

# Temperature effect on embryonic development in fish eggs



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## Abstract

The captive breeding of Koi Carp (*Cyprinus carpio carpio*) was successfully carried out at the Directorate of Coldwater Fisheries Research, Bhimtal, India. Induced breeding trials conducted on the fish revealed that the fish can be naturally spawned Low temperature using sGnRH analogue and dopamine antagonist (Ovaprim). Spawning was observed eighteen hrs after the injection at low temperature ( $16 \pm 2^{\circ}\text{C}$ ). The fertilized eggs were adhesive and transparent with diameter ranging between 0.8 mm to 1.10 mm. The incubation period was 120 hours and 84 hours at temperature  $15-18^{\circ}\text{C}$  (April) and  $20-26^{\circ}\text{C}$  (August) respectively. The hatchlings were transparent and measured 3.45-4.75 mm, with a large oval head, a well defined yolk sac and short tail. The yolk got fully absorbed within 2-3 days and by this time mouth formation was complete and the larvae started exogenous feeding. Present study, may be useful in standardizing the ex-situ breeding protocols for Koi carp under lower temperature.

## Introduction

Ornamental fish is often used as a generic term to describe aquatic animals kept in the aquarium hobby (Livengood et al 2009). Ornamental fishes form an important commercial component of aquaculture providing for aesthetic requirements and upkeep of the environment (Swain et al 2008). USA is the largest importer of ornamental fishes followed by Europe and Japan. The emerging markets are China and South Africa. Over US \$ 500 million worth of ornamental fish are imported into the USA each year (Anonymous. 2006). India's share in ornamental fish trade is estimated to be less than 1 % of the

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global trade. The major part of the export trade is based on wild collection. The overall domestic trade in this field cross Rs 1000 lakh and is reportedly growing at the rate of 20 per cent annum (NABARD).

Common carp ( *Cyprinus carpio* ) is one of the most important cultured fish in the world. More than 2. 7 million tonnes of common carp were produced in 2000 (FAO, 2002). Koi carp is ornamental variety of domesticated common carp (*Cyprinus carpio*) that are kept for decorative purpose in outdoor ponds or water gardens. They belong to the family Cyprinidae and the order Cypriniformes. It is one of the most popular and favorite ornamental fishes amongst all ornamental fish species and it has high market value for its excellent color. The color and scale pattern of the species is highly variable. It may look like big gold fish, distinguishing for its barbels at the sides of the mouth and for its size (Ghosh et al 2012). They are delicate and are very peaceful towards occupants and hence well suited to aquarium. There is various colour variations in koi carp like white, black, red, yellow, blue and cream. Like all cyprinides, koi carp is also a egg layer. They produce adhesive eggs. This species exhibits gonochorism, external fertilization with varied spawning frequencies (Balon 1990) and considered as batch spawner (Kalilota et al 1993). They grow up to 100 cm length with an elongate body measuring 3 to 4 times less in height than length. In their natural habitat, koi carp live up to 15-24 years (Kuroki, 1981).

Considering the importance of koi carp, information on the early life history of a fish is very important for optimization of its large scale seed production, culture and management practices, therefore, this study was carried out to

highlight some aspects of the early life history, the development biological clock of koi carp in relation to low temperature.

## Material and Methods

The fishes were purchased from Lucknow Local market during 2012. In the same day, the fishes were transported to the Fish farm, Directorate of coldwater Fisheries research (DCFR), Bhimtal. At the farm after disinfection, all fishes were reared in a cemented pond. The fishes were fed with floating pellets containing crude protein 28%, crude fiber 11. 1%, and carbohydrate 33% (Table 1). After proper acclimatization and maintenance, the healthy and mature breeders (90-550g) were selected according to sexual dimorphism and transferred to hatchery shed in FRP tank of size 200cm X 200cm X 30cm with flow through arrangement of water system. The females are usually easier to identify, as belly of a mature female is generally larger, whereas male's remains streamlined and more torpedo shaped (Mihalache et al 2011). The sex ratio of the spawners was kept at 2: 1 for male and female. The breeding programme was carried out using salmon Gonadotropin releasing hormone analogue and domperidone injection (ovaprim, Syndel laboratories INDIA Pvt. Ltd). Brooders were administered hormone @ 0. 6 ml per kg body weight to female and 0. 3 ml per kg body weight to male intra peritoneal in the evening hours. The breeders set were released into FRP tank of 3000 L capacity having provision for flow through water system after the hormonal administration. Aquatic macrophyte (Hydrilla) was introduced into breeding tank for hiding purpose as well as holding adhesive eggs (Haniffa et al 2006). Translucent netting at the top also provided in order to observe to observe spawning behavior of fish. The egg hatching and larval

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rearing upto yolk sac absorption was taken up in the same tank that was used for spawning. The fertilization rate was counted by collecting random light microscope with digital camera (Nikon ECLIPSE E100). Samples of the eggs before fertilization and developmental time was rounded to nearest minutes until morula stage and then to hours. In present study, the developmental stages were divided into embryonic and larval development upto yolk sac absorption. The embryonic stage occur inside the egg shell and ends at the hatching. While, larval phase occur as egg hatches and ends when the larvae become capable of exogenous feeding. The water quality of hatchery was measured for temperature, pH, electrical conductivity (EC), total dissolved solids and dissolved oxygen by HANNA HI 9828.

## Results

There are few reports on breeding of koi carp in low temperature (Watson et al 2004; Ghosh et al 2012). present study spawning was noticed after 18 hours of hormone injection. The fertilized eggs of koi carp were foun to have adhesive, demersal and sticky to substratum (i. e. hydrilla). They were 0. 8-1. 10 mm in diameter, rounded and due to the adhesive nature of the egg, considerable debris adhered to the capsule of the egg. As the egg envelope is thick, transparent and sticky, observations on the developmental stages are difficult (Kovac, 2000). The eggs were deposited singly and were adhesive throughout the incubation period. The incubation period of eggs depends largely on water quality parameters such as salinity and temperature (Kuo et al 1973; Lio et al 1975). In the present study, the water temperature was 15-18 ° C during April and 20-26 ° C during August, under

these conditions, eggs hatched out in 120 and 84 hours after fertilization respectively.

Although a true metamorphosis is not generally described for fishes, the term hatchling, larvae and post larvae are used to indicate different stages of development from hatchling to fingerling stage (Bogline et al 1992). In present study, the embryonic development was divided into zygote, cleavage, blastula, gastrula and hatching period (Table 2, 3 & Fig 1). The cleavage was meroblastic and the first division (2 celled stage) occurred 1 hours after fertilization, followed by second cleavage 1 hour 35 minutes after fertilization. The 16 celled stage was reached 2 hours 20 minutes after fertilization. Subsequent cleavage increased cell number and reached morula stage. At this stage, a cap like structure was seen over the animal pole, which gradually increases in size the blastoderm further spread over the yolk and the formation of germinal ring around yolk was clearly visible within 15 hours after fertilization. The yolk invasion completed after 32 hours and 13 minutes after fertilization. The head and tail ends of the embryo became distinguishable during yolk plug stage. Yolk invasion was over and the blastopore was almost closed. The notochord was clearly seen at 46 hours and 16 minutes after fertilization. Further, embryo was elongated and encircled the whole yolk material within 48 hours after fertilization. At this stage, the anterior posterior axis was distinguishable in broader cephalic region with distinct forebrain and narrow end as tail region. At 76 hours after fertilization cephalic region became prominent, optic lens starts differentiating and mesodermal somites (16-18) were highly visible. A heart beat (80-91) per minutes were noticed at this stage. The caudal region

started detaching from yolk and head further elongated in size showing all parts of brain, heart, lens and 22-25 somites after 101 hours after fertilization. The beating of heart intensified 130-140 beats per minutes and tail showed rhythmic movement on both side one by one. At 109 hours after fertilization lens fully formed and pectoral fin bud was clearly visible. In final stage of embryonic development, the growing embryo occupied the entire previtelline space. The lashing movements, which gradually become vigorous and egg capsules, were weakened and ruptured. The embryo ruptured the egg shell by the continuous movement and hatched out at 120 hours after fertilization at  $16 \pm 2^{\circ}\text{C}$ . The hatchlings were transparent and measured 3.45-4.75 mm, with a large oval head, a well defined yolk sac and short tail. The yolk got fully absorbed within 2-3 days and by this time mouth formation was complete and the larvae started exogenous feeding

## Discussion

Temperature is one of the most decisive environmental variables affecting embryonic development in fish eggs (Bermudes and Ritar, 1999; Kamler, 2002; Yang and Chen 2005). Within a viable range, incubation temperature strongly affects the rate of embryonic development of fish. Generally, lower temperature retards the rate of embryonic development and higher temperature accelerates it (Marangos et al., 1986; Pepin, 1991; Mihelakakis and Kitajima, 1994; Hart and Purser, 1995; Das et al 2006). The results of present showed that water temperature has a strong effect on development rate and hatching success of koi carp. In present study, the fertilized eggs of koi carp were found yellowish, adhesive and demersal. Haniffa et al (2007)

and Ghosh et al (2012) found similar results in koi carp and common carp. Two celled, four celled, eight celled and sixteen celled stage were found 60, 95, 120 and 150 minutes after fertilization respectively. Similar findings were reported by Ghosh et al 2012 in koi carp. They found two celled, four celled, eight celled and sixteen celled stage with in 80, 110, 140 and 170 minutes after fertilization at 17 – 20 °C respectively. However, Haniffa et al (2007) reported that same series occurred at 60, 90, 110 and 140 minutes after fertilization at 26 – 28 °C. In common carp, it took 30, 80, 100 and 120 minutes after fertilization at 26 °C for same series (Balon 1995). The initiation of gastrula stage was noticed at fifteen hours after fertilization of egg at  $16 \pm 2$  °C. Similar results was reported by Ghosh et al (2012) in koi carp. However, Haniffa *et al.* (2006) the same stage in koi carp at 7. 30 to 11. 40 minute after fertilization at 26-28 in summer season. Balon (1995) observed initiation of gastrulation of *C. carpio* occurring 6 hrs and 30 mins after fertilization of the eggs at 26-28 °C. This variation might be due to low water temperature and species difference.

Changes in the pattern of the entire structure of an organ in relation to the environment are decisive for evaluating the developmental patterns of species (Balon, 1999; Mahmud et al 2012). The early development of fish is strongly affected by incubation temperature (Mahmud et al 2012). Generally, lower temperature retards the rate of embryonic development of fish and higher temperature accelerates it (Saka *et al.* , 2001). In present study period the ambient temperature was low and fluctuating which may delay the embryonic and larval development of koi carp. A comparative study on



the study of embryonic development of koi carp at different temperature is listed below (Table 3). In present study, embryo hatched out in 144 hrs after fertilization at  $16 \pm 2^{\circ}\text{C}$  which was similar to the findings Watson et al (2004). They reported the time required to hatch the embryo of koi carp in 5-7 days at  $20\text{-}24^{\circ}\text{C}$ . Similar results were obtained by Ghost et al (2012). However, the results of present study vary from Haniffa et al 2007, who found 72-73 hours are needed for hatching of Koi carp. This can be attributed to different physical condition of brood fish and lower temperature of water at the time of breeding.

In conclusion, Koi carp can be easily matured and bred successfully under low water temperature captive conditions similar to carp. The descriptive investigation into the embryonic development and temperature tolerance should provide valuable information about the ability of the species to handle low temperature condition. As there are no commercial approaches of induced breeding and seed production of koi carp in the colder regions of the country but there is high demand of this ornamental fish for its colorful and attractive appearance. Hence, In spite of the long incubation period, the captive breeding, embryonic development protocol described herein should provide a base for future studies on koi carp and help in achieving conservation and commercial goals.

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