the end of the gastrula stage. The notochord could beclearly seen 14 hours after fertilization, with identifiable somites, the beginning of lens and heart formation being almost completed. 19 hours after fertilization, the caudal region detached from the yolk mass and became free. In the final stage of the embryonic development, the growing embryo occupied the entire previtelline space; it exhibited frequent twitching movement by lashing the tail against the egg capsule.

B) Larval stage

i) Pre larval Phase

After a few seconds pause, this movement suddenly culminated in a violent jerk breaking of the pre-vitelline membrane and the hatchling emerged, tail first. Hatching occurred 24-25 hours after spawning at water temperatures of 28.5-30 ^{0}C and the hatchlings were transparent. The most important characteristics of pre-larval stage were the existence of yolk sac and it was almost round in shape. This yolk sac was located in anterior and ventral of the body. At the beginning of the pre-larval phase mouth, anus and digestive tube was like straight pipe. Head was smaller than body, eyes were big. The hatchlings become 4566.9µmin total length within 48 hrs after fertilization. At this developmental stage, they have no swim bladder, mouth or vent. They breathe by absorbing oxygen through the fine blood capillaries that surround the yolk sac still attach to the gut. The head of the hatchling was noticed above the yolk sac and the brain was clearly visible. After 56-58hrs of fertilization, small yellowish colour airbladder formation was occurred and it was fully formulated within 10 to 11days of fertilization. 6-8 hours after hatching, the fin folds were seen continuously around the tail. This stage was completed by fully absorption of yolk sac. Pigmentation was observed at the head parts of larvae. Formation of pectoral fin was observed.

ii) Post larval Phase

2 days later, the hatchlings swam freely, the mouth began to function and the next day, the larvae started exogenous feeding. By day 3 the hatched larva had completed most of its morphogenesis, and it continued to grow rapidly. Prominent changes during the next day included the inflation of the swim bladder and the continued anterior dorsal protrusion of the mouth. Rasbora larvae as the general appearance may differ from mature in many ways in both outer and inner structure. 5 days later the larvae started exogenous feeding with green water in two times per day and it was continued till 6 days. 7days to 10 days the larvae was fed by the first stage of Artemia. The pigmentation became spread on their body parts also.

Formation of zygote occurred within 5 mins in Rasbora which is also similar to *Brachidanio rerio*. First cleavage was observed in Rasbora after 30mins of fertilization whereas the same was observed in *B. rerio* and *Danio aequipinnatus* after 50 and 45 mins respectively. This variations may be due to both intrinsic and extrinsic factors. Hatching of *B. rerio* occurred after 40-42 hrs on the contrary in Rasbora it occurred after 48 hrs, which is little higher. In case of *D. aequipinnatus* complete yolk sac was absorbed after 6 days but in case of Rasbora it takes about 11 days [9, 10].

Conclusions

The study on embryonic and larval development of *Rasbora daniconius* definitely helps the breeders for its propagation under captive condition. The information generated in this study will also help the researcher those who are interested in the study of fish on embryonic and larval development.

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