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Induced Breeding of Grass Carp (*Ctenopharyngodon idella*) and Silver Carp (*Hypophthalmichthys molitrix*) Using Ovatide as Synthetic Hormone at National Fish Seed Farm (Nfsf) Manasbal, Kashmir, J&K

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Abstract

In the present study economically important and fast growing food fishes grass carp (*Ctenopharyngodon idella*) and silver carp (*Hypophthalmichthys molitrix*) were successfully spawned with Ovatide (combination of GnRH analogue with dopamine antagonist pimozide) in Kashmir. The preparations were administered by an intramuscular injection of single dose of 0.7 and 0.8-0.9 ml/kg body weight for female grass carp and silver carp and a single dose of 0.35 and 0.4-0.45 ml/kg body wt. body wt. for male fishes respectively. After dosing, the fishes were immediately carried to breeding pools in the Chinese Hatchery. After 14-16 hours of dosing spawning took place. After 10-12 hours of spawning, twitching movement started. Hatching occurred after 20-30 hours of fertilization at 24-26°C. Fecundity of grass carp and silver carp were recorded as 70000- 80000 and 1-1.10 lac eggs/kg body wt. of fish respectively. The fertilization percentage of grass carp and silver carp were recorded as 80.03% and 78.12% respectively. The hatching percentage of grass carp and silver carp were recorded as 70.10% and 69.71% respectively. And the fry survival percentage of grass carp and silver carp were recorded as 15. 21% and 14.56% respectively. The source of water was Manasbal Lake that favored most of the water quality parameters for spawning, hatching and survival of fry than ground water.

Keywords: Induced spawning; Brooder; Ovatide; GnRH; Pimozide; Fecundity; Grass carp; Silver car; Chinese hatchery

Introduction

Among the most significant advancements in the field of aquaculture during recent times is the development of techniques to induce reproduction in fish. These techniques have allowed farmers to profitably breed and raise species that do not naturally reproduce in captivity and to manipulate the timing of reproduction to suit production cycles. Some species will not readily breed in captivity due to environmental or culture conditions that are different from those found in nature, such as water temperature or substrate type. These conditions may cause stress or may not provide the signals needed to complete the reproductive process. Numerous hormones have been used to induce reproduction. Two methods have emerged over the past few years that seem to offer the best chance for success at the least expense. They are injection of a GnRH analog with dopamine antagonist and injection of gonadotropin. Chinese carps are quick growing economic food fishes extensively cultured in ponds in China, Japan and some of the South-East Asian countries. Grass carp is herbivorous and consume vegetation and increase natural food production in the pond by nutrient recycling and fecal production [1,2] On the other hand silver carp is predominantly a phytoplankton feeder and as such efficiently converts the food into fish flesh resulting in high yields. With a view to study the cultural possibilities of these carps in Indian waters and their compatibility with the cultivated species in India, namely the Indian major carps, experiment consignments of fingerlings of the Chinese grass carp (*Ctenopharyngodon idella)* and the silver carp (*Hypophthalmichthys molitrix*) were brought in 1959 [3]. Major breakthrough achieved in induced breeding of Indian major carps by administration of fish pituitary hormone by Chaudhuri and Alikunhi [4,5] Alikunhi et al., [6] and the technique has been further developed for attaining commercial production of fish seed of these economic species [3,4]. In the case of Chinese carps, successful spawning of the Grass carp and Silver carp by hormone injection has been reported by Aliev [7], Alikunhi et al. [8], Chaudury et al. [5] and Nayeem et al. [9]. In recent years, Human Chorionic Gonadotropin (HCG) has received some attention as a substitute for pituitary but has met with little success,except in the breeding of silver carp.

Lutinising releasing hormone (LH-RH), a mammalian hypothalamic peptide, has the capacity to release gonadotropin from pituitary gland. The Chinese report on successful use of mammalian based LH-RH analogue (D-Ala$_6$, Pro$_9$, Net) for induced breeding of carps created worldwide interest on the use of LH-RH for breeding various species of fish. A major breakthrough in fish breeding research was the finding that dopamine, acts as an inhibitory factor for synthesis of gonadotropin.

When LH-RH was used alone, without Pituitary Gland, spawning failure clearly indicates that dopamine blocks the action of LH-RH on the secretion of gonadotropin. Thus blocking of dopamine action with some antagonists like pimozide, potentiate the action of LH-RH resulting in successful spawning. There has been considerable research in India on spawning of carps with ovatide and ovaprim. Kaul and Rishi [10], reported the successful spawning of mrigal. Najar et al. [11], reported successful spawning and larval rearing of

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snow trout *(Schizothorax niger Heckel*) in Kashmir Himalaya with the application of ovatide. Khan, et al. [12], reported the successful spawning of rohu and mrigal with ovaprim (LH-RH analogue) at Fish Hatchery Islamabad, Pakistan. Ovatide is a new highly potent compound containing a synthetic peptide analog to the naturally occurring salmon GnRH –D.Arg^6 Pro Net. It also contains a dopamine antagonist pimozide, whereas GnRH analogs stimulates the pituitary to release gonadotropins and trigger the process of reproduction, the dopamine antagonist inhibits the release of dopamine and make sure that the secretion of gonadotropin is not inhibited. The use of ovatide, thus, constitutes the latest and the most advanced technology employed for induced breeding of fishes and production of high quality fish seed. A single dose of ovatide given intramuscularly to the brood fish leads to the production of an increased number of eggs through complete spawning with high fertilization and hatching percentage. The present study is the outcome of seed production trials carried out at National Fish Seed Farm, Manasbal (NFSFM), Kashmir on grass carp (*Ctenopharyngodon idella*) and silver carp(*Hypophthalmichthys molitrix).*

Materials and Methods

A total of 42 fishes consisting 21 grass carps (14 males and 7 females in 2:1 ratio) and 21 silver carps (14 males and 7 females in 2:1 ratio) were used for this study conducted from 20th July upto ist August and 15 July up to 10th of August 2014 respectively. The total wt. of female grass carps and silver carps were calculated as 15.504 and 16.949 kg respectively. For breeding, hatching and spawn collection, Chinese Circular Hatchery was used, which has four components for different operations: a) breeding pool or spawning pool, b) hatching pool or incubation pool, c) spawn collection pool and d) overhead storage tank. All the four components were meant for different operations like breeding, hatching, spawn collection and supply of water respectively. Hypodermic 2 ml syringe having 0.1 ml graduations with a needle no. 22 was used.

Selection of brooders

(1). Healthy, disease free, fully mature ripe fishes of the age group of 4-5 years (2-5 kg wt.) was selected. (2). Good characteristic traits of grass and silver carps of both sexes were selected. Secondary characteristics of both sexes of grass and silver carps that showed full maturity of these fishes during selection are (Table 1)

Care of brooders

Brooders of both grass and silver carps were reared in composite culture prior to inducing breeding. Grass carp liked clear and fresh water but their excreta proliferated large quantities of plankton. In order to control reproduction of plankton and water, planktivorus silver brood carps were reared with grass carp. But before a day to induce breeding, all the males and females were separated and were put in separate cemented raceways for conditioning. During this time feeding was stopped and special care was taken while drag netting and handling of brooders for induced breeding to avoid injuries to fishes. Brooders of grass carps were extensively fed with green fodder in the form of aquatic weeds like Vallisneria, Ceratophyllum and Hydrilla at the rate of double the wt. of their body so as to make them fully mature.

Method of injection

The fishes were held firmly and weighed cautiously, a calculated amount of single dose of ovatide injection to both sexes of grass carps and silver carps were given intramuscularly in the region of the caudal peduncle above the lateral line. The needle was inserted under the scale with hypodermic 2 ml syringe through to a depth of about 1.5 cm and injected the fluid slowly. Males of grass carp and silver carp were injected 0.35 and 0.4-0.45 ml/kg body wt., whereas females of grass carp and silver carp were given 0.7 and 0.8-0.9 ml/kg body of fish respectively.

Time of injection

The time of injection depends upon the water temperature. Due to low temperature in Kashmir valley as compared to other regions of India, a slightly high dose of ovatide injection was administered in the evening around 4-5 pm.

Handling and transfer of Brooders

After dosing, fishes in the 2:1 (male: female) ratios were immediately transferred to breeding pool of Chinese Hatchery in plastic bucket in the evening time (4-5 pm). Each plastic bucket carried 100 liters of water and about 10 kg parent fish. No anesthesia was given during transfer of brood fishes, as the place where dosing of ovatide was done was in close vicinity to Chinese hatchery. Acclimatization was maintained to avoid fish mortality.

Breeding and spawning

After single dose of injection to both males and females, they were put in the ratio of 2:1 (male: female) in the breeding pool of Chinese hatchery especially in the evening time. Then after about 4-5 hours, showering and water jets were started so as to create circular water motion, soon after about 7-8 hours of showering they got excited and showed sexual play. Males started to chase females and forced them to lay eggs. Spawning took place after 14-16 hours of injection at temperature 24-26°C which heavily depended on temperature. Eggs were washed with the solution of potassium per manganate, the colour of eggs were whitish muddy. The eggs of both grass and silver carps were demersal in nature, semi-pelagic were also seen.

Counting of eggs

The number of eggs was measured by volumetric method. A beaker was used in which number of eggs was counted in triplicate and the average of the three estimations was taken to know the numbers of eggs per beaker. Multiplying this with the number of beaker measured, total number of eggs was calculated.

Hatching and incubation

Hatching and incubation mainly depends on temperature, because of low temperature in Kashmir valley comparing to other regions of India , hatching period was recorded as 20-30 hours after fertilization at temperature 24-26°C. Newly hatched larvae remained in the circular incubation pool for four days until yolk was absorbed. 3 days later in incubation pool baby milk powder (lactogen) were started and on 5th day they were transferred to nursery ponds for further rearing. Water quality parameters during experiments are given in Table 2.

Calculation of fertilization rate, hatching rate and fry producing rate

When fish eggs have developed to the middle gastrula stage (after 16-30 hours of fertilization), about 100 eggs were collected with small net at random, they were put into a white dish and the eggs such as turbid eggs, white eggs, empty eggs and rotten eggs with naked eyes were given up. For more accuracy another method was operated to distinguish the fertilized and unfertilized eggs by simply dipping the

Species	Characteristics of the female	Characteristics of the male
Silver carp	Females with soft, distended belly and pink-red genital papilla, released ova when subjected to gentle pressure on the abdomen.	Released milt when subjected to gentle pressure on the abdomen Pectoral fin was rough.
Grass carp	Pectoral fin was soft. Abdomen round bulged with reddish fleshy vent. Ova oozed out on pressing abdomen.	Pectoral fin was rough, milt oozed out when pressed on abdomen.

Table 1: Secondary characteristics of both sexes

DO(mg/L)	6.5
pH	7.9
Water Temperature(^{0}c)	24-26
Nitrate(mg/L)	0.3
Alkalinity(mg/L)	97.8
Co_2(mg/L)	9.8
Iron(mg/L)	0.05
Hardness(mg/L)	179
Phosphorous(mg/L)	0.09
Calcium(mg/L)	159
Carbonate(mg/L)	26.3
Bicarbonate(mg/L)	153
Total carbonate	181
TDS(mg/L)	588

Table 2: Phsico-chemical parameters of Chinese hatchery water.

eggs in a dilute $KMnO_4$ in a petri dish. The unfertilized eggs got colored whereas the fertilized eggs remained unchanged. Calculation of the fertilized eggs by percentage.

$$\text{Fertilization rate\%} = \frac{\text{no. of fertilized eggs}}{\text{total no. of eggs}} \times 100$$

$$\text{Hatching rate\%} = \frac{\text{no of hatched fry}}{\text{no. of fertilized eggs}} \times 100$$

But in production, it was trouble-some to obtain the accurate figures of the actual hatching rate, therefore, it was important to calculate the fry producing rate in production.

$$\text{Fry stocking rate\%} = \frac{\text{no. of fry stocked in ponds}}{\text{no. of fertilized eggs}} \times 100$$

Results

Results of this study showed that successful induced spawning in grass carp and silver carp were achieved by using a single dose of ovatide. Certain drugs and different analogues of LH-RH are being tested for breeding fishes with varying degree of success. However, it was only when the dopamine inhibitory activity in the synthesis of gonadotropin was demonstrated that the reason behind the spawning failures became clear. Investigations have now clearly shown the potentiated actions of analogues when they are combined with dopamine antagonists like pimozide or domperidon. Based on the extensive research on Chinese carp, Peter, et al. [13] defined a new method of breeding called the Linpe in which LH-RH analogue is combined with a dopamine antagonist. It is major breakthrough in the history of aquaculture. Ovaprim and Ovatide are new drugs developed essentially on this new method combining releasing hormone with dopamine antagonist. Earlier studies conducted in India, Nandeesha et al. [14] and Khan et al. [12] have clearly demonstrated superiority of ovaprim in induced spawning of major carps.In this study both the grass carp and silver carp were successfully induced to spawn injected with single relatively high dose of ovatide (Tables 3 and 4). All the females and males of grass carp and silver carps (Total 42) showed positive response to ovatide and the ovulation of female fishes were 100%. The females of grass carps and silver caps were given 0.7 and 0.8-0.9 ml/kg body weight, whereas males were given 0.35 and 0.4-0.45 ml/kg body wt. respectively. In the present study grass carp showed full maturity and were ready to induce from 20th July upto 1st Aug 2014 whereas silver carps became fully mature and were ready to induce from 15th July upto 10th Aug.2014. Both males and females were put in 2:1 ratio in Chinese hatchery. The spawning and hatching periods for both grass carp and silver carp were prolonged and recorded as 14-16 hrs after injection and 20-30 hrs after fertilization at temperature 24-26°C respectively (Table 5). After 3 days in incubation pool, baby milk powder (lactogen) were started and on 5th day they were shifted to nursery ponds for further rearing.

The results obtained showed that both the grass carps and silver carps are not difficult to spawn with ovatide and the results obtained were satisfactory (Table 6). Though the number of eggs spawned by these carps in this study were relatively low than previously reported, but fecundity, fertilization and hatching to fry were comparatively high. The low fecundity may be due to low temperature and particularly not finding their natural productivity and the artificial feed given were not up to their standards, so these conditions might lower the nutritional intake of the brood fishes and finally might be cause of low fecundity.

Conclusion

The use of induced hormone "ovatide" was first time used in Kashmir valley at NFSF Manasbal to Chinese carps and the results obtained from the above experiment clearly demonstrated that the Ovatide was found effective in inducing spawning in Kashmir valley. It was also found that a slightly high dose of ovatide injection comparing to other regions to both grass carp and silver carp showed positive response. Although fecundity was low, fertilization and hatching period too was prolonged, yet fertilization, hatching and fry survival percentages were good. It

Females of the following fish species	Dosage of spawning agents
Catla	0.4-0.5 ml/kg body weight
Rohu	0.3-0.4 ml/kg body weight
Mrigal	0.25-0.3 ml/kg body weight
Fringe-lipped carp	0.3-0.4 ml/kg body weight
Catfish	0.6-0.8 ml/kg body weight
Silver carp	0.4-0.7 ml/kg body weight
Grass carp	0.4-0.8 ml.kg body weight
Bighead carp	0.4-0.5 ml.kg body weight
Mahseers	0.6-0.7 ml/kg body weight
Males of all species of carps	0.1-0.3 ml/kg body weight
Males of catfish	0.15-0.4 ml/kg body weight

Table 3: Dosage of ready-to-inject spawning agents (Ovaprim, Ovatide,WOVA-FH, etc.).

Fish species	Dose of ovaprim(ml)
Catla catla	0.4-0.5
Labeo rohita	0.3-0.4
Labeo rohita	0.4
Cirrhina mrigala	0.25-0.3
Cirrhina mrigala	0.4
Hypophthalmichthys molitrix	0.4-0.7
Ctenopharyngodon idella	0.4-0.8
Aristichthys nobilis	0.4-0.5
Hypophthalmichthys molitrix	0.6
Schizothorax niger	0.3-0.5
Ctenopharyngodon idella	0.7(ovatide)
Hypophthalmichthys molitrix	0.8 -0.9(ovatide)

Table 4: Dosage of Ovaprim-C and ovatide for carps at different locations.

	Grass carp	Silver carp
Parameters	Ovatide Treatment	Ovatide Treatment
Fecundity((body wt. of fish^{-1})	70000-80000	1-1.10 lac
No. of females treated	07	07
Totalwt. of females(kg)	15.504	16.949
Temperature(^{0}c)	24-27	24-26
Spawning period(hours)	14-16	16-18
Total no. of eggs	1240320	1864390
Total no. of fertilized eggs	992628.096	1456461.468
Hatching period(hours)	20-30	20-30
Total no. of hatchling	695832.295	1015299.289
Fertilization percentage	80.03	78.12
Hatching percentage	70.10	69.71
Total no. of fry survival	150978.733	212060.79
Percentage of fry stocked	15.21	14.56

Table 5: Effect of Ovatide on grass carp and silver carp at NFSF Manasbal.

Shape	Circular
Diameter inner circle(m)	1.25
Diameter outer circle(m)	4
Depth of water(m)	0.75
Volume(L)	5107
Rate of water flow(m/sec)	0.2-0.4
Input of water (L/sec)	8
Duck mouth input pipe(nos.)	16
Nursing time(hour)	96
Egg loading capacity(lac)	30

Table 6: Incubation pool structure used for induced breeding at NFSF Manasbal.

was also seen use of ovatide reduced handlings of brood fish due to the single injection given to both the sexes simultaneously. These not only well decrease/avoid post spawning mortality of fish but also increase spawning response.

Further trials are now essential to standardize use of dosage and to gather additional information on the eggs and hatchlings produced through Ovatide treatment, such as their size, rate of growth, survival etc.

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