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Evaluation on propagation of common carp (*Cyprinus carpio* Lin.) with hormonal and natural stimuli

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Abstract

The success of breeding of common carp (*Cyprinus carpio*) using hormonal inducement and environmental stimuli was evaluated considering different sex ratios, and natural and artificial substrates. A total of 18 females (weighing 250 to 2200g) divided into 6 treatments were investigated. A successful spawning was observed in all the treatment groups, only 66.66% female responded successfully to LHRH-A combined with dompheridone and 83.33% female in natural stimuli. Females induced with LHRH-A and dompheridone found prompt ovulation than that of natural stimulation. A significant variation ($F=7.45$, $P< 0.05$) was found among the different treatment groups. The number of eggs released appear to depend on body weight ($t=15.72$, $P<0.05$), sex ratio ($t= 7.96$, $P< 0.05$) and percentage of ovulated females ($t=5.34$, $P< 0.05$). Although environmentally stimulated females released more eggs than injected female ($t=5.18$, $P< 0.05$) but their survival rate was similar ($t= 1.77$, $P< 0.05$). Comparison between the two approaches under the conditions of AIT hatchery shown that both are suitable for spawning induction in common carp. However, environmental stimulation is advantageous because of the less labor and lower cost required for ovulation.

Key words : Common carp, Propagation, Hormone

Introduction

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.* 1988) and now spread over almost all the sub-tropical and tropical countries of the world.

There is a wide range of breeding practice of common carp e.g. natural, semi-artificial and complete artificial condition. The special characteristic of this species is its adhesive egg which is usually attached to a substrate. Effective breeding program is an essential requirement to viable commercial operation of a hatchery. Complete intervention of the reproductive cycle is a prerequisite for the development of an efficient breeding program (Gjerde 1986). Intensified

management of breeding fish is required both in reducing contamination and maximizing the demand of seed production. At the higher stage of breeding technology, fertilization rate and quality of egg also increased e.g. subsequently rate of egg hatching was enhanced to about 95% under controlled environment (Dwevedi 1986).

For semi-artificial practice the environmental stimulation was practiced by taking nominal measures like substrate application, keeping male and female together, creating required dark photo period and maintaining ambient water temperature. Isolation of eggs, hormonal treatment etc. are also used together with environmental stimulation. Now-a-days the most common and popular hormonal treatment is 'Linpe method', which is based on the GtH released by a super active analogue of piscine gonadotropin releasing hormone (sGNRHa) combine with dopamine receptor antagonist (Peter *et al.* 1988).

The main objective of the present study was to evaluate the breeding performances of common carp using different hormonal and natural stimuli.

Materials and methods

Broodstock management

Initially broodfish were kept in a earthen pond having water area of 0.2 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. Three weeks prior to spawning externally matured 57 female and 48 male carps were segregated and kept to a net cage ($10x10x2m^3$ size) suspended into the pond. Pelleted feed was administered two times daily at the rate of 1-2.5% body weight of the total biomass during the reared period. Fertilization of the pond of suspended cage was done two times (1st week and 4th week) at that period. One day prior to propagate, 18 females and 27 males were finally selected examining their stage of ripeness among the collected brood and transferred them to octagonal breeding tanks having $3.75m^2$ surface area with a flow through system. Ripe females were recognized by palpating the females abdomen which is bulging and soft to touch and swollen genital papilla. Males, on the other hand deep-pit-like vent and more slender body and ooze out milt when gently pressed in the body. The selection rate for female and male fish was 31% and 57% respectively.

Experimental design

The breeding design is shown in Table 1. In this experiment, the number of treatment was six comprising mainly the effect of injection, male-female ratio, and the substrate. In treatment I and II fish were hormone injected with plastic

substrate in each case but only difference was sex ratios. As the same way treatment III and IV comprised of natural leaves as substrate and fish were also hormone injected with different sex ratios. On the other hand, in treatment V and VI fish were tried to induce environmentally without injection using same substrate in different sex ratios. Amount of substrate was same (3 kg) in each treatment group.

Table 1. Experimental design

Treatments	Experimental unit	Stimulation	Substrate	Sex ratio (F:M)
I	Octagonal tank	Hormonal	Plastic	1:1
II	,,	Hormonal	Plastic	1:2
III	,,	Hormonal	Natural leaves	1:1
IV	,,	Hormonal	Natural leaves	1:2
V	,,	Environmental	Plastic	1:1
VI	,,	Environmental	Plastic	1:2

Hypophysation

Two types of hormones were used, these are Suprefact (LHRHa) and Motilium (Dompheridone). Only females were injected an intramuscular injection one time with a dose of 10mg suprefact and 10 mg motilium per kilogram of female. Before injection, stock solution was prepared diluting 1 ml of suprefact to 10 ml solution.

Breeding

Injected and non-injected broodfish of both sexes are transferred to the shallow octagonal concrete tanks ($2.0x2.0x0.25m^3$) in the hatchery for breeding and manipulating them in six tanks considering the sex ratios and substrate mentioned in design. The breeding tank is filled to about 0. 18m of freshwater. Spawning nests made of tree leaves and plastic materials are placed in the tank bottom spreading properly. A gentle flow of water is supplied in the breeding tank from biofiltered 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. As per expectation hormone induced breeders spawned the following morning within 6.00-8.00 hrs after injection but environmentally stimulated breeders were bred after 24 hrs (30.00-31.00 hrs after injection) than usually expected. The fish are allowed to spawn completely about 4-6 hrs after breeding start then

they are removed and records were kept of the weight of individual females and the total weight of the eggs released. The breeders then transferred to the small circular tank for further observation. 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 are transferred to the small hapa, made of mosquito net placed within small circular tank for incubation and primary nursing, where for sufficient oxygen, biofiltered recirculatory 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 48-59 hrs. Ten hours after hatching, the substrates was removed from the hapa. Hatchling rate of eggs was determined to count the hatchlings in a sample of particular amount of water and by measuring the total volume of water of individual hapa. Survival rates of hatchling are recorded 48 hr after hatching through counting of hatchling in a sample measuring about 20 ml of water .

Water quality parameters

Temperature and dissolved oxygen content of incubation tanks were monitored during the incubation period with the help of a oxygen meter.

Statistics

Analysis of Variance (one way ANOVA) was used to compare the significant differences among the treatment groups and to find out the least differences between the treatments, the critical analysis (CD) was done. Also Students t-test was used to compare the difference between factors involved.

Results and discussion

Tables 2 and 3 shows the overall performance of common carp breeders subjected to induce spawning during this investigation. Eight out of twelve and five out of six-females responded successfully to LHRH-A combined with dompheridone (66.66%) and environmental stimulation (83.33%) respectively. The overall success was 77.22%. Initial egg release occurred after 6 hrs interval in all injected females. Although most of the hormone treated female spawned successfully within the expected period but the percentage of success was lower than that of the environmentally stimulated females. Due to sub-optimal conditions in the fish pond and lack of certain environmental cues, fish can not spawn always at all in captivity or response in hypophysation even if their ovaries have completed successful vitellogenesis.

Table 2. Spawning performance of common carp

Treatments	Hour of treatment	Hour of initial egg release	Spawning ratio	Body weight of female before / after spawning	Fecundity (No of eggs)	Fertiliz-ation rate(%)
I	0.45am	8.00am	1/3	2.05/1.90	96,922	88.32[b]
II	0.45am	7.00am	1/3	3.20/2.90	176,761	87.60[b]
III	0.45am	7.00am	3/3	2.55/2.30	22,670	81.60[c]
IV	0.45am	7.00am	3/3	2.65/2.40	113,505	89.70[b]
V	0.45am	6.00am	2/3	2.65/2.30	249,309	91.00[b]
VI	0.45am	6.00am	3/3	2.65/2.20	338,559	83.00[a]

Means followed by same superscript letters vertically are not significantly different ($p< 0.05$)

Yaron and Levavi-Zermonsky (1986) cited that, vitellogenesis in the Israeli population of the common carp is completed by February, when follicle diameter reaches 1mm, however, at this time of year not all female carp will ovulate in response to either hypophysation or GnRH+MET (Drori unpublished). Another most important factor is the selection of suitable female carp including examination of abdomen softness by palpation or measuring fish maximal circumference (Rothbard 1981, Horvath and Tamas 1984). These method, though easy to perform, adopting accurately require much skill and experience. The eggs retained in unspawned females may be of poor quality also (Rothbard 1981, Horvarth and Temas 1984) and the rate of processes involved in oocyte final maturation and ovulation in fish depends on the ambient temperature (Yaron 1995).

Table 3. Hatching performance of common carp

Treatments	Hatching time (hours)	Total hatching number (after 8 hours)	Hatching rate (%)	Number of hatchling at harvest	Survivability (after 8 hours)
I	48	64,800	75.70	24,400	37.65
II	50	137,472	88.78	53,028	38.57
III	52	17,200	92.97	400	2.33
IV	51	84,811	83.30	69,799	82.30
V	59	144,599	64.00	51,875	36.00
VIa	59	109,951	39.00	30,800	28.00
VIb	59	56,201	20.00	1,120	2.00

The fecundity for injected and non-injected females varied between 0.02 to 0.17 and 0.25 to 0.34 million respectively with an average range of 0.02-0.34 million. A significant variation ($F=7.45$, $P<0.05$) was observed among the treatments in respect of number of eggs released by the females. Production of eggs in treatment-VI differ significantly ($P< 0.05$) from all other treatments groups and no variation was found among the rest of the treatments except treatment-III. Due to lack of replications in each treatment and some systems error in on set of experiments, it could not be able to find out the suitable treatment and factors responsible for more success of ovulation. Even than, an attempt was made here to find out the extent of difference between the treatment groups considering individual factor effect. A comparison of pairing one or two males with each female indicated that fecundity was consistently higher when two males present ($t=7.96$, $P<0.05$). The number of eggs released appear to depend on the body weight ($t=15.72$, $P<0.05$) of the fish used in these experiment and oocyte maturation and ovulation (100% success) in common carp were potentiated when natural leaves used to serve as spawning substrate for them. In present experiment, number of eggs production by the environmentally stimulated female were significantly higher ($t=5.18$, $P<0.05$) than that of the injected female and survival rate was similar ($t= 1.77$, $P<0.05$). A significant variation ($t=5.34$, $P<0.05$) of the percentage of ovulated females with respect to eggs released was observed also. Although 100% ovulation success performed by the females in treatment-III, but their fecundity and average survival of hatchling from eggs were remarkably low. The causes of lower number of egg production is unknown but a tremendous drop in average survival of hatchling was done due to insufficient oxygen (Table 4) in the system as air stone was out of order at the period of incubation. On the other hand, in treatment-VI, about half of the total eggs were kept in the same tank along with the substrate and another half transferred to the tank V as the same manner with the parents for incubation. In treatment-V, the lowest hatchling at harvest indicate cannibalistic nature of common carps.

The fertilization rate was found more of less similar for all the treatment groups and ranged between 81.60 to 91.00%. Lin *et. al.* (1986) observed from several experiments that fertilization rates of ovulated oocyte for common carp were consistently high (90%), hatchling rates were consistently high (90%) and that fry were normal. A wide range of survival rate (2.00-82.30%) of hatchling in all treatment groups after 48 hours observation found poor survival rate in both approaches, may be due to some unsuitable condition for incubation of eggs in the shallow tank with insufficient oxygen is responsible for poor hatching rate and survivability of hatchlings.

Table 4. Temperature and dissolved oxygen content in incubation tank

Treatments	Hours	Temperature (^{o}C)		Dissolved Oxygen (mg/l)	
		Feb. 25 1996	Feb. 26 1996	Feb. 25'96	Feb. 26'96
I	8.00	27.5	25.7	7.2	6.8
	16.00	32.0	31.7	5.5	7.1
II	8.00	27.5	25.7	6.6	6.3
	16.00	32.0	31.7	5.6	7.0
III	8.00	27.5	25.7	1.5	0.4
	16.00	32.0	31.7	0.9	1.1
IV	8.00	27.5	25.7	6.8	7.0
	16.00	32.0	31.7	7.1	7.4
V	8.00	27.5	25.7	6.7	7.1
	16.00	32.0	31.7	7.0	7.1
VI	8.00	27.5	25.7	7.7	7.5
	16.00	32.0	31.7	7.4	7.4

In this experiment adopting 'Linpe method', induced ovulation and spawning has proven to be successful with common carp. Utilizing low doses of dompheridone plus sGnRH-A analogue may be considered as the cost effectiveness of the 'Linpe method' but less labor and lower cost required for hypothalamic manipulations through environmental stimuli. On the other hand, 'Linpe method' has advantages than other hormonal induced method in terms of cost effectiveness of labor and reduced stress on broodstock as fish generally have to be handled only once to give a single set of injections. Comparison between the two approaches under the present physical facilities available shown highly effective and reliable technique in induced ovulation and spawning of cultured freshwater fish .

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