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Aquaculture 255 (2006) 536–541
Aquaculture
www.elsevier.com/locate/aqua-online

Thermal dependence of embryonic development and hatching rate in *Labeo rohita* (Hamilton, 1822)

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Received 9 September 2004; received in revised form 14 January 2006; accepted 17 January 2006

Abstract

The present study was designed to assess the embryonic development of *Labeo rohita* fertilized eggs incubated at four different temperatures (26, 31, 33 and 36 °C) until hatching. Fertilization was carried out at ambient temperature (26 °C) and was considered as 0 h of embryonic development. Developmental stages were monitored by sampling embryos in different temperatures at particular intervals. Highest hatching percentage and least time for attaining each ontogenic stage were observed at 31 > 33 > 26 > 36 °C and were significantly different ($p < 0.05$). The lowest hatching percentage and maximum time duration for attaining a given ontogenic stage were observed at 36 °C; this treatment also resulted in malformed embryos. Our study demonstrated that incubation temperature significantly ($p < 0.05$) influences hatching duration, hatching rate and survival of *L. rohita* eggs.

Keywords: Hatching rate; Embryonic development; Temperature; *L. rohita*

1. Introduction

Emission of greenhouse gases and carbon dioxide is expected to increase global mean temperature by 1.5 to 4.5 °C over the next half century (Hounghton and Woodwell, 1989) and its impact on freshwater and marine fish is immense, as most of the fishes are poikilothermic in nature. Therefore, the rate of their biological functions is critically dependent on environmental temperature. Thermal tolerance of aquatic animals is also dependent on acclimation temperature and duration of acclimation (Manush et al., 2004). Over the years, attention has been focused on the thermal tolerance of embryos and larvae (Houde, 1989; Pepin, 1991). Embryos and larvae are more sensitive to temperature changes than adult fishes (Brett, 1970). It is evident that embryos of temperate species are more sensitive to temperature than embryos of tropical species (Hokanson and Kleiner, 1974; Irvin, 1974; Budington et al., 1993). It is reported that upper lethal temperatures of embryos, larvae (Subasinghe and Sommerville, 1992) and adults (Allanson and Noble, 1964) of the freshwater Mozambique tilapia (*Oreochromis mossambica*) vary in the range of 2 °C among different life stages.

Embryonic development is a complex process in which cellular differentiation and proliferation occur simultaneously though their rate is different (Gould, 1977; Hall, 1922). Temperature directly influences the developmental rate and development is faster at

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doi:10.1016/j.aquaculture.2006.01.013

increasing temperatures. However, this increase of developmental rate of embryos with increase in temperatures occurs only within the acceptable thermal limits (Cossins and Bowler, 1987; Atkinson, 1996). In general, thermal limits are narrower for early stages and reduced survival of embryos and juveniles can occur at temperatures within the tolerance range for adults (Elliott, 1981; Cossins and Bowler, 1987). Acclimation temperature or thermal history may also affect the temperature tolerance of embryos.

India is the second largest producer of fish in the world from inland aquaculture after China (FAO, 2001). Intensification of freshwater aquaculture practices in India has increased the production of Indian Major Carps, Rohu (*Labeo rohita*), Catla (*Catla catla*) and Mrigal (*Cirrhinus mrigala*). Indian Major Carps contribute 90% to the total aquaculture production (FAO, 2001). *L. rohita* is one of the preferred Indian Major Carps, widely distributed throughout India. With huge potential for growth, *L. rohita* is considered a major component of fish culture. Ever since the success of induced breeding in Indian Major Carps, attempts have been made over the decades to improve the production of carps through selective breeding and hybridization in India (Chaudhuri, 1971). Temperature is considered one of the main environmental factors (Gadomski and Caddell, 1991) influencing hatching percentage and survival of embryo and larvae. Therefore, the present study was designed to assess the embryonic development of *L. rohita* eggs at four different incubation temperatures. Thus, results from this study on the effect of temperature on hatching rate and rate of embryonic development may be useful for commercial fish propagation in the varied climatic conditions in India. This laboratory work may serve as a prelude to the possible effects of global warming and thermal challenge on embryonic development in enclosed freshwater bodies.

2. Materials and methods

2.1. Experimental animals

Ten pairs of *L. rohita* male (0.87±0.05 kg) and female (1.36±0.05 kg) broodstock were induced to breed by injecting with Ovatide (Hemmo Pharma, Mumbai) at the rate 0.3 ml/kg for females and 0.2 ml/kg for males. Ovatide is a synthetic fish hormone, which contains 20 μg of Gonadorelin A (D Arg^{6} Pro-NHET^{9} Des-Gly^{10} sGnRH) and 10 mg of domperidone/ml. Subsequently, spawners were maintained in separate breeding hapas or tanks and were taken out for stripping after 12 h. In natural conditions, Indian Major Carps breed in rivers or enclosed water bodies. In our study, the embryonic development of fertilized eggs was monitored in laboratory conditions at different temperatures, which necessitated stripping procedures. Eggs and milt from all the broodstock were collected by gentle stripping into clean and dry porcelain trays and pooled together to ensure genetic variability. Pooled eggs and milt were mixed gently by using a quill feather for effective fertilization in each tray. Fertilized eggs were water hardened by mixing with clean and well aerated freshwater at ambient temperature (26 °C).

2.2. Experimental design

Pooled swollen or water-hardened eggs (approximately 600 eggs) were distributed uniformly into a temperature controlled Thermo Lab (GFL 1070, Germany) each equipped with four different chambers containing 3.5 l of water (having individual temperature control at a precision of 0.01 °C) at four test temperatures (26, 31, 33 and 36 °C). A uniform flow rate of 0.5 L min^{-1} was maintained in incubation chamber until hatching. Fertilized eggs were kept in suspension by using a sieve made of bolting silk. Continuous aeration was provided in the chambers to maintain optimum dissolved oxygen level (>5 mg L^{-1}) and the pressure of air blower was adjusted as to maintain eggs in suspension. A fixed photoperiod of 12L : 12D (Light : Dark) was maintained with light exposure from 6.00 to 18.00 h. Eggs were taken out from each chamber at various time intervals and ontogenic stages were observed under a light microscope (Olympus, Japan) coupled with a camera. Each temperature treatment was repeated under similar conditions four times (considered as replicates) using fertilized eggs from separate brood stock.

2.3. Statistical analysis

Data on embryonic development of *L. rohita* embryos incubated at four test temperatures were analyzed by one-way analysis of variance (ANOVA) using a standard statistical package (SPSS, version 11.0). Tukey's multiple range test was carried out for post hoc mean comparisons ($p<0.05$).

3. Results

Data pertaining to the effect of incubation temperature on ontogenic developmental stages are presented in

Table 1
Time (in hours) for attaining different ontogenic stages of *L. rohita* eggs (until hatching) at four different incubation temperatures (26, 31, 33 and 36 °C)

Developmental stages	Time (hours) for embryonic development at different temperatures			
	26 °C	31 °C	33 °C	36 °C
2 cell stage	$0.22^{a}\pm0.03$	$0.21^{a}\pm0.02$	$0.24^{a}\pm0.03$	$0.49^{b}\pm0.02$
4 cell stage	$0.35^{a}\pm0.02$	$0.28^{a}\pm0.01$	$0.34^{a}\pm0.03$	$0.62^{b}\pm0.05$
8 cell stage	$0.46^{a}\pm0.02$	$0.36^{a}\pm0.02$	$0.45^{a}\pm0.04$	$0.71^{b}\pm0.08$
16 cell stage	$1.47^{a}\pm0.11$	$0.48^{b}\pm0.03$	$0.84^{b}\pm0.21$	$1.37^{a}\pm0.05$
32 cell stage	$2.52^{a}\pm0.20$	$1.23^{b}\pm0.10$	$1.60^{b}\pm0.21$	$1.86^{ab}\pm0.13$
Morula stage	$2.68^{a}\pm0.18$	$1.75^{c}\pm0.17$	$1.82^{bc}\pm0.14$	$2.48^{ab}\pm0.14$
Yolk plug formation	$4.45^{a}\pm0.15$	$2.31^{b}\pm0.09$	$2.67^{b}\pm0.21$	$3.48^{c}\pm0.11$
Yolk sac elongation stage	$5.81^{a}\pm0.11$	$3.93^{b}\pm0.16$	$4.00^{b}\pm0.21$	$4.43^{b}\pm0.21$
6 somite stage	$7.95^{a}\pm0.19$	$4.91^{b}\pm0.15$	$5.68^{b}\pm0.17$	$7.21^{a}\pm0.47$
Tail bud formation	$10.52^{a}\pm0.10$	$7.70^{b}\pm0.21$	$7.96^{b}\pm0.34$	$9.02^{ab}\pm0.65$
Tail formation	$11.80^{ac}\pm0.23$	$10.58^{d}\pm0.20$	$11.03^{cd}\pm0.30$	$12.75^{a}\pm0.26$
Commencement of hatching	$12.75^{a}\pm0.17$	$11.50^{a}\pm0.17$	$11.81^{a}\pm0.29$	$14.93^{b}\pm0.63$
Hatching completed	$13.82^{a}\pm0.17$	$12.89^{a}\pm0.30$	$12.97^{a}\pm0.23$	$15.35^{b}\pm0.29$

Different superscripts (a, b, c) in the same row indicate significant difference amongst different incubation temperatures ($p<0.05$). Values are expressed as mean±SE ($n=4$).

Table 1 and Fig. 1. Embryos of *L. rohita* completed their development in the least time at 31 °C followed by 33, 26 and 36 °C. Maximum duration of hatching at 36 °C was significantly ($p<0.05$) longer than the other temperature treatments (Table 1). Data pertaining to the duration of embryonic development of *L. rohita* fertilized eggs

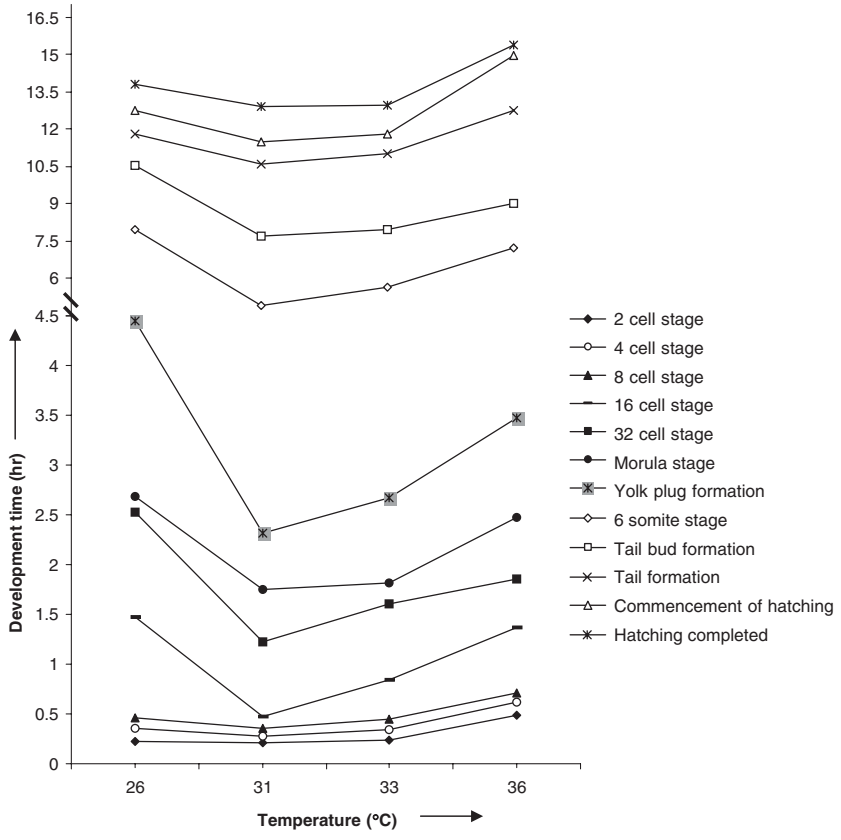

Fig. 1. Developmental stages of *L. rohita* eggs incubated at different temperatures (26, 31, 33 and 36 °C).

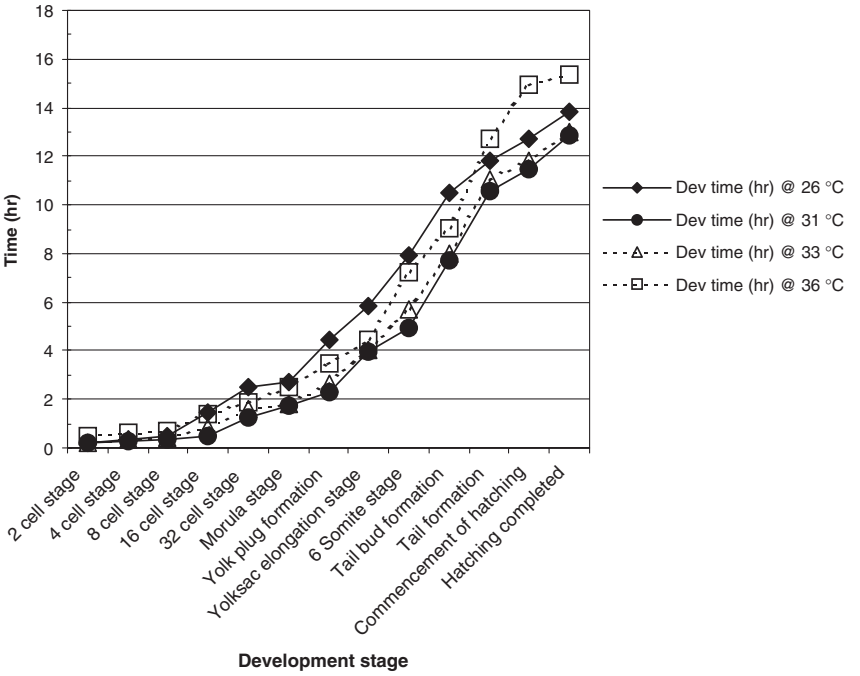

Fig. 2. Time (hours) for embryonic development of *L. rohita* eggs at different incubation temperatures (26, 31, 33 and 36 °C).

incubated at four different temperatures is represented in Fig. 2. However, the embryos incubated at 36 °C hatched with gross morphological abnormalities, which resulted in a drastic reduction in the rate of survival. Minimum duration for hatching and times to reach different life stages were observed at 31 °C followed by 33 and 26 °C except at the two-cell stage (Table. 1). However, hatching percentage at 33 °C was significantly ($p<0.05$) less than at 26 and 31 °C (Fig. 3). At the time of hatching, yolk absorption was evident until 33 °C and the size of yolk sac at three different temperatures (26, 31 and 33 °C) was 2.01±0.018, 2.00±0.01 and 1.8±0.023 mm, respectively.

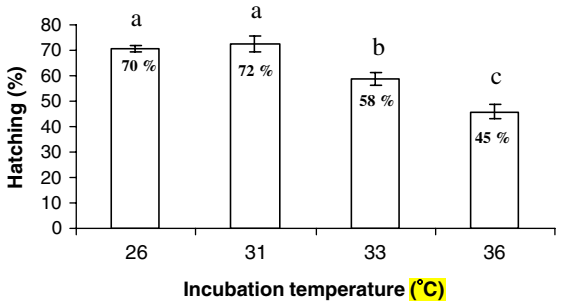

Fig. 3. Hatching percentage of *L. rohita* eggs at four different incubation temperatures (26, 31, 33 and 36 °C). Different superscripts (a, b, c) indicate significant difference amongst different incubation temperatures ($p<0.05$).

4. Discussion

Knowledge on the effects of temperature during embryogenesis is a prerequisite for successful hatchery production of carps. Both organogenesis and somatic growth are controlled by enzymatic activities. Embryonic development of ectotherms mainly depends on the differential expression of certain genes and temperature (Ojanguren and Brana, 2003). At the point of hatching of embryos may be monitored in respect to yolk absorption, circulation system, gill development, ability to swim and readiness to eat as reported in eleuthroembryos (Luczynski et al., 1984). In the present study, yolk sac absorption was faster at higher incubation temperature (33 °C). However, hatching cannot be defined as a physiological stage (Luczynski and Kolman, 1987). The relationship between incubation time and temperature has been reported for other tropical finfishes; *Carnax mate* (Santerre, 1976), *Polydactylus sexifilis* (Santerre and May, 1977), *Mugil cephalus* (Walsh et al., 1991). From our study, the optimal temperature for incubating *L. rohita* eggs is recorded at 31 °C considering rate of development and hatching percentage, which is higher than earlier reports on *L. rohita* (Ponnuraj et al., 2002). This may be due to adaptive response of *L. rohita* embryos evolved over the years due to global warming and climatic changes. The lowest hatching percentage and gross morphological abnormalities at 36 °C suggest

that the thermal limit for embryonic development of *L. rohita* is below 36 °C. However, some of the embryos reared at 36 °C reached relatively advanced developmental stages in a short time (Table 1), in spite of gross abnormalities. Embryonic development in fishes is dependent on many factors in addition to temperature, pH and dissolved oxygen. Minor variation in dissolved oxygen is very likely at higher incubation temperatures (36 °C) in spite of external aeration, which could be a reason for early hatching of premature embryos at 36 °C. Overall results suggest that early embryonic stages are steno thermal in comparison to the advanced ontogenic phases of *L. rohita*, as reported from our earlier findings (Das et al., 2004; Chatterjee et al., 2004) and agrees with similar studies in other species (Elliott, 1981; Cossins and Bowler, 1987).

From the point of fertilization until hatching, low temperatures retard and high temperatures accelerate embryonic development (Lasker, 1964; Blaxter, 1981; Pepin, 1991; Hart and Purser, 1995; Hamel et al., 1997), which is consistent with our findings at four different temperatures (26, 31, 33 and 36 °C). Time required to reach a given ontogenic stage was progressively longer at 26 °C presumably due to lower metabolic rate and embryonic development. Poor hatching percentage and formation of malformed embryos in later stages at 36 °C suggest that this rearing temperature is well above the tolerance limit for development of *L. rohita* eggs or may be due to the lack of adequate enzymes involved in hatching (Reddy and Lam, 1991). Higher hatching rate at 26 and 31 °C suggests that these temperature ranges are most suitable for incubation.

Overall results suggest that 31 °C is the ideal temperature for egg incubation of *L. rohita* 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. rohita* is recommended between 26 and 31 °C. This study reveals that *L. rohita* embryos can accommodate climatic changes due to global warming up to 33 °C, without hampering the reproduction and embryonic development.

Acknowledgements

We acknowledge the financial support from the Board of Research in Nuclear Sciences (BRNS Sanction No. 99/36/22/BRNS), Department of Atomic Energy (DAE), Government of India (Grant No 089) for this research work. The first author is thankful to BRNS, DAE, Government of India for providing senior research fellowship during this research work. Our sincere thanks are due to Mr. Wakhde, Officer Incharge, Khopoli fish seed farm, Government of Maharashtra for providing the broodstock and facilities in the seed farm for carrying out this research work.

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