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DIFFERENTIAL EMBRYONIC DEVELOPMENT IN ROHU (*LABEO ROHITA*) DURING NORMAL AND LATE BREEDING SEASON UNDER TARAI CONDITIONS OF UTTARAKHAND

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ABSTRACT : The present paper deals the comparison of embryonic development of rohu (*L. rohita*) in normal and late breeding season under Tarai conditions of Uttarakhand with the objective to ascertain the role of most important environmental factor i.e. temperature. The study incorporates the morphological aspects of embryonic development labile to temperature. The fertilized eggs of rohu of the hatchery conditions were used for the study. The present study reveals that the different stages of embryonic development is completed within less time during the normal breeding season as compared to late breeding season. Although, the initial stages of embryonic development takes place within similar time of course in both the conditions of normal breeding season and late breeding season, moreover, the later stages of embryonic development complete within less time in the normal breeding season as compared to late breeding season.

Key words : Embryonic development, Normal and late breeding season, Rohu (*Labeo rohita*).

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. This synchronization, associated with endogenous processes, regulates spawning season and thereby ensures seed production when environmental conditions remain most suitable for their survival. Thus, freshwater carp farming in India relies almost exclusively on larvae production from captive fish under culture condition, particularly during their natural spawning season (Gupta and Rath, 2006).

L. rohita is widely cultured throughout India owing to its high commercial value. Successful induced spawning and multiple breeding in captivity by hypophysation have been achieved in the fish. Moreover, improved brood husbandry earns some degree of control for advancing FOM and spawning time, even 3-4 weeks prior to normal spawning season, when induced with GnRH based hormone under indoor hatchery condition (Naeem *et al*, 2013). As a result, the carp culture farm can begin fry culture earlier in the production season and thereby be able to stock grow-out systems with larger rohu earlier in the season. Such stocking procedures usually result a higher return to the aquaculture industry. Late spawning in carp also provides an opportunity to attain gonadal maturation repeatedly for multiple induced spawning to facilitate higher seed production per unit body weight.

Temperature is the main environmental factor governing the development of fish eggs. It determines certain morphological feature, hatching rate and the behavior of larvae. Temperature is known to influence the efficiency of yolk utilization. Environmental factors may be used during gametogenesis to manipulate fish spawning time in order to get viable gametes on a year-round basis (Chemineau *et al*, 2007).

Induced breeding is a method in which exogenous hormones are injected into the body of mature parent fish for induction of breeding. Induced breeding through hypophysation has been the common practice since the development of the technology in 1957 by Chaudhuri and Alikunhi at Central Inland Fisheries Research sub station, Cuttack (Odisha), several synthetic commercial formulations of purified salmon gonadotropin and dopamine antagonists such as ovaprim, ovatide and wova-FH have also been successfully used in recent years. Environmental and hormonal manipulation of ovulation in the fish have become of practical importance in the fish farming industry for two main reasons: to solve the problem of spawning asynchrony which necessitates frequent brood stock handling and for accelerating or delaying gametogenesis in captive broodstock, spawning may be scheduled to yield fry whenever needed. Use of exogenous hormones is an effective way to induced reproductive maturation and produce fertilized eggs. Ovaprim and ovatide are a kind of analogue of salmon gonadotropin releasing hormone (sGnRHa) with a dopamine blocker. The use of sGnRHa resulted in successful stimulation of ovulation in some cyprinids and catfishes (Sahoo *et al*, 2007). The main objective of this study aimed to examine the variation in embryonic development in rohu during the different seasons *i.e.*, monsoon and post-monsoon and to detect the influence of water temperature on eggs hatching as well as on larval growth in rohu.

MATERIALS AND METHODS

Experimental fish

The experiment was conducted on female gravid rohu of +2 age, collected from brood stock ponds during two different times i.e., monsoon and post-monsoon. The experiments were conducted in Instructional Fish Farm of College of Fisheries, Pantnagar. Fully matured rohu female (average body weight: 1.2 kg) and 2 males (avg body weight: 800 gm) were selected based on the external secondary sexual characters from farm pond of College of Fisheries.

Selection and handling of broodstocks

Good quality and well-matured male and female brood fish were collected and transferred to the cemented holding tanks of the hatchery. The sex ratio of one female to two males were used.

Hormone injection

The fry production in hatcheries is through induced breeding and of seasonal activities during monsoon months. The brood fish were weighed and dosage of Ovatide solution was calculated according to Nandeesha *et al* (1990a) by the following formula :

Quantity to be injected (ml) = Weight of brood fish (kg) × Dosage of Ovatide (ml)

Hormon solution was injected in a single dose. The female is given the 0.25ml/kg body weight and after 5-6 hrs males are given Ovatide dose 0.25ml/kg and female 0.5ml/kg. The water current in the circular pole is created at speed 0.3-0.4m/second. After spawning, eggs were collected for further study.

Fertilization

Soon after release of eggs and sperms fertilization takes place. Micropyle closed after fertilization and egg absorbed water to increase in diameter, water hardening occur to prevent polyspermy. About 39 minutes after fertilization cleavage starts.

Incubation and hatching

As the breeding were conducted in the circular spawning tanks with 2m diameter, hatching occurred after 18hrs (NMS) and 22 hrs (LMS). The newly hatched larvae were kept in the circular spawning tanks with optimum circulation of water and physiochemical parameters then development is observed under microscope.

In general, temperature and DO level play an important role during the breeding season, metabolism and survival of fish. Water quality parameters were observed during normal and late breeding season. DO level was same in both seasons (4-6 mg/L). Ambient temperature was 28.35^{0}C and 30.45^{0}C and water temperature was 26.30^{0}C and 28.23^{0}C in normal and late breeding season respectively.

RESULTS AND DISCUSSION

Embryonic development in rohu was observed from 0 hours to 78 hours and results shows that development of blastodisc started in 4 hours (NBS) and 6 hours (LBS). Aggregation of blastomeres and differentiation of morula stage appears between 6 hours (NBS) and 8 hours (LBS). Development of embryo began in 11 hours. Differentiation of head region is completed in the developing embryo within 9 hours (NBS) and 10 hours (LBS). Body somites and brain vesicles were seen in 12 hours (NBS) and 14 hours (LBS). Well differentiated brain parts became visible in 18 hours (NBS) and 20 hours (LBS)/(photoplate-1), followed by differentiation of eye ball, branchial and vascular bed within 24 hours (NBS) and 26 hours (LBS), Actively functional digestive system was observed within 48 hours (NBS) and 50 hours (LBS), while yolk sac absorption, fin development and chromatophores were found on the head behind the eyes within 78 hours (NBS) and 86 hours (LBS)/(photoplate-1). The fertilized eggs were found adhesive, sticky, demersal and orangish-yellow in colour. The average diameter of egg was 0.8 mm. The fertilized eggs had a spot (blastodisc) on one pole and were readily recognizable through naked eye. A cap like structure was seen over the animal pole, which gradually increased in size. The yolk plug was identified by the completed invasion of the yolk by gradual spreading over the germ layer. At this stage the head and tail end of the embryo was clear and distinguishable. The embryo was elongated and encircled the yolk material. Both tail and head ends were clearly differentiated (Fig. 4 A&B). The twisting movements, which gradually became vigorous and egg capsules were weakened and ruptured. The embryo ruptured the egg shell by the continuous lashing

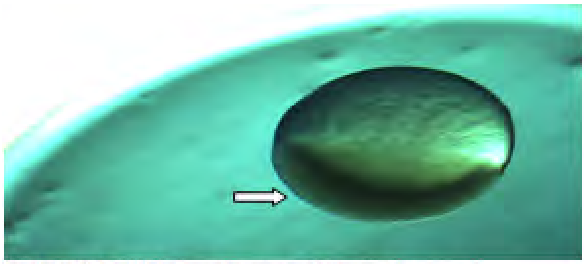

Fig. 1-A (NBS-4Hr) : Development of blasto-disc (⇨).

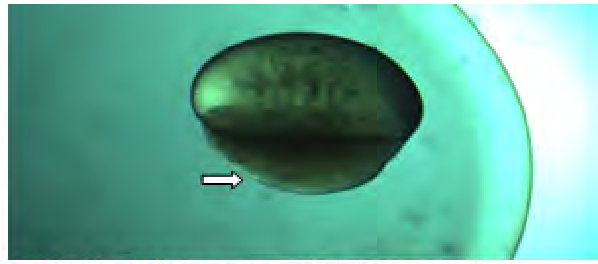

Fig. 1-B (LBS-6Hr): Development of blasto-disc (⇨).

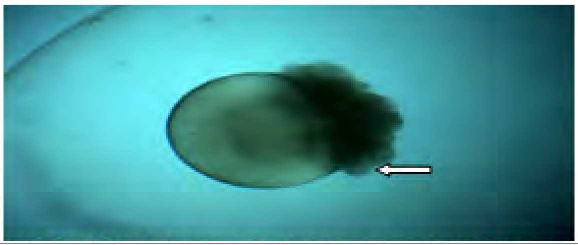

Fig. 2-A (NBS-6Hr): Aggregation of blastomeres and differentiation of morula stage (⇦).

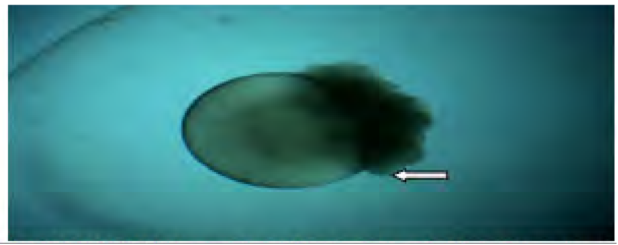

Fig. 2-B (LBS-8Hr): Aggregation of blastomeres and differentiation of morula stage (⇦).

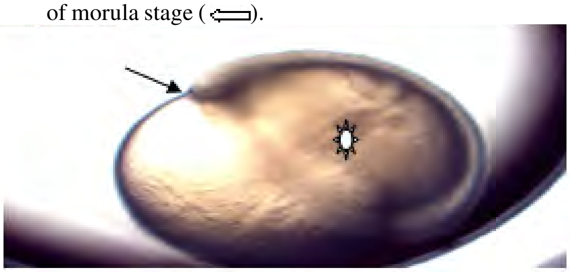

Fig. 3-A (NBS-9Hr): Developing embryo with well differentiated head region (☼) hanging over yolk-sac (➔).

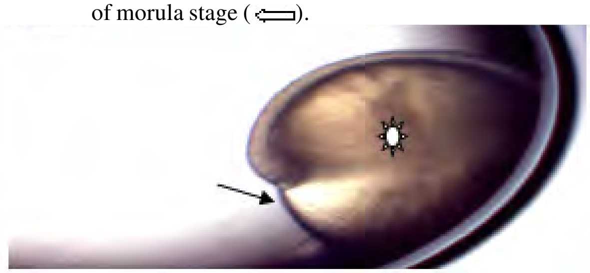

Fig. 3-B (LBS-10Hr): Developing embryo with well differentiated head region (☼) hanging over yolk-sac (➔).

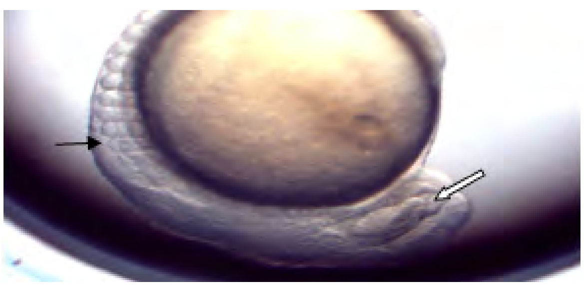

Fig. 4-A (NBS-12Hr): Embryo with differentiation of body somites (➔) and brain vesicles (⇦).

Fig. 4-B (LBS-14Hr): Embryo with differentiation of body somites (←) and brain vesicles (⇨).

Fig. 5-A (NBS-18Hr): Hatchling showing well differentiated brain parts (➔).

Fig. 5-B (LBS-20Hr): Hatchling showing brain parts under differentiation (←).

Photo Plate 1 : Embryonic development in *L. rohita* during normal and late breeding season (0 to 20 hrs).

movement. Hatching took place at 27⁰C in NBS and 29⁰C in LBS. The newly hatched spawn measured 2.0 mm just after hatching. Newly hatched larvae (2.4 mm) were slender, straight and transparent, gradually tapering towards the tail. Yolk sac attached to the body. The heart of the larvae were functional in between head and the anterior margins of the yolk.

During normal breeding season, the embryonic development take place within 78 hrs and during late monsoon season, it take place about 86 hrs. The time difference between normal monsoon season and the late monsoon season is about 8 hrs. The above result shows that development in rohu is better and more efficient during the normal breeding season in comparison to late monsoon season because in normal breeding season, gonads are filled with good quality of eggs having good potential of development but in late monsoon season more number of spent phases present in gonads having inferior quality of eggs and poor potential of development. This study is useful for investigating a fish stock in short and in terms of Fishery biology, it is important to know the egg and larva because these kind of studies are important to determine the spawning seasons and areas, to determine the temporal changes of the spawning season, to predict the ovulating mature stock size, to predict the rate of death of at the end of spawning period and to examine the relation with environment. In the present study, we succeeded in obtaining the larvae of *L. rohita* in NBS and LBS, but in LBS the embryonic development was delayed and yolk absorption process was slow. Due to delayed yolk absorption, organogenesis was also delayed. The fertilized eggs were round, transparent, demersal and adhesive. The colour of the fertilized eggs were orangish-yellow.

The length of the newly hatched larvae of *L.rohita* was 2.4 mm. However, Miah *et al* (2009) found that the newly hatched larvae of Calta (*Catla catla*) were from 4.2 to 4.7 mm in length. The difference of egg diameters was due to seasonal variability and brood size of major carp. The size of the swollen fertilized eggs may vary widely even within species. Therefore, it could not be used as a diagnostic character for distinguishing one from another (Basak *et al*, 2014).The apparent deviation in the size of hatchlings of rohu from that of catla might be related with the size of these species which is much larger than the *L.rohita*. In the larval stage, the development of caudal fin bud carried out in 18 hrs (NBS) and 20 hrs (LBS) after hatchling in rohu was similar to *C. mrigala* (Yesmin *et al*, 2014). The development of the ventral and horizontal embryonic fin fold of *C.mrigala* was more prominent which was similar to this study. In the larval stage 12 hours after hatching, body somites and brain parts appered, which were similar to *C. mrigala* and *Catla catla* (Parichy *et al*, 2009).

Ovatide has been used to successful spawning of many commercially cultured fish including Indian major carps (Marimuthu *et al,* 2009). In the present study, Ovatide dose at 0.25ml/kg to male and 0.5ml/kg to female was sufficient for spawning in rohu in both monsoon and late-monsoon months. However, the responsiveness of post-monsoon female rohu in terms of egg production, quality of eggs, embryonic development and hatchlings were significantly lower when compared with the response parameters in monsoon bred rohu. Late induced spawning in captive rohu often encounters with reduced spawning performances and devaluation of final product. This variability in maturational status influences the progress of final oocyte maturation in rohu and thereby may be responsible for deteriorations in the production as well as quality of eggs. A similar observation was made earlier in silver carp during the post spawning period, when oocytes were subjected to in-vitro induction for final oocyte maturation (Dasgupta *et al*, 2009). The factors implicated as likely determinant of spawn production were egg size and hatchling length. Smaller eggs with late embryonic development produced during post monsoon experience increased mortality as reported in Rainbow trout (Faulkner *et al.*, 2008). Interestingly, the post monsoon embryos showed higher developmental failure particularly between embryonic streak and tail bud stage than that noted in monsoon embryos. Such failure may lead to the manifestation of certain abnormalities in later stages of development in carp. Teji *et al* (2006) reported that the release of post mature eggs and genetically impaired eggs might be responsible for such developmental failure as evident in Silver carp and White amur. Although larger eggs produce significantly bigger hatchlings. The embryonic and larval development of rohu were studied at an ambient temperature of 28°C-30°C and water temperature of 26°C-28°C. The rate of larval development varied in other species. This variation is thought to be temperature dependent, the higher the temperature the quicker was the development.

This study clearly demonstrates that ovatide is efficient for spawning in rohu in both normal and late breeding season. However, late spawning in rohu resulted in late and delayed embryonic development. Use of ovatide prior to spawning is enhance reproductive performance in terms of quantity and quality of egg and larvae productions. Thereby, the rohu fry production could be done as late as October, allowing public and private hatcheries to produce rohu fingerlings ensuring a reliable

Fig. 6-A (NBS-24Hr): Hatchling showing differentiated eye ball (⟶) and well differentiated branchial (⟹) and vascular bed of vessels particularly dorsal aorta (⟼).

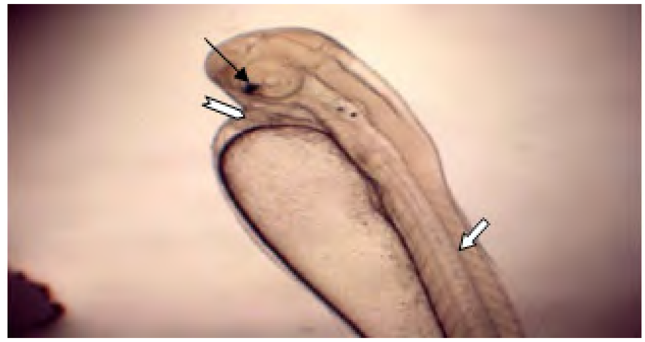

Fig. 6-B (LBS-26Hr): Hatchling with differentiated eye ball (⟵) and but branchial (⟹) and vascular bed of vessels particularly dorsal aorta (⟼) is not well differentiated.

Fig. 7-A (NBS-48Hr): Hatchling showing well developed and functional digestive system as by presence of ball of waste material (⟶) and smaller size yolk sac (✱).

Fig. 7-B (LBS-50Hr): Hatchling showing digestive system under development (⟶) and larger size of yolk (✱).

Fig. 8-A (NBS-68Hr): Hatchling showing actively functional digestive system by presence of fibrous waste material being solid (⟶) and rudimentary yolk sac (✱).

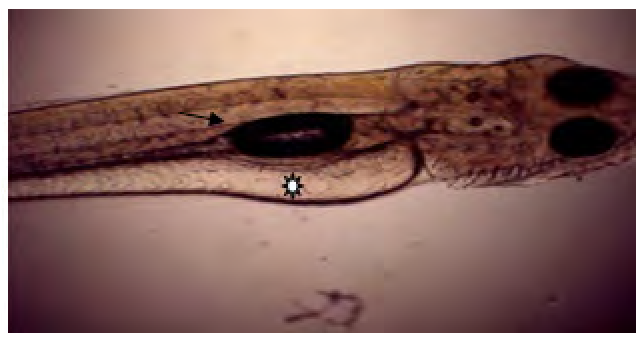

Fig. 8-B (LBS-74Hr): Hatchling showing functional digestive system by presence of ball of waste material (⟶) and visible yolk sac (✱).

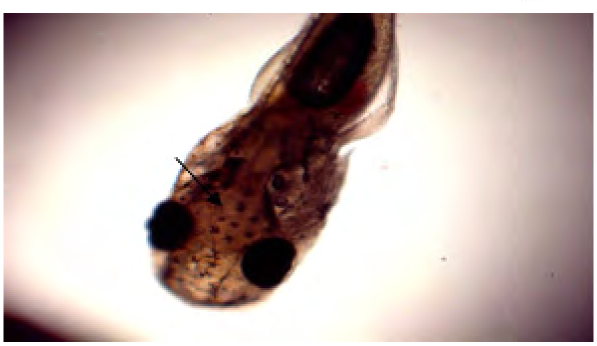

Fig. 9-A (NBS-78Hr): Hatchling showing complete yolk sac absorption, fin development and intense body pigmentation (⟶).

Fig. 9-B (LBS-86Hr): Hatchling showing presence of rudimentary yolk sac (✱), fin development and poor body pigmentation (⟶).

Photo Plate 2 : Embryonic development in *L. rohita* during normal and late breeding season (24 to 86 hrs.

and steady source of stocking materials for the grow-out system earlier in the season.

REFERENCES

Basak S K, Basak B, Gupta N, Rahman S M M and Haque M M (2014) Embryonic and Larval Development of Silver Barb (*Barbodes gonionotus*) in a Mobile Hatchery under Laboratory Condition. *IOSR J. Agri. Vet Sci.* **7,** 81-90.

Chemineau C M, Fostier A and Zanuy S (2007) Broodstock management and hormonal manipulations of fish reproduction. *Gen. Comp. Endo*. **165**, 516-534.

Dasgupta S, Sarkar SK, Niranjan S and Bhattacharya S (2009) Variation in spawning responses, egg and larvae production from induced rohu (*Labeo rohita*) during pre-monsoon seasons: relationship with hormonal changes and oocyte responsiveness during final maturation. *Aquaculture* **290**, 320-326.

Faulkner S G, Welz M, Tonn W M and Schmitt D R (2008) Effects of Simulated Blasting on Mortality of Rainbow Trout Eggs. *Trans. Am. Fish. Soc.* **137**, 1–12.

Gupta S D and Rath S C (2006) Carp breeding and seed production. In: Ayyappan, S. (Editor), Handbook of Fisheries and Aquaculture. DIPA, *I. Con. Agri. Res.* 248–264.

Marimuthu K, Haniffa M A and Rahman A (2009) Spawning performance of native threatened Spotted Snakehead Fish, *Channa punctatus* (Actinoptergii: Channidae: Perciformes), induced with ovatide. *ACTA Ich. et. Pis.* **39,** 1-5.

Miah M L, Harun M S, Rahman M M, Haque M R and Hossain M A (2009) Study on the embryonic and larval development of an endangered species of bata (*Labeo bata*). *Int. J. Sustain. Crop prod.* **4,** 72-82.

Naeem M, Zuberi A, Ashraf M, Ahmad W, Ishtiaq A and Hasan N (2013) Induced breeding of *Labeo rohita* through single application of ovaprim-C at Faisalabad Hatchery, Pakistan. *Afri. J. Biol.* **12,** 2722-2726.

Nandeesha M C, Rao K G, Jayanna R, Parker N C, Varghese T J, Keshavanath P, Shetty H P C (1990a) Induced spawning of Indian major carps through single application of Ovaprim. In: Hirano R, Hanyu M (eds). The second Asian Fisheries Forum, *A. Fish. Soc. Philippines* 581-585.

Parichy D M, Elizondo M R, Mills M G, Gordon T N and Engeszer R E (2009) Normal Table of Post-Embryonic Zebrafish Development: Staging by Externally Visible Anatomy of the Living Fish. *Dev Dyn. Dec.* **238,** 2975–3015.

Sahoo S K, Giri S S, Chandra S and Sahu A K (2007) Effect of Ovaprim doses and latency period on induced spawning of *Clarias batrachus*: Observation on larval deformity. *Indian J. Experiment Biol.,* **45,** 920-922.

Teji K T and Thomas K J (2006) Observations on the morphological abnormalities in induced bred larvae of some freshwater fishes. *Indian J. Fish*. **53,** 353-358.

Yesmin R, Sume S A, Haque M H, Sultana N and Khan G Q (2014) Induced breeding of endangered Striped Dwarf Catfish (*Mystus vittatus*) and its embryonic and larval development. *Res. Agric. Livest. Fish.***1,** 1-9.