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Induced breeding of telescopic eye gold fish (*Carassius auratus auratus*) using synthetic hormone (WOVA-FH)

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Abstract
The present study was aimed to perform induced breeding of Telescopic eye gold fish (*Carassius auratus auratus*) in captivity by administering WOVA-FH hormone at different doses. The study was conducted during the period from January-March, 2018 at Chennai, Tamil Nadu for 3 months. All the breeding pairs were bred including control. Significant (<0.05) difference were observed in the success rate of spawning at different dose among treatments and in control. WOVA- FH at a dose of 0.7ml/kg and 1.4ml/Kg was found to be effective for male and female respectively with 74% fertilization rate, 82 % hatching rate and fecundity of 1185-1205 eggs/female. Variability in hormone dose induced resulted in variability in latency period (5-9hrs), fecundity (767-1205 eggs/female), fertilization rate (46-74%) and hatching rate (52-82%). The positive response to synthetic hormone (WOVA-FH) with considerable fecundity, fertilization and hatching rate makes it possible to conduct the breeding program of this species commercially and the method can be applicable for commercial culture.

Keywords: *Carassius auratus auratus*, telescopic eye gold fish, WOVA-FH, induced breeding, synthetic hormone

1. Introduction
Aquarium keeping is popularizing and is the second largest hobby in the world [1]. This hobby has a long history dating back to several centuries. Introduction of civil aviation after the Second World War expanded the hobby to a global industry [2]. Ornamental fish keeping is now emerging as an important commercial activity. The ornamental fish sector is a small even though the vital part of an international fish trade. It contributes positively to rural development in many developing countries [3]. The Contribution of India to the world ornamental fish trade is merely at a tune of US$ 1.7 million, which is rather sparse considering the vast US$8 billion global market that has been growing at an average annual rate of 9% [4]. Organized trade in ornamental fish depends on an assured and adequate supply of demand, which is possible only by mass production. The Induced breeding technique is a convenient way for artificial propagation and mass breeding. Induced breeding is a method of ripening fish gonad to breed in confined water using stimulating agent and during off breeding season. A common method used for induced breeding in fish is an administration of the pituitary extract from a mammal or a fish or use of synthetic hormone [5].
C. auratus auratus fish is the most common variety of fancy goldfish and popularized due to its special feature, telescopic eye [6]. This fish has a broad head and a split caudal fin that is moderate in length and slightly forked for its short and stubby body. They are also available with a couple of other tail fin styles: veil tail, broadtail, and a butterfly tail. The *C. auratus auratus* exist in many different colours and with both metallic and nacreous scale types. The black/white strain is Panda Telescope and chocolate strain has orange pompoms. The popular Black Moor Goldfish is a black version of the *C. auratus auratus* [7].
They are frequently reproduced in large aquariums and outdoor ponds as well. Telescopic gold is not listed on the IUCN (International Union for the Conservation of Nature and Natural Resources) red list. If proper management steps are not taken, this variety of gold fish may become extinct as a result of inbreeding depression or other problems [8]. The goldfish is the highest traded variety and becoming increasingly important not only from the standpoint of the ornamental fish trade but also as experimental test animals because of its ability to adapt itself

successfully to environmental conditions [9]. The present study was undertaken to develop the induced breeding technique for *C. auratus auratus* (Black moor) using synthetic hormone to meet the high demand.

2. Materials and Methods

2.1 Collection and Conditioning of Brood fish

The present experiment was conducted at the Advance Research Farm Facility (ARFF), Fisheries College & Research Institute, Ponneri, Chennai, India. Brooders (15.0±2.0 gm; 8.0±1.5Cm) of *C. auratus auratus* (n = 100) were purchased from ornamental fish market located at Kolathur, Chennai and transported in oxygenated polyethylene bags. Before releasing into acclimatization tank, fishes were quarantined in 2ppm $KMnO_4$ solution.

2.2 Brood Stock Maintenance

Brood fish were stocked in circular Fibre Reinforced Plastic (FRP) tanks (2ton capacity) at 50 nos./tank. Fishes were fed with supplementary feed (38% protein) at 5% of body weight twice a day, Along with supplementary diet, brooders were fed earth worm on every alternative day to enhance maturity in captivity. The brood-stock was conditioned in 2 month period with intense feeding.

2.3 Experimental design

In the present study, mature brooders (20-32 gm) were induced using synthetic hormone WOVA-FH at different doses (Table 1) to optimize the ideal dose for induced breeding. Females were induced by injecting two doses while males were given a single dose of hormone during second/resolving injection of the female. Fishes were injected intramuscularly to avoid stress.

Table 1: Dosage of WOVA-FH used in Experimental Trials

Treatment	Sex	Dose of WOVA-FH ml/kg B.W		
		1st dose	2nd dose	Total dose given
Control	Female	No Inducement		
	Male			
T1	Female	0.5	0.5	1.0
	Male	-	0.5	0.5
T2	Female	0.7	0.7	1.4
	Male	-	0.7	0.7
T3	Female	1.0	1.0	2.0
	Male	-	1.0	1.0
T4	Female	1.2	1.2	2.4
	Male	-	1.2	1.2

2.4 Preparation of spawning tank

FRP tank (1ton) was used as spawning tank for Induced breeding of fish. The tank was disinfected before using by washing with chlorine and rinsing using Potassium permanganate. FRP tank was filled (1ft) with fresh RO water and continuous aeration provided. Spawning tanks were laid with artificial polyethylene strips (breeding mops) to use as substrate to attach eggs. The brooders were released into FRP tank at 2:1(M: F) ratio after injecting.

2.5 Dilution / Preparation of Hormone

Synthetic hormone WOVA-FH (Biostadt India Ltd) was obtained from a local medical shop. By diluting hormone in distilled water the concentration of WOVA-FH was reduced to 0.0125ml in each unit of the 1 ml syringe (40 units) the concentration standardized for 25g fish.

2.6 Selection of Male and Female

Mature healthy *C. auratus auratus* brooders (20-32 g) were selected based on secondary sexual character viz. size and colour pattern. Female can be easily identified by swollen belly, whereas in male body is streamlined and torpedo shaped. Mature males exhibit tubercles on the head and pectoral fins [10].

2.7 Estimation of Hormone efficiency

The efficiency of hormone-induced was estimated based on parameters like fecundity, fertilization rate, hatching rate and survival of larvae [11]. Fecundity was measured by random sampling, and calculating the total number of eggs in 1 spawning mop and multiplied with total numbers of mops in the tank. The fertilization rate of eggs was determined by randomly observing one spawning mop and counting fertilized eggs having intact nucleus of the total eggs released. Hatching rate was calculated by comparing the numbers of larvae and fertilized eggs. Ova diameter and egg development stages were measured using trinocular microscope (NLCD-120E, Lawrence & Mayo) with digital display.

2.8 Water Quality parameters

During the experimental period, water quality parameters in brood-stock, spawning and larval rearing tank were maintained at an optimum level (Table 3) as required for gold fish. The water quality parameters such as Dissolved Oxygen, Temperature, pH, Ammonia and total dissolved solids were analyzed periodically using APHA [12].

2.9 Statistical analysis

Statistical analysis of obtained data was carried out using SPSS software (version 20 for windows). Significant differences between treatments were compared by One-way ANOVA. Duncan's multiple range tests ($p<0.05$) was used to describe significant differences within and between treatments.

3. Results

3.1 Breeding behaviour

Spawning activity was observed 4 hrs after the second dose of injection. The courtship behaviour was observed at the bottom of the breeding tank. Active participation was seen by both male and female. During mating splashing of water was observed frequently with dorsal fins seen above the water surface. Males release the milt by rubbing their body against the female by aligning on either side of the female. The adhesive eggs were deposited on submerged spawning mops and were fertilized externally. Gold fishes are generally not good parents and they do not exhibit parental care, hence brooders were separated from spawning tank soon after spawning.

Courtship behaviour was observed after 1-8 hours of hormone injection and breeding was performed 5-9 hours after the 2nd dose of injection. Latency period varied significantly between the doses of WOVA-FH. Spawning took place as early as after 5 hrs of injection in T4; 7 hrs in T3, 8 hrs in T2 and 9hrs T1.

The result showed delayed latency period with the low dose WOVA-FH induced groups than other groups. Fertilized eggs of *C. auratus auratus* are adhesive, demersal and spherical in nature. The eggs of black moor became translucent as development progressed. The diameter of the fertilized egg capsule ranged between 0.9 mm and 1.1 mm, with yolk sphere of about 0.5-0.9 mm.

Fig 1: Length and weight measurement of brood fish.

Fig 2: Injecting hormone Intramuscularly.

Fig 3: Courtship behaviour.

Fig 4: Attachment of eggs on Spawning mops.

Fig 5: Free swimming hatchlings.

Table 2: Results of Induced breeding trials of *C. auratus auratus* using WOVA-FH

Treatment	Avg Size of the fish		Latency Period	Fecundity	Fertilization Rate	Hatching rate
	Female	Male				
Control	21.4 ±0.5	16.8±0.5	12	767.33±11.8^{b}	46.6±0.88^{a}	52.66±1.76^{a}
T1	20.8±0.5	17.1±0.5	9	868.33±11.6^{c}	65.3±1.45^{c}	69.0±0.57^{c}
T2	20.1±0.5	17.5±0.5	8	1189.00±15.3^{e}	74.0±1.52^{d}	82.0±0.57^{d}
T3	21.2±0.5	16.5±0.5	7	982.33±7.4^{d}	73.0±0.57^{b}	79.3±0.88^{d}
T4	20.9±0.5	18.0±0.5	5	670.33±9.2^{a}	52.3±1.20^{b}	63.6±2.88^{b}

Note: Values with different superscripts in a column differs significantly at p>0.05 (n=3)

3.2 Water quality parameters:

Water quality parameters play a major role in captive breeding at an optimum range which duplicates water quality as similar to the usual environment. This results in ease of breeding. In all the experimental tanks water quality parameters were maintained at optimum range given in the Table 3. Captive breeding was observed in all the treatments and control trials indicating that water quality parameters were conducive for gold fish breeding.

Table 3: Water quality parameters recorded during the experimental period

Parameter	Optimum range
Temperature (^{o}C)	20-24
pH	7.2-7.8
Dissolved oxygen(mg/l)	4.8-5.4
Free CO2(mg/l)	Nil
Total dissolved solids (mg/l)	40-130

3.3 The efficiency of hormone

The efficiency of hormone was calculated and observed parameters like Fecundity, Fertilization rate and Hatching rate. Fecundity in induced fishes was found to be significantly (p<0.05) higher in T1 (868.33±11.6), T2 (1189.00±15.3) and T3 (982.33±7.4) than control, except T4 (670.33±9.2). The fertilization rate of egg observed in the study was, 65.3±1.45, 74.0±1.52, 73.0±0.57 and 52.3±1.20 % corresponding to 0.5, 0.7, 1.0 and 1.2 ml/ kg body weight of fish using WOVA-FH (Table 2). Results indicate that high fertilization occurred on using hormone dose of 0.7 and 1.0 ml/ kg body weight of male and 1.4 and 2.0 ml/ kg body weight of the female. The hatchlings were released within 50-56hrs at 22.8 ^{0}C. In all the 4 treatments hatching rate was found to be significantly (p<0.05) different from control. High hatching rate was observed in T2 (82.0±0.57) and T3 (79.3±0.88) followed by T1 (69.0±0.57) and T4 (63.6±2.88).

Table 4: Egg and embryonic development stages in breeding of *C. auratus auratus*

Time elapsed form Spawning (Hours)	Developmental stage observed
0.40h	1-cell
0.55h	2-cell
1.19h	4-cell
1.40h	8-cell
2.15h	16-cell
2.03h	32-cell
2.33h	64-cell
3.04h	Early high blastula
3.35h	Late high blastula
4.24h	Flat blastula
6.13h	Early gastrula
7.45h	Middle gastrula
10.27h	Late gastrula
11.39h	Blastopore closure
16.15h	optic vesicle formation
22.42h	Lens formation
25.50h	Heat beat begins
31.02h	Circulation begins
32.04h	Coelom formation/Pectoral limb bud
33.08h	Major vein formation/Pigmentation
34.53h	Caudal median fin fold formation
38.01h	Fin ray formation
65.33h	Hatch out

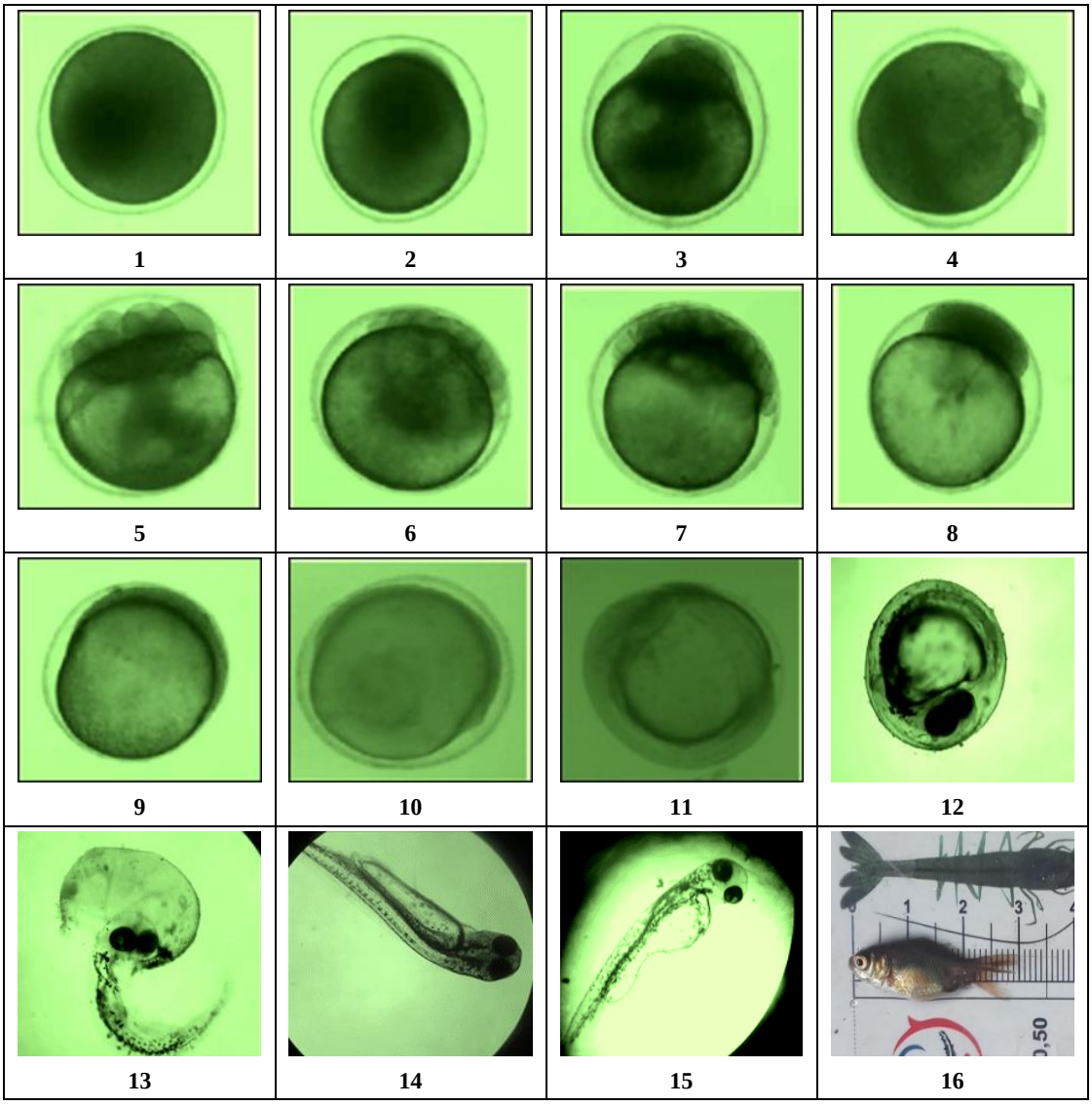

Fig 6: Embryonic development of *C. auratus auratus* (1) fertilized egg (2) blastodisc (3) two cell (4) four cell (5) eight cell (6) sixteen cell (7) thirty two cell (8) sixty four cell (9) morula (9) blastula (10) gastrula (11) "C" shape embryo (12) Eyed ova (13) hatchling jerking out of egg shell (14) Developed hatchling (15) Absorption of yolk sac in hatchling (16) 40 day old fry.

4. Discussion

The induced breeding technique is viable to meet the continuous demand of ornamental fish year round. Synthetic hormones are widely used for induced breeding and have good degree of success [13]. In the experiment, *C. auratus auratus* was successfully bred in captivity by administration of WOVA-FH. The present study indicates that using WOVA-FH at 0.7 ml/Kg body weight for male and 1.4 ml/Kg body weight for the female is the ideal dose for inducing *C. auratus auratus* in captivity. Spawning response of *C. auratus auratus* in the present study was comparable to that of the major carps where 95-100% breeding response is easily achieved [14].

Latency period was observed to be prolonged in control than the treatment groups. Spawning was earlier, in fishes after 5 hrs of inducing hormones of 2nd injection in high dose T4, followed by T3, T2, and T1 after 7hrs, 8hrs and 9hrs respectively. Similar observations are made by El-Hawarry [15] in silver carp induced with different analogues with domamine antagonists. Sridhar and Vijayakumar [16] noted 5-6hrs of latency period in *Ompok bimaculatus* (butterfish) when induced with Ovaprim. Similar observations were made by Pandey *et al*. [17] and Behera *et al.* [18] in *L. rohita and L. bata* respectively when induced with synthetic hormones. The present study indicates an inverse relationship between the dose of hormone and latency period. Similar observations by Mangesh *et al*. [19] in the induced breeding trial of *Hemichromis bimaculatus* has been studied.

The present study shows that hormone inducement increases fecundity and fertilization and hatching rate compared to control. Fecundity, fertilization and hatching rate was found to be different among the treatments. Fecundity and fertilization rate was observed to be in the range of 46-74% and 52-82%, similar results are observed by Yamamoto [20] and Jagtap [21] in gold fish when induced by HCG and Ovaprim. Minimal fertilization in T4 at a high dose may be due to early milt release and in lower dose as a result of late inducement in the male. Similar findings were reported by Mangesh *et al*., [19] in *H bimaculatus*. In the present investigation WOVA-FH at 0.7 ml/Kg body weight has given best result compared to other treatments, similarly Rahaman *et al*., [22] reported highest fecundity, fertilization and hatching rate in Comet gold fish by inducement of ovaprim at 0.7 ml/kg body weight with double dose to female. Rahaman *et al*., [22] and Salar *et al*., [23] achieved high production by giving double dose to female in gold fish and common carp it shows that potentiating action of the releasing hormone is high in some drugs when given in two doses [24]. Similarly in the present study, two doses of injection gave better results. The size and colour of fertilized eggs of *C. auratus auratus* were found to be similar to that of common carp, *Cyprinus carpio* [25]. As like Helen and Battle [9] observations in gold fish, eggs were very much adhesive in nature and were 0.8- 1.1 mm in diameter and hatch in approximately 60-65 hours at 25 ^{0}C. The present study revealed that the colour of fertilized eggs was transparent initially and changed to creamy as the embryonic development proceeded similar observations were made by Haniffa *et al.* [27] in Koi carp. The yolk sac was fully absorbed after 60to 65 hrs at 24-25 ^{0}C in the *C. auratus auratus* and this was observed to be similar to comet gold fish [22] and Koi carp [27].

5. Conclusion

The present study concludes that fecundity, fertilization and hatching rate can be improved by inducing hormone. The induced breeding experiment proved that WOA-FH inducement at dose of 0.7 ml/kg for male and 1.4ml/Kg for female is ideal to get better production than natural breeding. This investigation standardized ideal dose of WOVA-FH hormone for induced breeding of *C. auratus auratus.* This technology helps to get production year round without any crop gap, hence helpful for the farmers to get high production with the least input cost and better maintenance.

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7. References

1. Anna Mercy TV, Eapen Jacob, Raju K. Thomas. Studies on the reproductive behaviour of the common catopra, *Pristolepis marginata* Jerdon (Nandidae–Perciformes) under captive conditions. Current Science. 2003; 84(11):1468-1473.
2. Tissera K. Global trade in ornamental fishes-1998 to 2007. Paper presented at International Aquashow, Cochin, Kerala, India, 2010.
3. FAO. The ornamental fish trade. Production and commerce of Ornamental fish: technical-managerial and legislative Aspects. Globefish research programme, Rome, 2010; 102:134.
4. Sudha C, Gokula V. Induced spawning of rosy barb *Puntius conchonius* by *Natrum muriaticum*. IJAPBC. 2015; 4(4):801-804.
5. Sudha CC. Study on induced breeding in ornamental fish, *Poecilia sphenops*. European Journal of Experimental Biology. 2012; 2(4):1250-1255.
6. Hsin-Yuan Tsai, Mariann Chang, Shih-Chieh Liu, Gembu Abe, Kinya G Ota. Embryonic Development of Goldfish (*Carassius auratus*): A Model for the Study of Evolutionary Change in Developmental Mechanisms by Artificial Selection. Developmental dynamics. 2013; 242:1262-1283,
7. Marshall E. Ostrow, Goldfish (Barron's Complete Pet Owner's Manuals), Barron's Educational Series. Inc, 2003.
8. Andrews DC. An Interpet Guide to Fancy Goldfish. Interpet Publishing. IUCN. 2002; 1(902389):64.
9. Helen I, Battle HI. The embryology and larval development of the goldfish (*Carassius auratus*) from Lake Erie. Department of Zoology, University of Western Ontario, 1939.
10. ReddY PSR, Subramanian R, Baskar IS, Sheno YAS, Elambarithy B. Induced breeding of goldfish, *Carassius auratus* with human chorionic gonadotropin. In: Special Publication Carp Seed Production Technology (P. Keshavanath and K. V. Radhakrishnan, eds.) College of Fisheries, Mangalore. 1990; 2:21-23.
11. Haque MR. Management of an ideal carp hatchery in Quality Assurance in Induced breeding. Department of Fisheries, Jessore, Bangladesh, 1997, 31-37.
12. APHA (American Public Health Association, American Water works Association and Water Environmental Federation). Standard Methods of Examination of Water and Wastewater, 21st edition APHA, Washington. 2012, DC 20001-3710.

13. Harvey BJ, Hoar WS. Theory and practice of induced breeding in fish, 1979.
14. Basavaraja N, Mahesh T, Gireesha O, Malleshappa HD, Varghese TJ. The technology of Indian major carp seed production in Karnataka. Aquacult. Asia. 1999; 4(1):16-20.
15. Waleed El-Hawarry N, Nemaatallah BR, Shinaway AM. induced spawning of silver carp, *Hypophthalmichthys molitrix* using hormones/hormonal analogue with dopaime antagnonists. online J Anim Feed Res. 2012; 2(1):58-63
16. Sridhar S, Vijayakumar C. Cur. Sci, Nov.25, Article 1997; 27:1-4.
17. Pandey AK, Mohapatra CT, Kanungo G, Sarkar M, Sahoo GC, Singh BN. Ovatide induced spawning in the Indian major carps, *Labeo rohita* (Hamilton Buchanan). Aquaculture. 2002; 3(1):1-4.
18. Behera BK, Das P, Singh NS, Sahu AK. Captive breeding of an endemic medium carp Pengba, *Osteobrama belangeri* (Val.) with Wova-FH in Manipur. Journal of Aquaculture. 2010; 18:23-29
19. Mangesh Bhosale M, Felix S, Mahadevi, Aruna S, Cheryl Antony, Gopalakannan. Induced breeding of African Jewelfish *Hemichromis bimaculatus* (Gill, 1862) using gonadotropic signaling molecular analogue WOVA-FH., Journal of Entomology and Zoology Studies. 2017; 5(6):2547-2551.
20. Yamamoto KY, Nagahama Y, Yamazaki F. A method to induce artificial spawning of goldfish all through the year. Bulletin of Japanese Social Science of Fish. 1966; 32:977-983.
21. Jagtap HS. Comparative study on induction of spawning in goldfish (*Carassius auratus*) by prostaglandins & other inducing agents. Indian J Sci. Technol. 2011; 4(8):225-226.
22. Bazlur Rahaman SM, Zakaria Mahmud, Faysal Ahmed, Alokesh Kumar Ghosh, Wasim Sabbir., Induced Breeding, Embryonic and Larval Development of Comet Gold Fish (*Carassius auratus*). Electronic Journal of Biology. 2011; 7(2): 32-39.
23. Salar Dorafshan, Hossein Mostafavi, Bagher Mojazi Amiri. Induction of spawning in common carp Cyprinus carpio using pituitary extract and GnRH analogue in combination with Domperidon. Iranian Journal of Biotechnology. 2003; 1(4):213-217.
24. Peter RE, Sokolowska M, Nahornaik CS. Comparison of (DArg6, Trp7, Leu8, Pro9, Net) leutinizing hormone–releasing hormone (sGnRH-A), and (D-Ala6, Pro9, Net)-leutinizing hormone (LHRH-A), in combination with pimozide in stimulating gonadotropin release and ovulation in the gold fish, *Carassius auratus*. Can. J Zool. 1986; 65:987-991.
25. Woynarovich E, Horvath L. The artificial propagation of warm-water fin fishes: A manual for extension. 1984; FAO Fisheries Technical Paper No. 201 FIR/T201.
26. Haniffa MA, Benziger PSA, Arockiaraj AJ, Nagarajan M, Siby P. Breeding Behaviour and Embryonic Development of Koi Carp (*Cyprinus carpio*), Journal of Bio-Sci. 2006; 14:121-125.