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Comparison of the efficiency of different synthetic hormonal induction in the breeding of Common carp; *Cyprinus carpio* (Linn. 1758) in eco-carp hatchery

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Abstract
Common carp (*Cyprinus carpio)* is an important exotic major carp having high consumer preference for its nutritive values and organoleptic properties. Its faster growth rate and proper utilization of ecological niche makes it a suitable candidate species for aquaculture. A study was carried out to observe the effect of different inducing agents, viz. Ovatide, Wova –FH and Gonopro-FH on fertilization rate and hatching percentage of common carp, in eco-carp hatchery in a private fish farm at Sarakana village of Khordha district. The experiment was divided in three treatment groups i.e T_1 (Ovtide treated fish), T_2 (Wova-FH treated fish) and T_3 (Gonopro –FH treated fish). The fertilization was found 90% with Ovatide75.23% with Wova-FH and 88.54% withg Gonopro-FH. However hatching percentage was high (80%) in Gonopro-FH treatef fish, 69% with Ovatide treated fish and 45 % with Wova-FH. A comparison was carried out for fecundity, fertilization, and hatching rate during the induced spawning of *Cyprinous carpio* administered single dose of Ovatide, Wova-FH and Gonopro-FH. Gonopro-FH performed much better than Ovatide and Wova-FH. The results suggests that breeding and seed production of common carp with the inducing agent Gonopro-FH gives better result than other inducing agents in the form of massive scale seed production.

Keywords: common carp (*Cyprinus carpio*), Induced breeding, ovatide, wova-FH, gonopro-FH, fertilization rate, hatching percentage

1. Introduction
Fish culture in freshwater ponds was practiced in India as early as 350 B.C., though on a limited scale. Carp culture is common in India. India is blessed with huge freshwater resources consisting 3.15 million ha of reservoirs, 2.41 million ha of ponds and tanks, 0.798 million ha of beels, jheels and derelict waters and 1.24 million ha of brackish waters (Ayyappan, 2006) [4]. In addition to this, 0.195 million Ian of rivers and canals and 0.72 million ha of upland lakes can be put to different fish culture practices (Somvanshi *et aI*., 2007) [16]. Available statistics shows 'that only about 0.8-0.9 million ha of available water area (34%) under ponds and tanks has been put to use for aquaculture across the country (Ayyappan and Joykrushna, 2003) [3]. The fishery resources of India are one of the richest in the world, covering both marine and inland waters. The natural fishery resources, varying from temperate waters of Himalayas to sub-tropical and tropical waters south wards are supporting a wide variety of commercially important finfishes and shellfishes. India is the third largest farmed fish producer ranking second globally. The current fish production in India has reached 8.3 million tonnes, which expected to reach 12.5 million tonnes by 2025.
Through fisheries, aquaculture is the only answer to meet the nutritional demands of the increasing population since capture fisheries has its own limitations. As a consequence of adoption of scientific fish farming for production of carps, catfishes and freshwater prawns, the freshwater aquaculture sector now contributes more than two-third of the total inland production. The farming area of commercially important finfish and shellfish is persistently increasing throughout the country. Carps are among the commercially desirable and popularly cultured species in Asian and the Indo-pacific region. The fish, common carp *(Cyprinus carpio)* is a very widely cultured species among Cyprinid because of its high tolerance to environmental fluctuation. This fish originated from Central Asia (Jhingran *et al.*1985) [9] and now spread over almost all the sub-tropical and tropical countries of the world.

Generally common carp breeds in natural water bodies. But artificial breeding in commercial community and farm level is too much important for the successful expansion of aquaculture and farmers economic condition. Induced breeding is a technique whereby ripe fish breeders are stimulated by pituitary hormone or any other synthetic hormone introduction to breed in captive condition. The stimulation promotes timely release of sperms and eggs. The technique of induced breeding was first evolved in Argentina after producing pituitary extract by Houssay 1930 where viviparous fish was injected with the hormone to make premature birth. In the year 1934, Brazilians were succeeded in induced breeding by pituitary extract. This technique was also followed in America (Merlin & Hubs) and in Russia (Gerebilisky). In India the first experiment in induced breeding of fish was made by Hamid Khan in 1938 when Khan tried to induce spawn *C. mrigal* by the injection of mammalian pituitary gland (Khan,1938) [10]. Later H.L. Chaudhari succeeded in induced spawning on small carp species, *Esomus danricus* by administering the intra-peritoneal injection of catla pituitary gland. The first success in induced breeding of Indian Major Carps (*L. rohita* and *C. mrigala*) was in the 1957 by H.L Chaudhari and K. H. Alikunhi (Choudhuri H, Alikunhi KH.; 1957) [6] at Central Inland Fisheries Research Institute, substation, Cuttack (Orissa).

In tropical condition common carp attains sexual maturity within 1 year (Alikunhi, 1966) [1]. It spawns throughout the year in pond environment with two periods, one from January to March and the other during July to August. Spawning occurs in shallow marginal weed infested areas. It is a batch spawnner. For successful induced breeding it is necessary to select ripe brood fish (Penman and McAndrew, 1998) [14]. This carp is polyphagous in nature and in cultured ponds, feed on a wide variety of plant and animal matters (Pillay, 1993) [15]. The common carp was found to attain maturity when six to eight months old, the males about two months earlier than the females and at a smaller size. Mature specimens of both sexes occur throughout the year, being maximum during late January to March and July to August. This fish can breed with induction of pituitary extracts but the potency and the quality of the pituitaries used for preparing the extract became undependable, and because of this problem there is failure in spawning and also high stress on fish observed. So to overcome these problems several inducing agents i.e. Ovaprim, Ovatide, Wova-FH etc. are developed for successful induce spawning in fish with good percentage of fertilization. The present investigation was conducted to ascertain comparative efficacy of Ovatide, Wova –FH and Gonopro-FH on fertilization rate and hatching rate in in common carp (*Cyprinus carpio)*.

2. Materials and Methods

2.1 Study area and duration

The experiment was conducted at a private hatchery located Sarakana village of Khordha District from January 2015 to March 2015.

2.2 Brood stock managemen

Initially brood fish were kept in an earthen pond having water area of 0.5 ha with other species. At the advent of breeding season, the brood fish were checked for observing gonadal condition and manipulated them for better management aspects. To provide plentiful sustained supply of plankton and benthic organisms, brood ponds were fertilized at recommended dose using inorganic and organic fertilizers.

2.3 Selection of brood fish

Table 1: Selection criteria of the mature brood fish of common carps.

Male	Female
(a) Small in size	(a) Relatively large in size.
(b) Abdomen normal; not bulky like female.	(b) Abdomen bulging, elastic and soft
(c) Pectoral fins were rough.	(c) Pectoral fins were slimy.
(d) In the breeding season male usually tubercles on the head and gill plates.	(d) Tubercles absent
(e) Gently pressing on abdomen, male releases milt.	(e) Gently pressing on abdomen, eggs ooze out.

2.4 Formation of breeding pair

The present investigation was conducted by taking a total no of 24 brooders out of which 12 males and 12 females (1:1 ratio) as a breeding pair. Weight of all individual brooders sex wise were recorded by keeping the brood in a hand net with help of a balance. This is necessary to calculate the dose of hormone for its administration.

2.5 Conditioning of brooders

Equal number of breeding pairs were transferred to the three different spawning pools present in the eco-carp hatchery. The diameter of each spawning pool were 6m and height of the spawning pool were 2.5m (2.0m + 0.5m = 2.5m). The breeding pairs were kept for about 24 hours for conditioning under water showering. No feed was supplied during the period of conditioning. After 24 hours they were fed with GNOC and rice bran at 1:1 ratio per kg body weight of fish up to next three weeks.

2.6 Hormone Injection

The brood fish were weighed and dosage of the inducing agents were calculated by the following formula:

Quantity to be injected (ml) = weight of brood fish (kg) x dosage of synthetic hormone (ml)

The injection was given intraperitoneal (in the body cavity) at the base of pectoral fin and pelvic fin in the brooders at evening hours. Ovatide was given @ 0.2mi/kg body weight to female and 0.1mi/lg body weight to male kept in 1st spawning pool (Treatment-1). Similarly Wova-FH was given @ 0.5ml/kg body weight to all the brooders present in 2nd spawning pool (Treatment -2) and Gonopro-FH was given @ 0.5ml/kg body weight to female and 0.3ml/kg body weight to males in the 3rd spawning pool respectively (Treatment – 3).

Table 2: Breeding of common carp with different inducing agents.

Treatments	No. of female brooders (by wt. and no.)	No. of male brooders (by wt. and no.)	Inducing agent	Doses		Injection time
				Female (per kg body weight each)	Male (per kg body weight each)	
T_1	4.8 kg and 4nos	2.5 kg and 4nos	Ovatide	0.5ml	0.25ml	7.30AM
T_2	4.8 kg and 4nos	2.5 kg and 4nos	Wova-FH	0.5ml	0.5ml	8.00AM
T_3	4.8 kg and 4nos	2.5 kg and 4nos	Gonopro-FH	0.5ml	0.3ml	8.30AM

2.7 Breeding

The breeding tank was filled to about 0.19ml of freshwater. Spawning nests made of hydrilla and plastic materials were placed in the tank bottom spreading properly. A gentle flow of water was supplied in the spawning pool from bio filtered recirculating water system. Courtship behavior like swimming in pair smoothly, chasing females by the male and jumping little up the surface of the water was found before spawning. The presence of bubbles, milky water and fishy smell were the indications that spawning took place. Hormone induced breeders spawned within 6h - 8 h after injection.

The breeders then treated with 5ppm $KMnO_4$ solution and were transferred again to the rearing ponds. Utilizing the natural adhesiveness of common carp eggs, approximately 80-90% of fertilized eggs were attached to the substrate. Number of eggs were counted by weighing one sample of substrate where eggs are attached and to weighing total substrate of individual treatment, the fecundity was determined. Eggs were treated with malachite green by dipping the eggs attached to the substrate into the bucket filled with malachite green solution (2 ppm) to prevent bacterial and fungal infection before transferring them to the incubation tank.

Substrate with fertilized eggs attached were transferred to three different incubation pools having diameter 4m each and depth 1.2m each where for sufficient oxygen, bio filtered re-circulatory water with aeration systems were maintained. Quality of eggs, egg size and percentage of viable eggs were examined and on set of larval developmental stages were also observed regularly within 48-72hrs. 10-12 hours after hatching, the substrates were removed from the incubation pool.

2.8 Calculation of Fertilization rate, Hatching rate of the induced spawned breeding pair

Percent fertilization per female was calculated with the following formula

Total No. of egg counted Fertilization
= No. of fertilized eggs × 100 Percentage

Hatchability was determined by direct counting of the number of hatchlings of two days old and estimated as follows

Total No. of fertilized egg Hatchability
= No. of hatchlings (three days old) × 100

2.9 Water Quality Parameters

Water quality parameters such as temperature, pH, total alkalinity, total hardness, water transparency were recorded based on the standard methods given in APHA (1992) [2].

3. Results

The results of the trials are summarized as Fecundity, Fertilization and Hatching rates.

3.1 Fecundity

During the experiment it was observed that fecundity remains high for Ovatide treated fish (T_1) i.e. 7,50,000nos as compared to Wova-FH (T_2) and Gonopro-FH (T_3) treated fish (Table.3).

3.2 Fertilization rate

Fertilization rate with Ovatide was calculated as 90% while for Wova-FH it remained as 75.23% and 88.54% for Gonopro-FH. So Ovatide has 14.8% and 1.5% better results over Wova-FH and Gonopro-FH for fertilization rate of eggs (Fig.1).

3.3 Hatching rate

Although high fertilization rate was found with Ovatide treated fish group, hatching rate was highest (80%) with Gonopro-FH treated group. This group gave 11% and 35% better performance over Ovatide and Wova-FH for hatching rate (Fig.2).

Table 3: Details of induced breeding in *C.carpio* at 28±0.5°C

Treatments	Spawning time	Total no. of eggs produced (in lakhs)	Hatching time (from the time of hormone administration)
T_1	2.00PM	7,50,000	70hrs.
T_2	4.15PM	5,56,224	59hrs.
T_3	2.30PM	6,67,920	48hrs.

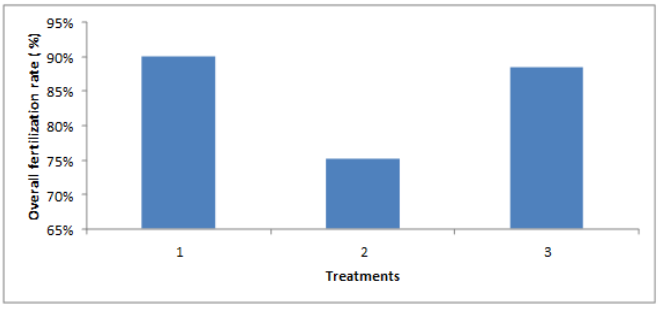

Fig 1: Effect of different inducing agent on fertilization rate (%) of *C. carpio*

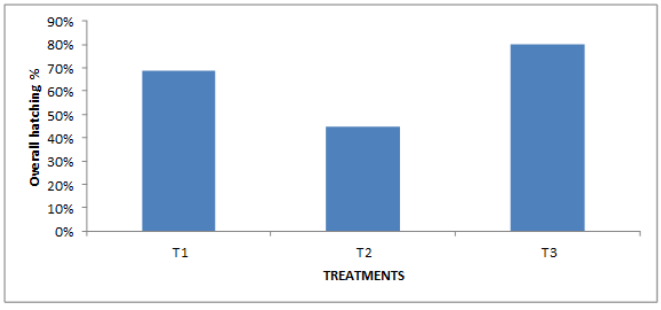

Fig 2: Effect of different inducing agent on hatching rate (%) of *C. carpio*

3.4 Water Quality Parameters

Different water quality parameters such as pH, dissolved oxygen (DO), total alkalinity, total hardness, water transparency, etc. were checked before as well as after spawning of the brooders. It was reported that before spawning the physico-chemical parameters of the water were p^H- 7.4, DO(mg/l) - 5.8,total alkalinity (mg/l)-120, Total hardness (mg/l)-135 and water transparency- 13.5. These parameters were shown in Fig.3.

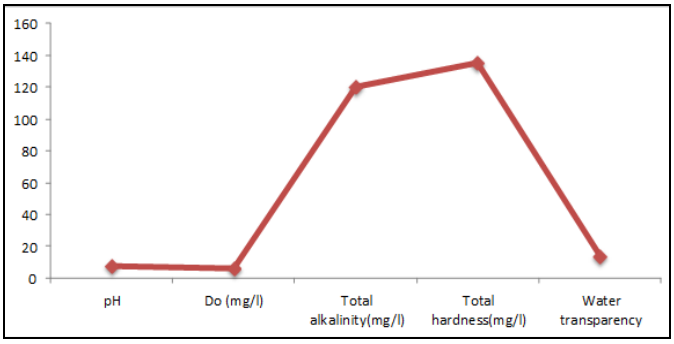

Fig 3: Physico-chemical parameters of spawning pool before spawning of the brooders

After spawning the parameters were p^H-6.7, DO(mg/l)-5.2,total alkalinity(mg/l)-113, total hardness (mg/l) -130 and water transparency -13.5cm. These parameters were shown in Fig.4.

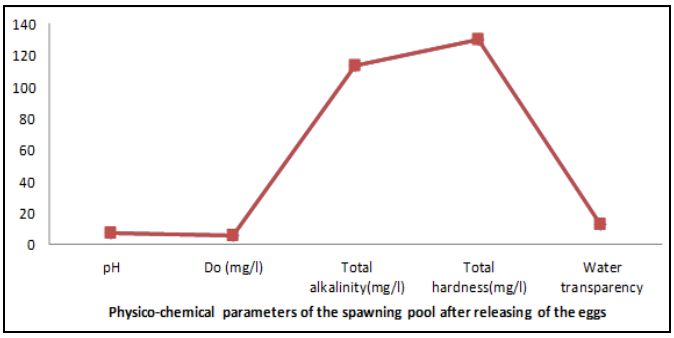

Fig 4: Physico-chemical parameters of spawning pool after spawning of the brooders

4. Discussion

The common carp was found to attain maturity when six to eight months old, the males about two months earlier than the females and at a smaller size (Parameswaran *et al*., 1972) [13]. This is one of the most important fish species of domestic fish farming. The artificial breeding of common carp has been carried out by efficient technological steps in hatcheries for decades. However, significant changes in fecundity occurred with respect to fish size. In this case, generally the larger fish gave a greater number i.e. when considering egg production. The relationship between fecundity (no. of egg production) and the body weight is thus proportional. The fecundity found from 1500 ± 200 to 7000 ± 1000 in each female (15000 ± 2000 to 70,000 ± 10,000 kg–1 total body weight). Freeman (1987) [8] reported that the female *C. carpio* deposits eggs - approximately 100,000 per kilogram of body weight. The slightly lower amount in egg number found in this study may probably be due to a smaller brood size. In our study the results obtained indicate that the fecundity of *C. carpio* differs significantly with different inducing agents and fecundity was high when induced with ovatide.

The fertilization rate and hatching rate are also affected by the seasonal variation that means with variation in temperature. Findings of the present study indicated that both the hatching rate and the fertilization rate vary with different inducing agents and also with temperature. Fertilization rate was high with ovatide treated group whereas hatching rate was high with Gonopro-FH treated groups.

The incubation period of eggs depends largely on water quality parameters such as salinity and temperature (Kuo *et al*., 1973; Liao, 1975) [11, 12]. In the present study the findings denoted that the incubation period or hatching time is varied significantly with different synthetic agents i.e. incubation period was lowest with Gonopro-FH treated group whereas it was highest with Wova-FH treated fish groups. The present study also indicated that incubation of eggs was also dependent on water temperature viz. in 28-32^{0}C hatching was within 2 days but in 20^{0}C hatching occurred in 3days.

Water quality parameters were considered as growth promoting factors within the optimum standard values. Brood pond management relating to fertilization and feeding was the prime consideration for producing quality broods and that in turn would allow the availability of good seed for successful aquaculture in the country. It is widely known that a complete brood stock diet is necessary to improve spawning quality and consistency. A high quality seed production demands in particular nutrition of brood stock which significantly affects fecundity and survival (Bromage *et al*., 1992) [5].

5. Conclusion

Based on results of this experiment, it can be concluded that Gonopro-FH use is more economical in commercial seed production of common carp, as it saves a considerable amount of time and avoids the excessive handling of brood fish. The positive response of common carp to Gonopro-FH indicated the higher potency of this synthetic hormone in inducing the fish more successful than other synthetic hormones in the hatchery. This study will be helpful to improve the brood stock, improve quality fish fry and increase the fry production rate in growout culture system.

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