

Effect of incubation temperature on embryonic development of *Labeo rajasthanicus*

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ABSTRACT

The present work aimed to investigate the effect of incubation temperature on the embryonic development of the minor carp *Labeo rajasthanicus*. To assess the embryonic development of *L. rajasthanicus*, eggs were incubated at five different temperatures (22, 26, 30, 34, and 38°C). Developmental stages were monitored by sampling embryos in these five different temperatures at particular intervals. The results showed that the eggs incubated at 38°C did not develop beyond the two-cell stage. The maximum duration for hatching and times to reach different life stages was observed at 22°C followed by 26°C. Hatching of eggs was observed after 20.27±0.29, 17.48±0.16, 15.03±0.13, and 17.13±0.20 hours of fertilization at 22, 26, 30, and 34°C respectively. The hatching time and time to attain different ontogenic stages were decreased with increased temperatures. This study demonstrated that incubation temperature significantly ($p < 0.05$) influenced hatching duration and ontogenic development in *L. rajasthanicus*.

INTRODUCTION

Temperature is an important environmental factor that influences the development of living organisms including fish. During embryonic development water temperature affect the organ, morphological and physiological development of the fish embryo and determine hatch success and incubation time (Geffen *et al.*, 2006; Kupren *et al.*, 2011). Incubation temperature may also have long-lasting effects on the development stages (Eriksen *et al.*, 2008). It has also been suggested that fish embryos have low thermal stability at early stages and then stability increases until the end of development, followed by a low stability again closer to hatching (Brooks *et al.*, 1997). Although the reason for this phenomenon is not clear, it has been suggested that changes in the pattern of gene

expression can be induced in cells and tissues by small temperature changes which could affect the development of the embryo (Macqueen *et al.*, 2007). The effects of the temperature increase during egg incubation could be both species and stage specific. In whitefish, *Coregonus lavaretus*, an increase in egg incubation temperature at the 4-cell stage resulted in more developmental abnormalities than when the increase in temperature occurred at the gastrula stage (Cinga *et al.*, 2010). *Labeo rajasthanicus* usually known as sarsi is one of the important minor carp native to south Rajasthan (India). This species has potential for inclusion in composite fish culture (Lal *et al.*, 2015). However, there is limited information about the optimum rearing condition, especially for temperature requirements of eggs and larvae.

Therefore, the aim of the present study was to investigate effect of incubation temperature on embryonic development of *L. rajasthanicus*.

MATERIALS AND METHODS

Collection of experimental fish

Active and disease free farm raised fishes were collected from farm ponds of Research Station, MPUAT, Udaipur. Fishes were treated with 5 % KMnO₄ to disinfect and were kept in F.R.P. tanks (2m x 1m x 1m) for conditioning. These fishes were fed with commercially formulated feed at 2-3 % of their body weight. Feeding was done twice in a day. First feeding was done in the morning time and second in the evening time.

Brood fish selection

Fully matured male and female fishes were selected for breeding purpose. The potential brood stock was selected on the basis of secondary sexual characteristics. Mature fish showed sexual dimorphism; pectoral fin of the male become rough, pointed and narrow genital papilla with freely oozing milt when slight pressure is applied on to the abdomen. Genital papilla was swollen and slightly pinkish in colour with the smooth pectoral fin. Abdomen of the female was bulgy.

Induced breeding

The mature brood stock of *L. rajasthanicus* was injected 0.2 ml/kg body weight of Gonopro-FH (selected through preliminary experiments). Injected brood fishes were divided in five groups stocked in FRB tanks (1x1x1 m) having different temperatures (22, 26, 30, 34 and 38°C). The eggs obtained from individual treatment were stocked (approximately of 1000 fertilized eggs) in glass aquarium for hatching. Water in aquaria was assigned to temperature treatments of 22, 26, 30, 34 and 38°C. The temperature was thermostatically controlled by electric heaters adjusted with thermostat. All the aquariums were aerated and kept in air conditioned room.

Embryonic development

The developmental stages of eggs were observed and recorded from fertilization to until every egg in each of the 5 water temperature treatments hatched. The dead eggs

were removed from the aquarium to prevent contamination of the water. Microscopic observations of samples of 4–5 eggs were made using a light microscope (Leica) and photographed with digital camera to determine the development stages. Based on the data obtained from those pictures and records of time, the hours of each development stage and hatching times from fertilization were calculated.

RESULTS

Description of embryonic development is presented in **Table (1)** and **Fig. (1)**. The fertilized eggs were demersal, adhesive and blue-green in colour. The average diameter of the egg was 3.14 ± 0.07 mm. embryonic developments until hatching for *Labeo rajasthanicus* was divided into 8 stages as described below:

Two cell stage: After fertilization, the first division occurred at 0.17 (22⁰C), 0.14 (26⁰C), 0.12 (30⁰C), 0.11 (34⁰C) and 0.17 hr. (38⁰C), and two cells were distinguishable in the animal pole. This stage lasts 0.10 to 0.12 hrs until the formation of four cells. However, at 38⁰C incubation temperature no further development was observed due to egg mortality.

Four cell stage: This stage was occurred after 0.22 to 0.29 hrs of fertilization (0.29-22⁰C, 0.25-26⁰C, 0.22-30⁰C & 0.24 hr-34⁰C) when four cells were formed. This stage lasts 0.12-0.15 hr. until the formation of eight cells.

Table 1: Characteristics of different ontogenic stages of *Labeo rajasthanicus*

Stage	Characteristics	Reference Fig. (1)
Fertilized egg	Egg spherical, blue-green zygote, demersal and adhesive	A
Two cell stage	Division of blastodisc into two blastomeres	B
Four cell stage	Four blastomeres	C
Eight cell stage	Eight blastomeres	D
Morula	Blastomere accumulate at animal pole	E
Gastrula	Yolk was invaded by blastoderm by spreading over it, embryonic shield was clearly visible	F
Appearance of Somites	Tail and head portions of embryo are distinguishable	G
Twitching stage	Twitching movements started	H
Hatching	4.19 ± 0.03 mm, light black in colour, straight, slender	I
Spawn	7.21 ± 0.06 mm in length and 0.34 ± 0.02 mg in weight	J

Eight cell stages: The formation of eight cells at different incubation temperature was observed at 0.44, 0.38, 0.34 & 0.36 hrs in 22, 26, 30 & 34⁰C, respectively. This stage lasts for 2.41 to 3.24 hrs until the formation of morula. During this period (2.41 to 3.24hrs) the fertilized eggs also passed through sixteen, thirty two, sixty four and multiple cell stages before the morula stage.

Morula: The morula stage was observed after 2.75- 3.68 hrs (3.68-22⁰C, 3.17-26⁰C, 2.75-30⁰C & 3.02 hr-34⁰C) after fertilization (**Table 2**). This stage lasts 6.9 to 9.45 hrs at different incubation temperatures until the formation of gastrula.

Gastrula: This stage was observed after 9.65- 13.13 hrs (13.13-22⁰C, 11.23-26⁰C, 9.65-30⁰C and 10.04 hrs-34⁰C) of fertilization. At this stage blastoderm started invading the yolk by spreading over it in the form of thin layer. The formation of germinal ring around yolk was clearly visible and that about half of the yolk occupied by blastoderm. Blastoderm covered three fourth of the yolk and embryonic shield was clearly visible.

Appearance of somites: The yolk plug was identified by completed invasion of yolk within 10.70- 14.15 hrs (14.45-22⁰C, 12.45-26⁰C, 10.70-30⁰C and 12.45 hrs -34⁰C) after fertilization by gradual spreading over the germ layer. At this stage the head and tail end of embryo was clear and distinguishable.

Table 2: Summary for attaining different ontogenic stages of *Labeo rajasthanicus* eggs at different incubation temperatures (*Values are expressed as mean ± SE*)

No	Stage	Time (hrs.) for embryonic development at different temperature				
		22 ⁰ C	26 ⁰ C	30 ⁰ C	34 ⁰ C	38 ⁰ C
1	Fertilized egg	0:00±0.00	0:00±0.00	0:00±0.00	0:00±0.00	0:00±0.00
2	Two cell stage	0.17±0.02	0.14±0.01	0.12±0.03	0.11±0.01	0.21±0.02
3	Four cell stage	0.29±0.01	0.25±0.01	0.22±0.08	0.24±0.01	*
4	Eight cell stage	0.44±0.03	0.38±0.13	0.34±0.01	0.36±0.04	*
5	Morula	3.68±0.11	3.17±0.09	2.75±0.05	3.02±0.02	*
6	Gastrula	13.13±0.19	11.23±0.11	9.65±0.17	10.04±0.07	*
7	Appearance of Somites	14.45±0.08	12.45±0.07	10.70±0.30	12.45±0.12	*
8	Twitching stage	16.44±0.18	14.30±0.09	12.44±0.06	13.40±0.31	*
9	Hatching	20.27±0.29	17.48±0.16	15.03±0.13	17.13±0.20	*
10	Spawn	61.53±0.31	52.15±0.25	48.41±0.19	50.03±0.61	*

*: Complete mortality of Eggs at two cell stage

Values with same superscripts in a column are not significantly different-Duncan's multiple range test, p<0.05

Twitching stage: This stage was obtained in 12.44 to 16.44 hr. At this stage embryo started occasional twisting movement. The tail gradually detached from yolk mas. The twisting movements, which gradually became vigorous and egg cell was weakened. This stage lasts for 2.59 to 3.83 hrs in different incubation temperatures until the hatching took place.

Hatching: The embryo ruptured the egg shell by the continuous movement. The hatching started at 15.03-20.27 hours (20.27-22⁰C, 17.48-26⁰C, 15.03-30⁰C and 17.13 hrs-34⁰C) after fertilization. Hatching was continued for 2-3.5 hrs because the entire embryo did not hatch out at a time. The newly hatched larvae measured 4.19±0.03 mm. After hatching,

the yolk-sac larvae were evenly distributed and positioned upright in the water column. The first larval movements occurred immediately after hatching, and the newly hatched larvae responded to water movements by swimming in a spiral fashion and short horizontal movements

DISCUSSION

In the present study, the two cells, four cells and eight cells stage of *Labeo rajasthanicus* were found within 0.11-0.17, 0.22-0.29 and 0.34-0.44 hr after fertilized, respectively. In *Labeo rohita*, the same series occurred at 35, 45 and 70 minutes after fertilization (Mookerjee, 1945). According to Rahman (1975) the same developmental stages appeared in case of *Anabus testidineus*, after 15, 20 and 45 minutes of fertilization, respectively. Morula stage was found in 2.75-3.68 hr after fertilization where Mookerjee (1945) observed the same stage in *L. rohita* five hours and 45 minutes after fertilization. The gastrula stage was observed in *L. bata* at 8.30 to 12.00 hours after fertilization of egg at 30.5°C. This variation might be due to species difference and temperature.

For successful hatchery production of carps, the knowledge on the effects of temperature during embryogenesis is a prerequisite. Both organogenesis and somatic growth are controlled by enzymatic activities. Embryonic development mainly depends on the differential expression of certain genes and temperature (Ojanguren & Brana, 2003). At the point of hatching of embryos may be monitored in respect to yolk absorption, circulation system, etc. (Luczynski *et al.*, 1984). In the present study, yolk sac absorption was faster at higher incubation temperature (34°C). However, hatching cannot be defined as a physiological stage (Luczynski & Kolman, 1987). The relationship between incubation time and temperature has been reported for other tropical finfishes; *Carnax mate* (Santerre, 1976), *Polydactylus sexifilis* (Santerre & May, 1977), *Mugil cephalus* (Walsh *et al.*, 1991). From our study, the optimal temperature for incubating *L. rajasthanicus* eggs is recorded at 26°C considering rate of development and hatching percentage, which is higher than earlier reports on *L. rohita* (Ponnuraj *et al.*, 2002). The complete mortality of eggs after two cell stage at 38°C suggest that the thermal limit for embryonic development of *L. rajasthanicus* is below 38°C. Overall results suggest that early embryonic stages are steno thermal in comparison to the advanced ontogenic phases of *L. rajasthanicus*, as reported in earlier studies (Elliott, 1981 and Cossins & Bowler, 1987).

From the point of fertilization until hatching, low temperatures retard and high temperatures accelerate embryonic development (Lasker, 1964; Blaxter, 1981; Pepin, 1991; Hamel *et al.*, 1997), which is consistent with our findings at five different temperatures (22, 26, 30,34 and 38°C). Time required to reach a given ontogenic stage was progressively longer at 22°C presumably due to lower metabolic rate and embryonic development. The developmental failure of embryos in later at 38°C suggests that this rearing temperature is well above the tolerance limit for development of *L. rajasthanicus*

eggs (Reddy & Lam, 1991). Overall results suggest that 26°C is the ideal temperature for egg incubation of *L. rajasthanicus* for faster embryonic development, better hatching percentage and least time duration for attaining given ontogenic stages. These results may be a prelude to effectively utilize the benefits of temperature on better hatching rate and reduced hatchery man-days and ultimately the cost of production in carp hatcheries. However, hatchery seed production of *L. rajasthanicus* is recommended between 26 and 30°C. This study reveals that *L. rajasthanicus* embryos can accommodate climatic changes due to global warming up to 34°C, without hampering the reproduction and embryonic development.

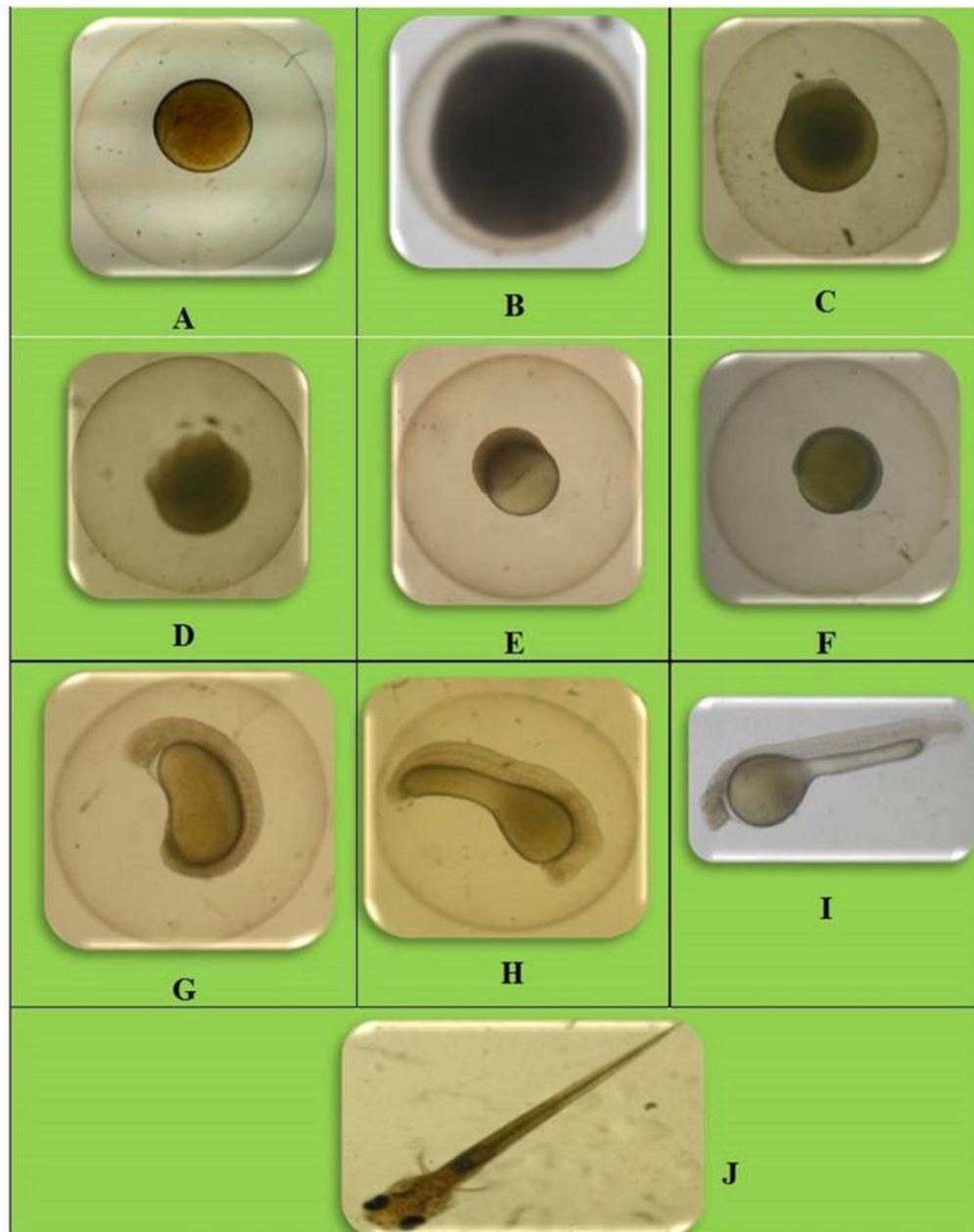


Fig.1: Fertilized egg & differential embryonic development stages of *Labeo rajasthanicus*

CONCLUSION

The mortality of embryos after two cell stage at 38°C suggest that this rearing temperature is well above the tolerance limit for development of *L. rajasthanicus* eggs. A higher hatching and hatchling survival rate at 22-30°C suggests that this temperature range is most suitable for incubation. Overall results suggest that 26°C is the ideal temperature for egg incubation of *L. rajasthanicus* for faster embryonic development, better hatching percentage and hatchling survival. Thus the hatchery seed production of *L. rajasthanicus* is recommended between 22 and 26°C.

ETHICS STATEMENT

The study including all stages of fish handling and rearing was approved by the University Zonal Research Committee, MPUAT Udaipur (India). The experimental fish *L. rajasthanicus* is not an endangered fish; hence the rules of the Govt. of India's Wildlife Protection Act of 1972 are not applicable for experiments on this fish.

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