

Differential Pattern of Embryonic Development of *Labeo rohita* and *Ctenopharyngodon idella* under Hatchery Conditions of Tarai Region of Uttarakhand during Late Breeding Season

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Abstract A comparative study of embryonic development of rohu (*Labeo rohita*) and grass carp (*Ctenopharyngodon idella*), the two most important cultivable species of Uttarakhand, was conducted to analyze the differences in the morphological development under the hatchery conditions of Tarai region of Uttarakhand during late breeding season (August). Embryonic development in rohu and grass carp was observed from 0 to 100 h in Chinese circular hatchery conditions at maintained water temperature of 28°C and dissolve oxygen 7.5–8 mg/l. A significant difference in the morphological development of embryos of both the fishes was recorded. While grass carp spawn shows better morphological development during breeding season, the rohu spawn shown far better morphological development in late breeding season. The details of the findings of the experiment has been presented in the paper.

Keywords Cultivable, Morphological development, Breeding season, Hatchery, Tarai region.

Introduction

Seasonality in reproduction is one of the most striking characteristics of fish, which is synchronized with seasonal changes in climate, day length and food availability. The synchronization, associated with endogenous processes, regulates spawning season and thereby ensures spawn (egg/fry) production during most suitable environmental conditions for better survival [1]. Thus freshwater carp farming in India relies almost exclusively on larvae production from captive fish under culture conditions, particularly during natural spawning season [2]. Rohu (*Labeo rohita*) and grass carp (*Ctenopharyngodon idella*) are the preferred species of freshwater aquaculture and also widely distributed.

Rohu (*Labeo rohita*), one of the most important major carp in India and the sub-continent, spawns naturally once in a year, during the monsoon season (June—September), in rivers [3]. Successful induced spawning and multiple breeding in captivity by hypophysation have been achieved in this fish [4—6]. On an average fecundity of rohu is estimated at 2.56 lakh per kg body weight.k

The grass carp (*Ctenopharyngodon idella*) is a herbivorous, freshwater fish species of the family cyprinidae, and the only species of the genus *Ctenopharyngodon*. It is a large cyprind native to Eastern Asia, with a native range from Northern Viet-

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nam to the Amur River on the Siberia–China border. It is a fish of large, turbid rivers and associated floodplain lakes, with a wide degree of temperature tolerance. Grass carp enters in reproductive condition during early summer season and spawn at temperatures of 20 to 30°C [20]. Breeding season varies from one geographical region to another and in India it is from March to August. Fecundity has been estimated at around 82,000 per kg body weight and the eggs are yellow to deep golden brown in color [7].

Embryonic development is a complex process in which cellular differentiation and proliferation occur simultaneously, though rate is different [8]. Temperature is the one of the important environmental factor with the largest influence on the development of different stages of fish [9, 10]. The rate of the biological functions of the fish is critically dependent on environmental temperature [2]. Embryos and larvae are more sensitive to temperature changes than adults [11]. Temperature directly influences the developmental rate and development is faster at increasing temperatures within the acceptable thermal limit [12, 13].

Considering the immense importance of seed production of these fishes during late breeding season, the study of their embryonic development is very important. In view of the existence of numerous findings presented above, the present investigation aimed at determining the effect of temperature and off breeding season on the embryonic development of these pride cultivable carps in India.

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Materials and Methods

The experiment was conducted on selected gravid spawners of rohu (*Labeo rohita*) and grass carp (*Ctenopharyngodon idella*) of + 2 year age group, collected from brood stock ponds of College of Fisheries, GBPUA & T, Pantnagar during late breeding season in the month of August. Pantnagar is situ-

ated in Tarai region of Uttarakhand. Tarai region is a wet moisture regime with high water level for most of the year. The prospective spawners were selected from earthen brood stock ponds reared since 5 months before the spawning to get better spawning results [14] following routine brood husbandry practices of Indian Major Carps. Brood raising is very important aspect of induced breeding operation. Care taken in raising a brood stock helps in the recruitment of healthy prospective fish, that prevents inbreeding depression and genetic drift in the off spring [14].

Sixteen males and sixteen females of rohu with average weight of 644 grams and 969 grams, respectively and twelve male and twelve female of grass carp with average weight of 815 g and 1325 g, respectively were used for induced breeding in the circular carp hatchery. The fully mature brood fishes of age between 2–3 years were selected based on the external secondary sexual characters [14]. The selected brood stock were administered with Gonopro FH, a synthetic breeding hormone, @ 0.5 ml/kg body weight of the female fish and 0.25 ml/kg of the male fish [14]. They were then allowed to spawn in the breeding tanks facilitated with artificial rainy and river condition with water of 28°C temperature and 7.5–8.0 mg/l DO. Spawning took place within 10 h after hormone injection.

Sampling of eggs was carried out at regular intervals using fine meshed scoop net and beaker. The eggs were then observed under the microscope to study the developmental stages and pictures were simultaneously captured using an over-mounted digital microscope camera connected to a computer. Observations were made more frequently during the initial stages of development at an interval of approximately 2 h and time lapse increased as the development proceeded. Observations of the developmental stages of fertilized eggs of respective fishes were taken at 2, 4, 6, 8, 10, 12, 16, 24, 36, 48, 60, 72 and 100 h.

Water quality parameters (temperature and DO) in brood stock pond and in incubation tank were also measured prior to brood selection and also that of incubation tank water, twice a day and the average value observed is presented in Table 1.

Table 1. Variation in water quality parameters observed during experimental period.

Fish species	Temperature (°C)		Dissolved oxygen (mg/l)
	Atmospheric	Water	
<i>Labeo rohita</i>	30	28	7.5–8
<i>Ctenophryngodon idella</i>	30	28	7.5–8

Results and Discussion

Embryonic development in rohu and grass carp was observed from 0 hours to 100 hours in Chinese circular hatchery conditions at maintained water temperature of 28°C and dissolve oxygen 7.5–8 mg/l. To increase the hatching period of the fertilized eggs of the experimental fishes for proper development of the embryo, a solution of harar (*Mirobolus indica*) @ 3–4 ppm water in hatching tank was mixed. The observation of development of embryo in different time period recorded during study period (Fig. 1) is as follows:

After 2 h of the fertilization, eggs of rohu were at blastula stage (8-cell) and in grass carp it was at 16-cell stage of blastula formation. At the completion of 4 h, the fertilized eggs of grass carp shows initiation of blastodisc formation while in rohu aggregation and differentiation of blastomeres was observed. After 6 h of the fertilization, fully formed blastodisc was observed in grass carp while in rohu the start of blastodisc formation was seen. At the completion 8 h, the experimental eggs of grass carp shows development of embryo while only in rohu, the appearance of embryo was observed. At 10 h, the developing embryo with appearance of head observed in grass carp while only developing embryo was seen in rohu. At 12 h of the experiment, the brain and somites cells were seen in developing embryo of grass carp while rohu shows differentiated somite cells and brain in developing embryo. At the end of 16 h, well developed somite cells with less yolk absorption is seen in grass carp whereas in rohu well developed somite cells with more yolk absorption was seen. At the completion of 24 hours, appearance of eye ball cavity was observed in rohu as well as grass carp. After completing 30 h, well

developed circulatory system was observed in grass carp while in rohu, a well developed brain with functional circulatory system was seen. Eggs of both the fishes were hatched within 36 h. Grass carp has less yolk absorption and development of brain part whereas in rohu, appearance of fin development and more yolk absorption was seen. At the completion of 48 h, well developed eyes and fins were seen in grass carp whereas in rohu well developed eye ball and organ differentiation was seen. At the end of 60 h, the development of digestive system was not yet initiated in grass carp whereas in rohu the initiation of development of digestive system has seen. At 72 h, the embryos of grass carp shows initiation of development of digestive system whereas in rohu developed digestive system evident by the presence of ball of waste material was seen. At the completion of 100 h, the digestive system has developed in grass carp and yolk sac still under absorption whereas in rohu fully absorbed yolk sac with functional digestive system was seen.

The observations recorded during present investigation shows that there was a significant difference in the development of embryos of grass carp and rohu. Both organogenesis and somatic growth are controlled by temperature and enzymatic activities. Embryos are more sensitive to temperature than adult fishes [11]. It was observed that early embryonic development (till 12 h) of embryo of grass carp was faster than rohu while after the development of somatic cells and brain the embryonic development in rohu picked up the pace and at 24 h both shows the same development stage (appearance of eye ball cavity). Embryonic development is a complex process in which cellular differentiation and proliferation occur simultaneously but their rate is different [8, 15]. Larvae of rohu hatched 1 h before grass carp at 32 h. Larval development of rohu was also noted much faster than the grass carp in further observations with fully absorbed yolk sac and functional digestive system at 100 h in rohu while yolk sac was not absorbed in grass carp till this time period. From the point of fertilization until hatching, low temperatures retard and high temperatures accelerate embryonic development [16], which is consistent with our findings.





























TIME	Rohu (<i>Labeo rohita</i>)	Grass Carp (<i>Ctenopharyngodon idella</i>)	TIME	Rohu (<i>Labeo rohita</i>)	Grass Carp (<i>Ctenopharyngodon idella</i>)
2 HOURS	 Blastula - 8 cell stage (→)	 Blastula - 16 cell stage (→)	12 HOURS	 Developing embryo with differentiated somites (→) and brain (→)	 Brain (→) and somites (→) of developing embryo
4 HOURS	 Aggregation and differentiation of blastomeres (→)	 Initiation of blastula formation (→)	16 HOURS	 Well developed somites (→) with more yolk absorption (→)	 Well developed somites (→) with less yolk absorption (→)
6 HOURS	 Initiation of blastula formation (→)	 Fully formed blastula (→)	24 HOURS	 Appearance of eye ball cavity (→)	 Appearance of eye ball cavity (→)
8 HOURS	 Appearance of embryo (→)	 Development of embryo (→)	30 HOURS	 Well developed brain (→) and functional circulatory system (→)	 Well developed circulatory system (→)
10 HOURS	 Developing embryo (→)	 Developing embryo (→) with the appearance of head (→)	36 HOURS	 Appearance of fin development (→) and more yolk absorption (→)	 Less yolk absorption (→) and development of brain parts (→)
48 HOURS	 Well developed eye balls (→) and organ differentiation	 Well developed eyes (→) and fins (→)	72 HOURS	 Developed digestive system evident by the presence of ball of waste material (→)	 Initiation of development of digestive system (→)
60 HOURS	 Initiation of development of digestive system (→)	 Digestive system not yet initiated	100 HOURS	 Fully absorbed yolk sac (→) with functional digestive system	 Developed digestive system and yolk sac (→) still under absorption

Fig. 1. Differential pattern of embryonic development of rohu (*Labeo rohita*) and grass carp (*Ctenopharyngodon idella*) under hatchery conditions of Tarai region of Uttarakhand during late breeding season.

Fish that have bred at least once in the earlier year have been proved better for breeding work, such fishes are known as professional brood stock [7]. Thus, results from this study on the effect of temperature on hatching rate and rate of embryonic de-

velopment may be useful for commercial fish propagation in the varied climatic conditions in India.

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