



Induced Breeding, Embryonic and Larval Development of Koi Carp in Recirculatory Aquaculture System

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ABSTRACT

Koi carp (*Cyprinus carpio*) is a colourful and economically valuable ornamental fish species, which has numerous strains. Even though culture of Koi carp is practiced at many tropical countries, there is a very little information on the seed production and embryonic development of the species especially in RAS. The present study was aimed to study the breeding, embryonic and larval development of Koi carp in RAS system. The matured brooders were administered with three synthetic hormone ovaprim, ovatide and WOVA -FH at 0.7 ml per kg of body weight. Comparatively WOVA-FH at 0.7 ml per kg showed better performance with higher number of ovulated eggs, fertilization and hatching rate than ovaprim and ovatide. For dose optimization, the matured brooders were administered with synthetic hormone WOVA -FH at three different doses viz., 0.3, 0.5 and 0.7 and 1.0 ml per kg of body weight at triplicate basis. The highest number of ovulated eggs (3380.5 ± 202.75), fertilization rate (90 ± 0.8) and hatching rate (88 ± 0.88) were observed in the brooders that were induced with 0.5ml per kg of WOVA -FH. The average diameter of the fertilized eggs ranged between 0.9 and 1.10 mm while the yolk sphere ranged between 0.6 and 0.8 mm. The larvae hatched out after 72 h of fertilization. The total length of the larvae ranged from 2.7 to 2.9 mm. This study aims to analyze the application of RAS technology for year-round seed production of Koi carp. Further this study aims in developing sustainable aquaculture practices to facilitate aquaculturists.

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Authors' Contribution

NK carried out the research work and drafted the manuscript. KKM narrated the work and executed the experiment. SKB reviewed the manuscript and checked the grammatical mistakes. MS analysed the data. SAS gave broad ideas in field sampling. ES reviewed the manuscript, condensed the matter and shared idea of analysis and interpretation part.

Key words

Koi carp, Induced breeding, Embryonic development, RAS

INTRODUCTION

Ornamental fishes fetch a higher price than the food fish in international markets because of their aesthetic value and high commercial value in the export trade. Koi carp, *Cyprinus carpio*, is a well-known decorative fish. It is a variety of the common carp (*Cyprinus carpio*) species, a native of South East Asia. Koi carp produced from China and Japan fetches high market value for its excellent colour pattern. New varieties of colours and colour patterns in Koi carp have been developed over recent years. The common colours are white, cream, red, blue, yellow and black. Koi carps generally live up to 15-24 years. Being hardy, it is

highly suitable for garden pools and aquarium keeping (Hussain *et al.*, 2014).

Aquaculture faces many obstacles related to water quality and waste management. The waste can cause environmental pollution if it is not treated before disposal. Therefore, the development of aquaculture innovation is fundamentally essential to develop into sustainable aquaculture practices. One of the more intriguing strategies for intensifying production while simultaneously reducing wastes is the development of recirculating aquaculture systems (RASs). These systems are designed to collect and remove waste products, uneaten feed, and bacteria from the tank where the fish live so that water can be recycled back into the system. Even though many ornamental fish and invertebrates have been induced to spawn in captivity, the larvae of only a few species have been successfully reared in captivity (Holt, 2003; Olivotto *et al.*, 2003). The bottleneck is the heavy mortality encountered in the larvae during the critical period of development, mostly noticed when the larvae change over from internal yolk storage to exogenous feed (Madhu *et al.*, 2012). Breeding is a complex process which depends on several factors. In addition to the other environmental and biological factors,

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the condition of brooders is the major factor determining the success.

Selecting the appropriate inducing agents will enhance the overall reproductive success. Based on the literature, for many fish species synthetic hormone, Ovaprim (M/s Syndel Laboratories, Canada) has been successfully used since 1990. After 1997, Ovatile (M/s Hemmo Pharma, Mumbai), an indigenous synthetic hormone which is more affordable than Ovaprim was used. Nowadays, WOVA-FH (M/s.Biostadt India Limited, Mumbai) is used as an inducing agent for many ornamental fishes. Hence, the present study was conducted using three commercially available GnRH synthetic hormone viz., WOVA-FH, Ovaprim and Ovatile. Literature on the seed production and embryonic development of Koi carp is very scanty. Considering these facts, the present study was undertaken on broodstock development, induced breeding and larval development of Koi carp using RAS for sustainable aquaculture practices.

MATERIALS AND METHODS

Collection and conditioning of broodfish

Experiments were conducted at Aquatic Rainbow Technology Park, Madhavaram, Chennai, India. The brooders (Female: 250±50 g; Male: 200±50 g) were collected from commercial ornamental fish market in Kolathur, Chennai and were transported in oxygenated polythene bags.

Brood stock maintenance

The broodfishes were segregated into males of 2+ years and females of 4+ years. They were reared separately in 2 cemented grow out raceway tanks (20-ton capacity) with RAS facility. The brooders were fed with commercial GROWFIN supplementary feed (1.8 mm) containing 38% protein, at 5% of body weight, twice a day. Along with the supplementary diet, brooders were also fed with earthworm and *Tubifex tubifex* (sludge worm) to enhance maturity under captive condition for 4 months. Then the brooders were separated based on the secondary sexual characteristics (Table I). Matured males were selected by observing freely oozing milt (Fig. 1a). Matured females were observed with bulged belly region, slightly swollen genital aperture, and oozing ova upon gentle pressure on the abdomen (Fig. 1b).

Preparation of spawning tank

For induced breeding of fish, FRP (fibre-reinforced plastic) circular tanks of 1-ton capacity with RAS facility were filled (1ft of height) with water. The tanks surface was laid with artificial polyethylene strips (breeding mops)

and nylon fibres. Nylon fibres act as a substrate to attach the eggs because Koi carp eggs are adhesive in nature.



Fig. 1. Procedure followed in the captive breeding of Koi carp using RAS facility.

Optimization of dosage for induced breeding

In the present study induced breeding trials were carried out using three different inducing agents to optimize the hormone on breeding performance. Ovatile, ovaprim and WOVA-FH were used as an inducing agent at 0.7 ml per kg of body weight (Table II). The synthetic hormone which showing better performance were administrated at 4 different doses viz., 0.3, 0.5, 0.7 ml, 1 ml per kg body weight intramuscularly for dose optimization. One control unit was also set up. Both males and females were induced by injecting single intramuscular injection, between the base of the dorsal fin and lateral line, in the evening (1730 h). After injecting the hormone, brooders were released at 2:1 (M: F) sex ratio into spawning tanks supplied with continuous aeration. The occurrence of spawning was monitored periodically by checking for the presence of eggs in the breeding hapa. Egg collection was done on the next day morning at 0530 h (Fig. 1e).

Table I. Sex identification characteristics in Koi carp.

Character	Male	Female
Size	Smaller	Larger
Body colour	Bright	Slightly dull
Body shape	Slimmer	Broader
Pectoral fin	Large, rough	Pointed, soft
Secondary sexual characteristics	Pink coloured vent Tubercles appears on the face Oozes milt	Vent will be rounder and pinker than male Tubercles absent Oozes eggs

Table II. Experimental design of dose optimization for induced breeding of Koi carp using different hormone in captive condition.

Treatment	Hormonal dosage	Latency period (h)	No of ovulated eggs (Per unit body weight)	Fertilization rate (%)	Hatching rate (%)	Breeding performance
Ovaprim	0.7 ml per kg	9.15	1450	53	49	Average
Ovatide	0.7 ml per kg	12.10	650	52	42	poor
WOVA-FH	0.7 ml per kg	7.35	2200	72	60	Good

Estimation of hormone efficiency

The efficiency of hormone was estimated based on parameters such as number of eggs spawned, fertilization rate and hatching rate. Number of spawned eggs was estimated by random sampling or by counting the eggs in each breeding mop or nylon fibres. The fertilization rate of eggs was determined by counting the egg having intact nucleus with yellowish colour. Fertilization rate and hatching rate was calibrated using the following formulae:

$$\text{Fertilization rate (\%)} = \frac{\text{Number of fertilized eggs}}{\text{Total no. of released egg}} \times 100$$

$$\text{Hatching rate (\%)} = \frac{\text{Number of hatched out eggs}}{\text{Total number of fertilized eggs}} \times 100$$

Data analysis

The data on number of ovulated eggs, fertilization, and hatching rates of different treatments were analysed by compared using one way ANOVA method using Statistical Package for the Social Sciences (SPSS) version 20.0. Duncan's multiple range tests was used to compare the significant differences within and between the treatments. The significance level was set at $p < 0.05$.

RESULTS AND DISCUSSION

In the present study, induced breeding trials were carried out using three different inducing agents ovaprim, ovatide and WOVA -FH at 0.7 ml per kg of body weight. Most carp species responded well at a dosage ranging between 0.4 and 0.70 ml per kg (Nandeesha *et al.*, 1990; Das, 2003). Comparatively WOVA-FH at 0.7 ml

per kg showed better performance with higher number of ovulated eggs, fertilization and hatching rate than ovaprim and ovatide (Table II). For dose optimization the matured brooders (250-300 g) were administered with the synthetic hormone WOVA-FH (M/s. Biostadt India Limited), Mumbai consisting of Synthetic Gonadotropin Releasing Hormone Analogue (SGnRH), Domperidone and Glycerol at four different doses 0.3, 0.5 and 0.7 ml, 1 ml per kg of body weight. The ideal dose of synthetic hormone WOVA-FH for induced breeding of Koi carp was standardized and the results are summarised in terms of number of eggs per female, fertilization and hatching rate are given in Table III. During the entire culture period, the optimum water quality parameters such as pH and total dissolved solids were maintained as required for Koi fish (Table IV). The spawning was observed, in all breeding trials with significantly varied latency period (The time between administration of inducing agent and initiation of spawning) of 7-12 h. Similarly, variation in the latency period with dose and hormone in induced fish breeding was observed in *Gonoproktopterus curmuca*, *Pethia manipurensis* and *Osteobrama belangeri* (Padmakumar *et al.*, 2014; Motilan *et al.*, 2014; Das *et al.*, 2016). This infers the effect of hormone and dose on the breeding performance in terms of pairing, courtship behaviour and spawning. In the present study, spawning activity was quicker at higher doses compared to lower doses; shows the difference in the mode of action and interaction of hormone. Similar observation was reported by Pandey *et al.* (2002) and Behera *et al.* (2007) in *Labeo rohita* and *L. bata*, respectively.

Table III. Induced breeding performance of Koi carp using WOVA-FH with different dose combination.

Treatment	Hormonal dosage	Latency period	No of ovulated eggs (Per unit body weight)	Fertilization rate (%)	Hatching rate (%)	Breeding performance
Control	No inducement	0.00	0.00	0.00	0.00	No spawning
Treatment 1	0.3 ml kg ⁻¹	9.25	1358±202.75 ^a	75±1.2 ^c	80±1.15 ^b	Partial spawning
Treatment 2	0.5 ml kg ⁻¹	8.15	3380±112.75 ^c	90±0.8 ^d	88±0.88 ^c	Complete spawning with high hatching rate
Treatment 3	0.7 ml kg ⁻¹	7.35	2336±338.29 ^b	85±1.4 ^b	79±1.2 ^b	Complete spawning
Treatment 4	1.0 ml kg ⁻¹	7.00	1579±202.75 ^a	70±0.3 ^a	69±0.88 ^a	Partial spawning

*Values in the same column with different superscript are significantly different ($p < 0.05$).

Table IV. Optimum water quality parameters.

Water quality parameters	Observed range in spawning tank
Temperature	24± 2°C
pH	7.2± 1
Dissolved oxygen	5.0± 0.1 mg/l
Ammonia	< 0.02 mg/l
Total hardness	150± 30mg/l
Total alkalinity	60 ± 5 mg/l

The significant difference ($P < 0.05$) was observed in egg output of the fishes bred with different hormone and doses. The better spawning was observed in WOVA-FH (0.5 ml per kg) and (0.7 ml per kg). Comparatively WOVA-FH at 0.5 ml per kg yielded (3380.5 ± 202.75) higher number of eggs. Further, results showed that Ovotide at 0.7 ml per kg produced lower number of spawned eggs. It was observed that fertilization rate was also varied significantly ($P < 0.05$) with different hormone and doses. The higher percentage of fertilization rate (90%) was observed in WOVA-FH (0.5 ml per kg). Lowest fertilization rate (52%) was observed in Ovotide (0.7 ml per kg). Rath *et al.* (2007) and Motilan *et al.* (2014) also reported better performance of breeding of IMC and *P. manipurensis* while administering WOVA-FH at 0.4-0.5 ml per kg of body weight. The egg output by the female also depends on the release of the gonadotropin releasing hormone which can be stimulated by synthetic inducing agents, which is evidenced by the several studies such as cherry barb (Sundarabharathy *et al.*, 2004), *P. manipurensis* (Motilan *et al.*, 2014) and *O. belangeri* (Das *et al.*, 2016). In the present study dose of different hormone affected the rate of fertilization apparently. Inducement of hormone at higher and lower doses causes poor fertilization that may be due to early milting and late inducement in males, respectively (Pandey *et al.*, 2002; Das *et al.*, 2016).

The higher percentage of hatching rate (88%) was observed in WOVA-FH (0.5 ml per kg) and lower percentage of hatching rate (42%) was observed in Ovotide (0.7 ml per kg). Further, results showed that hatching rate varied significantly with different hormone and dose administered. In a similar experiment, mature brooder was induced with three different dosages of ovaprim (0.4, 0.7 and 1.0 ml per kg) and it was found that Koi carp responds at all the three dosages but the best response was found at a higher dosage of 1.0 ml per kg (Ghosh *et al.*, 2012). Koi brood fish injected with synthetic hormone ovaprim at 0.2 ml per kg for male and 0.5 ml per kg for female with aquatic macrophytes (*Hydrilla verticillata*) for attachment and development showed the number of ovulated eggs, fertilization and hatching rate as 100%, 75.2%, and 83.3%, respectively (Malik *et al.*, 2014).

Several methods have been tried for induced spawning in fishes with various levels of success (Harvey and Hoar, 1979). Induced breeding of many Indian fishes was attempted by several workers however, the level of success varied based on the combination of the fish species and inducing agent (Ramaswamy and Sundaraj, 1969; JR *et al.*, 1992; Alok *et al.*, 1998). A study on different species of IMCs in comparison of spawning success with different inducing agents revealed that *Catla catla* responds more with Ovaprim, whereas, the results were better with Ovotide in *L. rohita* and *Cirrhinus mrigala* (Dhawan and Kamaldeep, 2004). Reddy and Mathur (2000) also reported similar results with ovotide in *L. rohita* and *C. mrigala* as compared to *C. catla*. Tiwana and Sudhanshu (2012) proved that Ovaprim gave 5.83 and 12.95% better performances over Ovotide and carp pituitary extract, respectively in terms of hatching rate of *L. rohita* eggs. The difference in effective dosage among different species was attributed to the varied levels of dopamine activity (Billard *et al.*, 1983; Richard *et al.*, 1986). Devi *et al.* (2009) attempted induced spawning and hatching of *O. belangeri* in its natural habitat using Ovotide (0.6 ml per kg for females and 0.3 ml per kg for males) and reported 95.0±1.05%

fertilization and $88.8 \pm 1.04\%$ hatching rates. A dosage of 1ml per kg body weight of Ovaprim was recommended for artificial spawning of *Clarias gariepinus* by Achionye and Israel (2012). Sharma *et al.* (2010) recommended 1 ml of Ovotide per kg body weight of female broodfish to be optimum among the three experimental doses, 0.6, 0.8 and 1ml per kg, for best breeding performance and egg quality in *Clarias batrachus*.

Comparing all the parameters, among the synthetic inducing agents used WOVA-FH at 0.5 ml per kg performed better in terms of number of spawned eggs, fertilization and hatching rate. Hence WOVA-FH at 0.7 ml per kg is the standardized hormone with standardized dose for induced breeding of Koi carp.

Embryonic and larval development

Changes in anatomical or morphological structure gives importance to study the difference between embryonic, larval and post-larval development (Kovac, 2000). The term hatchling, larvae and post larvae are used to indicate different stages of development from onset of hatching to fingerling stage (Boglinoe *et al.*, 1992) and is further divided into six stages namely embryo, hatchling, larva, post-larva, fry and fingerling and each stage was characterized by typical anatomical and physiological features (Jhingran and Pullin, 1985). In the present study, spawning was noticed 6-7 h after the injection and the eggs were hatched 68-72 h after fertilization at a water temperature of 25-26°C. The Koi carp eggs were spherical, demersal and adhesive in nature throughout their incubation period which made the observation of developmental stages more difficult. The diameter of the fertilized egg ranged between 0.9 and 1.10 mm while the yolk sphere ranged between 0.6 and 0.8 mm. The incubation period of eggs depends largely on water quality parameters such as salinity, temperature and pH. The embryonic developments were observed in three distinct phases *viz.*, cleavage, embryonic and larval development stages (Tables V and VI). These were observed using trinocular microscope NLCD- 120E, Lawrence and Mayo (Fig. 2).

Cleavage phase

Embryonic development lasted up to 12 h after fertilization of eggs. It consisted of the formation of perivitelline space, bipolar differentiation and first multiplication of cells, formation of blastula, early gastrula, late gastrula, beginning of epiboly, germ ring, and embryonic shield. The blastodisc was noticed within 30 mins after fertilization (Fig. 3B) and first two-cell division occurred in the animal pole (posterior side) at 0.55 h post-fertilization (h.p.f). The four-cell division

occurred at 1.20 h.p.f. The embryo attained an 8-celled stage, 16 -celled stage, 32-celled stage, 64-celled stage and 128-blastomeres stages within 2.45 h.p.f. The size of the blastomere reduced and appeared as cluster of solid cells called the morula. The blastoderm covered almost 1/3 of the yolk sac and formed dome like structure at 4.25 h.p.f. and entered to early gastrula stage. Germ ring was completed and Blastoderm started enveloping 3/4 of the yolk sphere at 7.45 h.p.f and entered into late gastrula stage. Blastoderm completely covered the yolk plug and epiboly came to an end at 11.40 h.p.f.

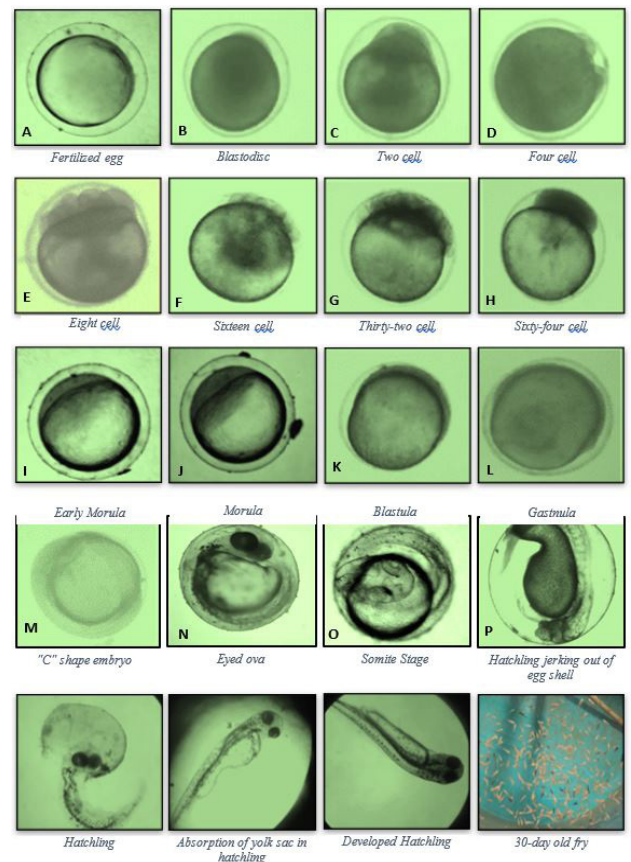


Fig. 2. Embryonic development stages of Koi Carp.

Embryonic phase

The initiation of the embryonic body (organogenesis) formation was observed when the blastomeres covered the whole yolk. The embryo reached 5 somite stage; the tailbud formed at the posterior end of the yolk mass. A pair of kidney shaped optic capsules was visible in the optic vesicle and the lens started to differentiate. As the embryo increased in size, anterior and posterior region became distinguishable as broader cephalic region with the distinct forehead and tail. When the embryo reached 10-12 somite stages,

Table V. Embryonic developmental stages of Koi off springs.

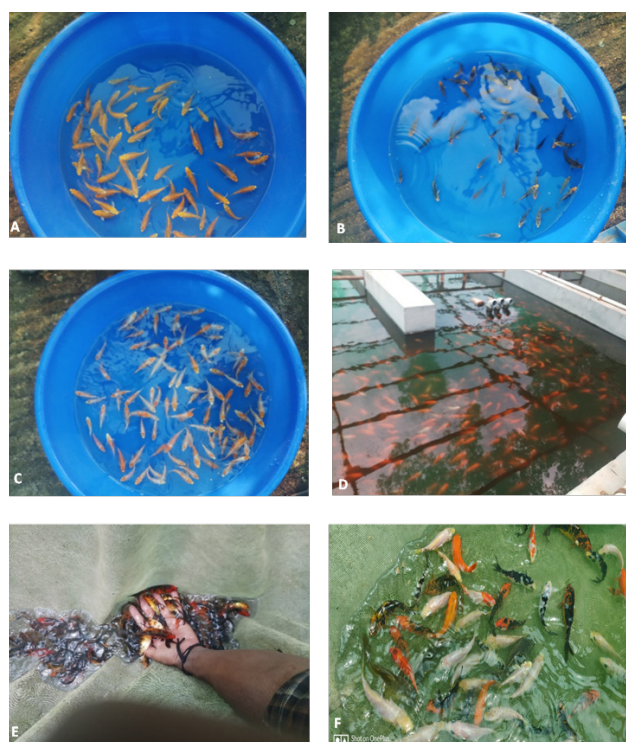
Stages	Time elapsed (H)	Developmental stages observed
Zygote period	0.00	The eggs were adhesive, demersal and spherical in shape. After the fertilization, the egg began to swell.
Cleavage period	0.55	At the stage of first cleavage the egg protoplasm moved towards the animal pole and divided the blastodisc into two equal blastomeres which is called 2-celled stage
	1.20	The second cleavage – 4 celled stage
	1.45	Third cleavage -8 celled stage
	2.03 h	Fourth cleavage-16 celled stage
	2.15 h	Fifth cleavage – 32 celled stage
	2.33 h	Sixth cleavage -64 celled stage
	2.45 h	After every division the size of the blastomere reduced and appeared as a cluster of solid cells
Blastula period	3.05 h	The morula entered the blastula stage and formed an ellipsoidal shape
	3.35 h	The line between the blastodisc and yolk became flattened out and wrapped around the yolk sac
	4.25 h	The blastoderm covered almost 1/3 of the yolk sac and formed dome like structure.
Gastrulation period	5.45 h	As a result of epiboly, germ ring was completed and Blastoderm started enveloping 1/3 of yolk sphere
	6.15 h	Blastoderm covered about 1/2 of yolk sphere
	7.45 h	Blastoderm covered about 3/4 of yolk sphere
Late gastrula stage	8.15 h	75% of the yolk was covered by blastomere
	9.45 h	80% of the yolk was covered by blastomere
	11.40 h	Blastoderm completely covered the yolk plug and epiboly came to an end. Tail bud appeared.
Embryonic phase	16.15 h	4-5 somite stage, Optic vesicle formation
	22.45 h	A pair of kidney shaped optic capsule was visible in the optic vesicle and the lens started differentiated. Tail bud also formed but not separated from the yolk sac
	24.10 h	As embryo increased in size, anterior and posterior region became distinguishable as broader cephalic region with distinct forehead and tail
	25.50 h	At 10-12 somite stages, the tail bud separated from the yolk sac and the heart beat began @ 70-80 beat / minutes
	31.05 h	At 19-20 somite stages, the pairs of optic vesicles became visible and the circulation began
	32.05 h	At 22-23 somite, stage 3/5 of the v-shaped myomeres encircled the yolk sac and gut started differentiated.
	33.05 h	Major vein formation, pigmentation occurred.
	36.20 h	Crystalline lens formed
	42.10 h	Embryonic fin fold formation, pectoral fin bud seen
	49.20 h	22-25 somite stage, lens fully formed, heart formed, embryo showed slight movement
	58.30 h	The embryo encircled the whole yolk sac, vigorous twitching movement
	63.15 h	Pectoral fin formation, A paired otolith could be seen in the optic vesicle
Hatching period	65.15 h	Xanthophore developed. At this point heart beat was much more rapid @130 beats/minute
	72.20 h	With the acceleration of body movement, larvae began to break the shell and tail emerged first.

the tailbud separated from the yolk sac and the heartbeat began @ 70-80 beat /minutes at 25.50 h.p.f. During the 13-19 somite stages, the optic vesicle became visible; gut started differentiated; major vein formation occurred;

pigmentation started; crystalline lens formed; pectoral fin appeared at 42.10 h.p.f. During 20-25 somite stages, the lens was fully formed, heart was formed, embryo showed slight movement at 49.20 h.p.f. At 72 h.p.f., the embryo

Table VI. Larval developmental stages of Koi carp.

Larval days	Length (mm)	Characters
1	2.7-2.9	Pigmentation was absent, Transparent with heavy yolk sac of about 1.60 mm, Devoid of mouth and anus, V-shaped myomeres were present.
2	2.9-3.1	Transparent, the mouth was not completely formed, the larvae still dependent on endogenous nutrients 1/2 of yolk sac absorbed, Rudimentary gill opening was differentiated, nostril started forming
3	4.0-4.2	Yolk sac was almost fully absorbed, Larvae started exogenous feeding, eye fully pigmented. nostrils were predominant.
4	5.0-5.2	All larvae started exogenous feeding. Functional mouth was observed. The digestive system was well developed.
7	5.9-6.1	Yellow pigments appeared on the body with silver bands on lateral sides and the mouth size was about 1.26 mm.
14	6.7-6.9	Fin rays started differentiated and the mouth size was around 1.35 mm
21	12-14	Various colour combination noticed on the body and the mouth size was around 1.47 mm.
35	24-32	Fin rays completely formed, started feeding artificial feed (0.6mm)

**Fig. 3. Larval development stages of Koi carp.**

occupied the entire space of the capsule, while pigmentation became more apparent during the pre-hatching stage. The wriggling movement of embryos was more visible at this stage, and tail wrapped completely around the egg. The eyes were pigmented. All the embryos were ready to hatch at this stage. The embryo had undergone rapid development and was clearly visible through the transparent egg membrane just prior to hatching with large eyes, and an orange yolk sac. After the 3rd day of incubation (72 h), the

embryo hatched out through the distal end of chorion by breaking the egg capsule with its active wriggling and the hatchlings emerged with tail first. The peak hatching took place between 06.00 to 08.00 h.

Larval metamorphosis

The newly hatched larvae measured 2.7-2.9 mm and were actively swimming near the corner of the water surface; devoid of mouth and anus. On the 3rd day post-hatch, the yolk sac was fully absorbed and the larvae started exogenous feeding. Jameson and Santhanam (1996) also reported that after 3 days, the larvae started exogenous feeding. During the 7th day post-hatch yellow pigmentation appeared in the body and the mouth size was 1.26 mm. After the 21st day post-hatch, various colour combinations appeared on the body. From the 35th day post-hatch, the larvae were fed with GROWFIN feed of size 0.6mm.

CONCLUSION

Koi carp is a colourful species which has numerous strains and it is an economically valuable ornamental species in aquaculture. Availability of quality seed is the basic and critical component of successful aqua culture practices. Induced breeding paves the way for continuous supply of seeds throughout the year. Result of the present study indicates that using WOVA-FH hormone at 0.5 ml per kg of body weight is the ideal dosage for inducing Koi carp using RAS system. This developed induced breeding technology would be helpful in producing year-round seed production with sustainability. The detailed description of embryonic development of Koi carp in this study will serve as baseline information for ornamental hatcheries due to its aquaculture importance.

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IRB approval

This research was carried out with the approval of University Students' Research Guidance Workshop Committee (Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, Tamil Nadu, India).

Ethical statement

This study was approved by the ethical committee of Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, Tamil Nadu, India.

Data availability statement

The data that support the findings of this study are available within the article.

Statement of conflict of interest

The authors have declared no conflict of interest.

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