Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(2): 1172-1176

Received: 02-01-2017
Accepted: 03-02-2017

Rajinder Kaur
Department of Fisheries Resource Management, College of Fisheries, G. B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India

RN Ram
Department of Fisheries Resource Management, College of Fisheries, G. B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India

Correspondence
Rajinder Kaur
Department of Fisheries Resource Management, College of Fisheries, G. B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India

Temperature's influence on the embryonic development of *Labeo rohita* (Hamilton, 1822)

Rajinder Kaur and RN Ram

Abstract
An experiment was conducted to study the effect of temperature fluctuations on the embryonic development of *Labeo rohita*. The freshly laid eggs collected from hatchery were exposed to different temperatures to assess its effect on their complete embryonic development. The egg development from hatching up to larval development was monitored closely in the laboratory. Eggs were exposed to variable ambient temperature (30-32 °C) and non-ambient temperature of **(**20-22 °C**)** to determine the effect of temperature on the developmental rate. All the aspects related to embryonic development were closely monitored under a microscope. It was found that egg hatchability was more at ambient temperature (30-32 °C) in comparison to the non-ambient temperature. Furthermore, the duration of egg incubation and the developmental rate increased with increasing temperature.

Keywords: *Labeo rohita*, temperature, embryonic development, eggs

1. Introduction
Despite the continued growth and contribution of aquaculture in the worldwide human consumption, there is very little knowledge about the precise mechanisms associated with the fertilization and egg activation in fish. Indeed, many commercially important species suffer the problems associated with fertilization, hatching and early embryonic development [1]. Over the last decade, our knowledge of fertilization and activation in mammalian eggs has grown tremendously. It is very important that lessons learned from the embryology field are swiftly being applied to the problems facing fish fertility [2].
Knowledge about fish development process is essential for life history studies and culture practices [3]. Embryology reveals many features related to the evolutionary relationship. Embryonic studies uphold phylogenetic development by mounting supportive proofs to decide the ancestral forms of an organism [4]. For example, it details about the evolutionary development by explaining many issues such as gill cleft which is present in the lower vertebrates (fish) is seen almost in all mammalian embryos during the early developmental stages [4]. In addition, this developmental phase of fish life is also useful in various experimental studies such as in doing aquaculture activities and toxicological studies. It is very important to carry on the proper study to characterize various embryonic and larval developmental stages to understand the biological clock and culture techniques of different species [2]. Life of an organism starts with coupling of male and female gametes. The zygote is formed as soon as the egg is fertilized by a sperm and then embryonic development starts which end up at hatching. The hatchlings further undergo organogenesis and appear like their parents and the larval stages end.
Temperature is the main environmental factor governing the development of fish eggs [5]. It determines certain morphological features, hatching rate and also the larval behavior [6]. The temperature requirement varies intra-specifically and inter-specifically for various developmental stages such as spawning, hatching, embryonic, efficiency of yolk utilization, larval and adult development [7, 8]. Due to higher Basal Metabolic Rate (BMR) and growth rate increases with increasing water temperature. Among the freshwater fishes, Indian major carp viz. *Catla catla* (Catla), *Labeo rohita* (Rohu) and *Cirrhinus mrigala* (Mrigal) contribute more than 80% to the total catch. Seed is one of the most important and critical inputs for fish culture. At present, India produces 38 billion fry per year. Rohu (*Labeo rohita*) is the most important and tastiest carp among the IMCs and it spawns once a year during the monsoon season, i.e. June-September, in rivers [9].
The aim of present study was to investigate the rate of embryogenesis in *Labeo rohita* under laboratory conditions to assess the effect of variation in water temperature on larval development.

2. Materials and Methods

2.1 Experimental fish

The experiment was conducted on female gravid Rohu (*L. rohita*) of +2 year age, collected from brood stock ponds during monsoon season in the month of August, 2013. The experiment was conducted in the Instructional Fish Farm of College of Fisheries, GBPUA&T, Pantnagar. Fully matured Rohu female with average body weight (BW) of 1.2 Kg ± 60 g and male (average BW of 800 ± 45g) were selected from the instructional fish farm of College of Fisheries. The selection of fish was done on the basis of external secondary sexual characters [10].

2.2 Selection and handling of brood stock

Mature and healthy brooders, one female and two males, were selected according to the sexual dimorphism for the breeding purpose. The females are usually easier to identify, as the belly of a mature female is generally larger and pectoral fins are rough whereas the male remains streamlined and pectoral fins are smooth in touch [10]. Healthy brooders are the prerequisite for the successful artificial propagation. These were then transferred to the cemented holding tanks of the hatchery.

2.3 Hormone

Ovatide was used as the hormone for induced breeding of fish. It was procured from the firm Hemmo Pharma, Mumbai and is available in liquid form in bottles. It is combined of GnRH analogue with dopamine antagonist pimozide. Recommended dose for Rohu female and male is 0.2-0.4 ml/kg and 0.1-0.2 ml/kg body weight respectively [10].

2.3.1 Calculation of hormonal quantity to be injected

Quantity to be injected (ml) = Weight of brood fish (kg) x dosage of Ovatide (ml)

2.3.2 Method of injection

The fishes were held firmly and weighed cautiously and a calculated amount of single dose as prescribed by manufacturer of ovatide injection to both sexes of Rohu was given intramuscularly. The needle was inserted under the scale with hypodermic 2 ml syringe through to a depth of about 1.5 cm and injected the fluid slowly. Males were injected @ 0.12 ml/kg body weight whereas females were given 0.35 ml/kg body weight of fish respectively.

2.3.3 Time of injection

The time of injection depends on the water temperature. The dose of ovatide injection was administered in the evening around at 5 P.M.

2.4 Handling and transfer of Brooders

After dosing, fishes in the 2:1 (male: female) ratios were immediately transferred to breeding pool of Chinese Circular Hatchery in plastic bucket in the evening time (5 pm). Each plastic bucket carried 50 litres of well-oxygenated water to reduce the stress. No anaesthesia was given during transfer of brood fishes, as the place where dosing of ovatide was done was in close vicinity to Chinese hatchery. Acclimatization was maintained to avoid fish mortality.

2.5 Breeding and spawning

After injecting the single dose of Ovatide hormone to both males and females of rohu, they were put in the breeding pool of Chinese hatchery especially in the evening time the ratio of 2:1 (male: female). Then after about 5 hours, showering and water jets were started so as to create circular water motion, soon after about 8 hours of showering they got excited and showed sexual play. Males started chasing females and forced them to lay the eggs. Spawning took place after 16 hours of injection at temperature 26-28 °C. Eggs were washed with the solution of potassium per manganate ($KMnO_4$); the colour of eggs was whitish muddy.

In general, environmental and physico-chemical conditions of water play an important role in breeding, hatching; embryonic and larval development. Water temperature plays a pivotal role in the metabolism and survival of fish eggs. Water quality parameters were observed during the development and were maintained properly.

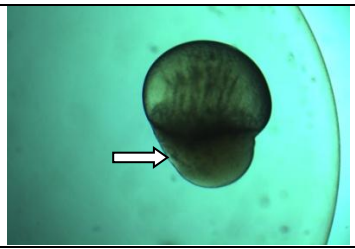
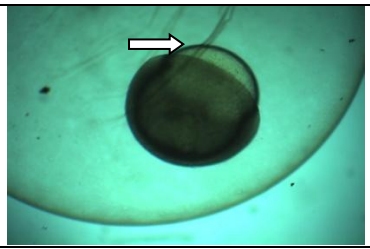
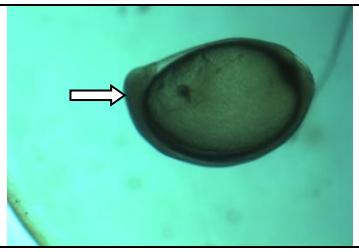
3. Results

3.1 Hatching and survivability

The eggs kept at non-ambient temperature showed a lesser hatching rate and survivability in comparison to the eggs kept at ambient temperature.

3.2 Embryonic development at ambient temperature (30-32 ºC)

Initiation of blastodisc formation occurred at 7hr after fertilization with further development at +9 hr (Fig. 1.1). At +11hr, embryo showed head region formation and at + 14 hr, body somite formation and brain development have started. After 22hrs of fertilization, the embryo was with well-developed body somites and brain vesicle was also formed. The hatching took place after twitching movement which weakened the embryo shell. In hatchlings, brain parts were under differentiation after 30 hrs of fertilization and also the initiation of eye formation was visible (Fig. 1.2). At +36hr, hatchling was showing differentiated eye ball and well differentiated branchial system. Well-developed eye ball, brain and vascular bed of vessels particularly dorsal aorta were visible in the hatchling at +46hr after fertilization. Caudal fin formation was seen in hatchlings after 54hrs of fertilization. The complete eye was developed in the hatchling after +60 hrs. After 70hrs of fertilization, development of the digestive system was seen and air bladder formation started after 79hrs. At +84hr, hatchling was with well-developed air bladder and rudimentary yolk sac with anal formation and development of caudal fin.

		
+ 7 hr (Initiation of Blastodisc formation)	+ 9 hr (Development of Blastodisc)	+ 11 hr (Developing embryo with head region formation)

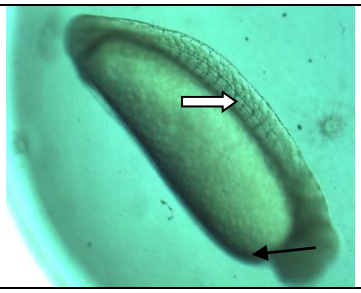
		
+ 14 hr (Embryo with somites formation and brain development started)	+ 22 hr (Embryo with differentiation of body somites and brain vesicles)	

Fig 1.1: Embryonic Development of Rohu At Ambient Temperature (30-32 ºc) Before Hatching

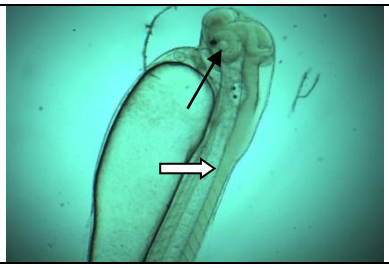
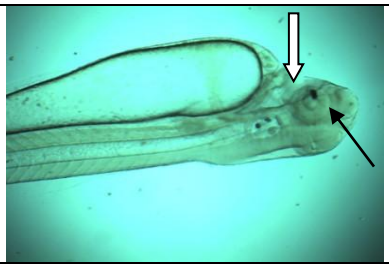
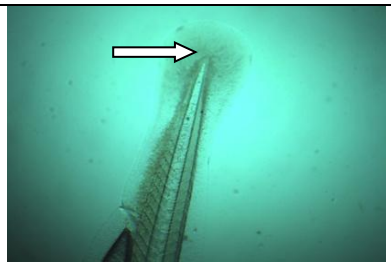
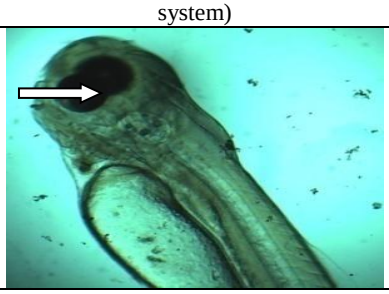
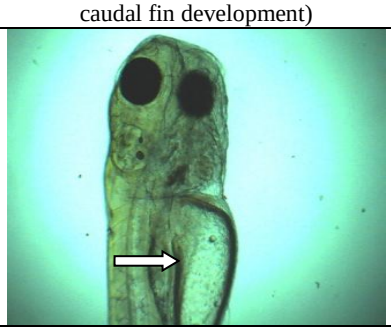
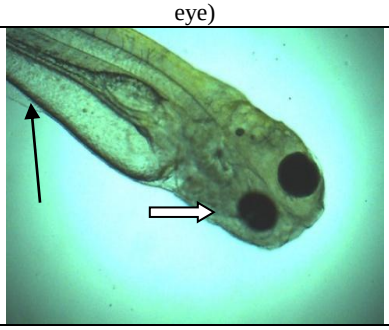
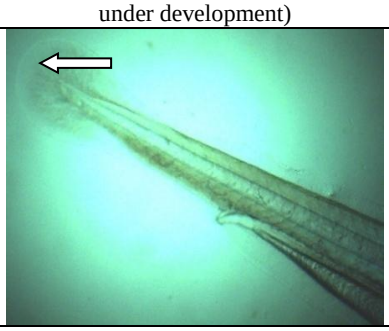
		
+ 30 hr (Hatchling showing brain parts under differentiation and initiation of eye formation)	+ 36 hrs (Hatchling showing differentiated eye ball and well differentiated branchial system)	+ 46 hr (Hatchling showing well developed eye ball, brain and vascular bed of vessels)
		
+ 54 hr (Hatchling showing initiation of caudal fin development)	+ 60 hr (Hatchling showing well developed eye)	+ 70 hr (Hatchling showing digestive system under development)
		
+ 79 hr (Hatchling showing Initiation of air bladder formation)	+ 84 hr (Hatchling with well developed air bladder, rudimentary yolk sac, anal formation and tail formation)	

Fig 1.2: Embryonic Development of Rohu at Ambient Temperature (30-32 ºC) After Hatching

3.3 Embryonic development at non-ambient temperature (20-22 ºC)

There was a start of blastodisc formation at 7hr after fertilization with further development at +9 hr (Fig. 2.1). At +11hr, blastodisc has fully covered the Yolk sac with initiation of embryonic body formation. Well differentiated head region was seen hanging over the yolk-sac at + 14 hr. At +22 hr, differentiation of body somites was seen and after 30hrs of fertilization, somites were well developed. After that twitching movement started and hatching had taken place. At +36 hr and + 46 hr, hatchling was with brain vesicle and differentiation of brain parts (Fig. 2.2). The hatchling was showing initiation of eye ball formation at +54 hr after fertilization. After 60 hrs of fertilization, hatchling was showing differentiated eye ball and well differentiated branchial system. The hatchling was showing digestive system under development and vascular bed of vessels particularly dorsal aorta after 70hrs of fertilization. At + 79 hr, hatchling showed complete eye ball formation and at +84hr, hatchling showed an elongated and decreased yolk sac, beginning of caudal fin formation and digestive system under development.

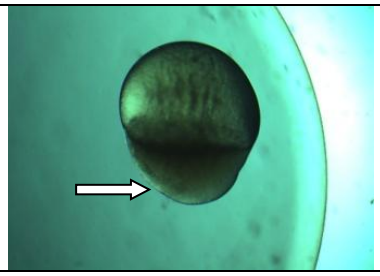
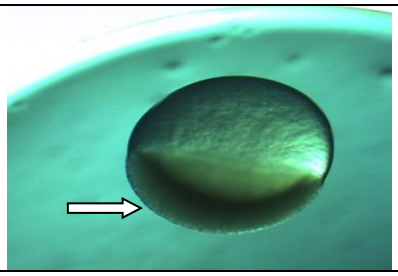
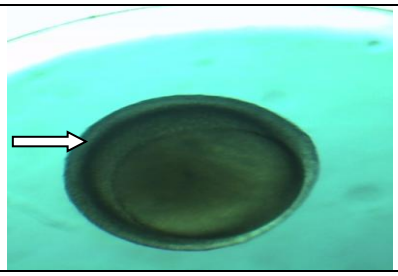
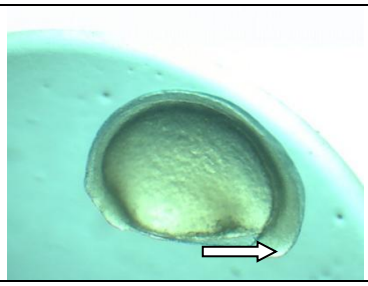
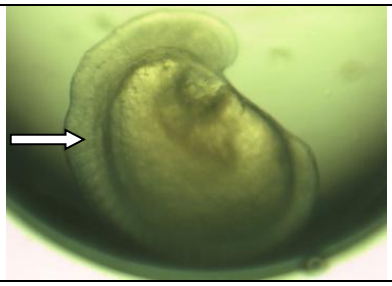
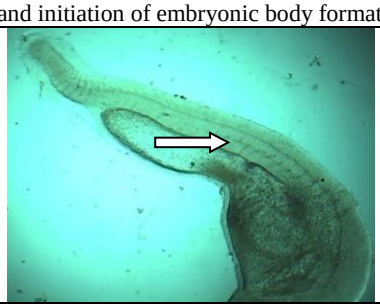
		
+ 7 hr (Start of Blastodisc Formation)	+ 9 hr (Development of Blastodisc)	+ 11 hr (Blastodisc has fully covered the Yolk sac and initiation of embryonic body formation)
		
+ 14 hr (Developing embryo with well differentiated head region hanging over yolk-sac)	+ 22 hr (Embryo with differentiation of body somites)	+ 30 hr (Embryo with somites development)

Fig 2.1: Embryonic Development of Rohu at Non-Ambient Temperature (20-22 ºC) Before Hatching

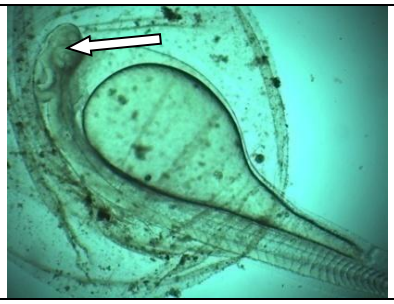
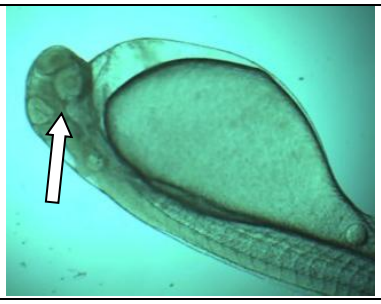
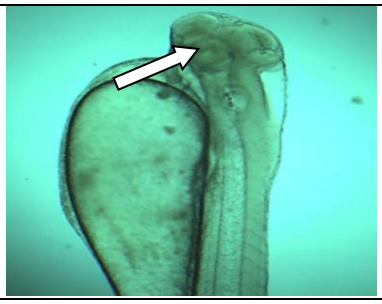
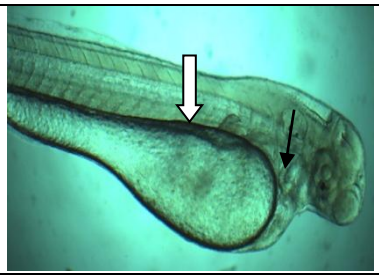
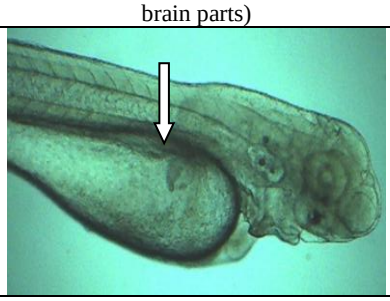
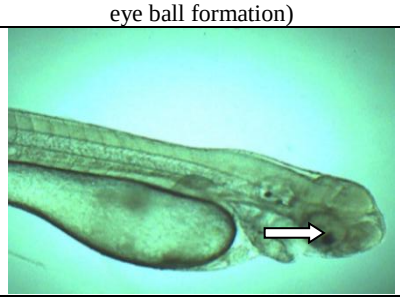
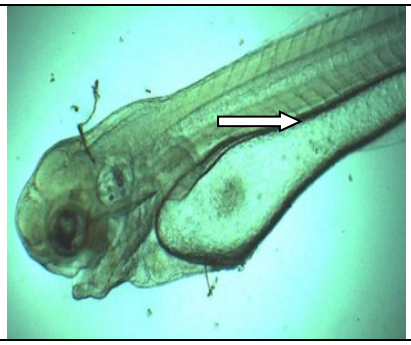
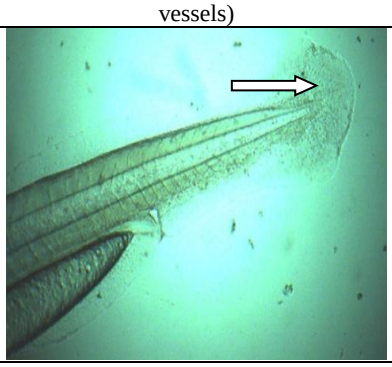
		
+ 36 hr (Hatchling with brain vesicle)	+ 46 hr (Hatchling shows well differentiated brain parts)	+ 54 hr (Hatchling showing initiation of eye ball formation)
		
+ 60 hr (Hatchling showing differentiated eye ball, well differentiated branchial system)	+ 70 hr (Hatchling showing digestive system under development and vascular bed of vessels)	+ 79 hr (Hatchling showing complete eye ball formation)
		
+ 84 hr (Hatchling showing elongated and decreased yolk sac, beginning of caudal fin formation and digestive system under development)		

Fig 2.2: Embryonic Development of Rohu at Non-Ambient Temperature (20-22 ºC) After Hatching

4. Discussion

The present study indicated that water temperature influences the embryonic development of *L. rohita* and hatchability of eggs directly [11, 12]. Ovatide has been used for successful spawning of many commercially cultured fish including Indian major carps[13]. In the present study, the ovatide dose at

0.25ml/kg to male and 0.5ml/kg to female was sufficient for spawning in Rohu. Temperature influences the embryo hatching and the level of their ontogenetic development. Lesser survival and hatching was observed [14] at temperature variation above and below the optimum range for embryonic development in *Leuciscus sp.* (Cyprinidae). The range of tolerated temperatures for the embryonic period may differ in particular populations and might reflect adaptations of different fish populations to specific local environmental conditions [15]. An increase in incubation temperature shortens the time until hatching [16, 17]. From the point of fertilization until hatching, low temperatures retard and high temperatures accelerate embryonic development and also reduce the survival rate of the spawn of *L. rohita* [18]. The ontogenic stage of hatched embryos, body length and volume of yolk sac depends on water temperature in quite a characteristic way. At other temperatures, the individuals that left the egg membranes usually show less advanced ontogenic development [11, 17]. The reason for faster hatching of embryos at higher temperatures is their increased mobility in warmer water and earlier excretion of the hatching enzyme. These characteristic behaviors of embryos incubated at higher temperatures were common in numerous fish species [19, 11]. According to a study by Das and his coworkers, 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 the ontogenic stages [20].

5. Conclusion

The study confirms that the temperature plays a vital role in the embryonic and larval development of *L. rohita* as there was a delay in the development of the embryo at non-ambient temperature in comparison to ambient temperature but it may have an adverse effect if the temperature exceeds the tolerable limit as the limit of optimum temperature ranges from 28-32 °C. 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.

6. Acknowledgement

The Authors are thankful to the Dean, College of Fisheries and Head, Department of Fisheries Resource Management, for rendering lab facilities for the conduct of this work. This work was carried as a part of course curriculum during the year 2013-2014.

7. References

1. Coward K Bromage NR, Hibbitt O, Parrington J. Gametogenesis, fertilization and egg activation in teleost fish. Reviews in Fish Biology and Fisheries. 2002; 12:33-58.
2. Rahman MM, Miah MI, Taher MA, Hasan MM. Embryonic and larval development of guchibaim, *Mastacembelus pancalus* (Hamilton). J Bangladesh Agril. Univ. 2009; 7(1):193-204.
3. Nica A, Cristea V, Gheorghe D, Hoha GV, Enache IB. Embryonic and larval development of Japanese ornamental carp *Cyprinus carpio* (Linnaeus, 1758). 2012; 116-120.
4. Aral F, Sahinoz E, Dogu Z. Embryonic and larval development of freshwater fish. Recent advances in fish farms. 2011, 83-94.
5. Nwosu FM, Holzlohnev S. Influence of temperature on eggs hatching, growth and survival of larvae of *Heterobranchus longifilis*. (Teleostei: Clariidae). J Applied Ichthyol. 2000; 16:20-23.
6. Bagenal TB, Braun E. Eggs and early life history. In methods for assessments of fish production in fresh water. T.B. Bagenal (Ed.) Oxford London: Blackwell Scientific Publication, 1978, 165-201.
7. Herzig A, Winkler H. The influence of temperatuire on the embryonic development of three Cyprinid fishes, *Abramis brama*, Chalealburnuschalcoidesmento and *Vimba vimba*. J Fish Biol. 1986; 28:171-181.
8. Blaxter JHS. Pattern and variety in development. In: W.S. Hoar and D.J. Randall (Eds). Fish physiology, Academic press, San Deigo, CA, 1988, 1-58.
9. Hora SL. Analysis of the factors influencing the spawning in carps. Proc. Natl. Inst. Sci. India. 1945; 11:303-312.
10. Thomas PC. Breeding and seed production of fin fish and shell fish. Darya publishing, Delhi, 2003.
11. Kamler E. Ontogeny of yolk-feeding fish: an ecological perspective. Reviews in Fish Biology and Fisheries. 2002; 12:79-103.
12. Rechulicz J, Ostaszewska T, Wojda R. Water temperature effects on incubation of ide (*Leuciscusidus* L.) eggs and selected parameters of the larvae. Acta Scientiarum Polonorum Piscaria. 2002; 1:35-46.
13. Marimuthu K, Haniffa MA, Rahman A. Spawning performance of native threatened Spotted Snakehead Fish, *Channa punctatus* (Actinoptergii: Channidae: Perciformes), induced with ovatide. ACTA Ich.et. Pis. 2009; 39:1-5.
14. Kupren K, Mamcarz A, Kucharczyk D. Effect of variable and constant thermal conditions on embryonic and early larval development of fish from the genus *Leuciscus* (Cyprinidae, Teleostei). Czech J Anim. Sci. 2011; 56(2):70-80.
15. Kupren K, Mamcarz A, Kucharczyk D. Effect of temperature on survival, deformations rate and selected parameters of newly hatched larvae of three rheophilic cyprinids (Genus *Leuciscus*). Polish Journal of Natural Sciences. 2010; 25:299-312.
16. Korwin-Kossakowski M. The influence of temperature during the embryonic period on larval growth and development in carp, *Cyprinus carpio* L., and grass carp, *Ctenopharyngodon idella* (Val.): Theoretical and practical aspects. Archives of Polish Fisheries. 2008; 16:231-314.
17. Kupren K, Mamcarz A, Kucharczyk D, Prusinska M, Krejszeff S. Influence of water temperature on eggs incubation time and embryonic development of fish from genus *Leuciscus*. Polish Journal of Natural Sciences. 2008; 23:461-481.
18. Ponnuraj M, Murugesan AG, Sukumaran N. Effect of temperature on incubation time, fertilization rate and survival of the spawn of Rohu, *Labeo rohita*. In: Venkataramani, B., Sukumaran, N. (Eds.), Thermal Ecology. BRNS. DAE, Mumbai (Pub), 2002, 430-433.
19. Blaxter JHS. The effect of temperature on larval fishes. Netherlands Journal of Zoology. 1992; 42:336-357.
20. Das T, Pal AK, Chakraborty SK, Manush SM, Dalvi, RS, Sarma K *et al*. Thermal dependence of embryonic development and hatching rate in *Labeo rohita* (Hamilton, 1822). Aquaculture.2006; 255:536-541.