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Full Length Research Paper

Induced spawning, fecundity, fertilization rate and hatching rate of Grass carp (*Ctenopharyngodon idella*) by using a single intramuscular injection of ovaprim–C at a fish hatchery Faisalabad, Pakistan

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In the present study, intramuscular injection of ovaprim-C was studied on the number of eggs/kg, fertilization rate and hatching percentage during April to June 2008 at Fish Seed Hatchery, Satyana Road, Faisalabad, Pakistan on Grass carp (*Ctenopharyngodon idella*). Fishes were spawned successfully following a single dose of injection of ovaprim-C (luteinizing hormone releasing hormone analogue) with 0.6 ml/kg for female and 0.2 ml/kg for male. Ova and milt were stripped simultaneously and mixture was stirred for 15 to 30 s during which fertilization occurred. Hatching occurred within 18 to 22 h after fertilization. Experiment was conducted in circular spawning tank with 2 m diameter. Regression analysis was applied to assess the body weight dependence of absolute fecundity (total ripe eggs in the ovary) and relative fecundity (number of eggs/kg). It was observed that body weight has positive influence on absolute fecundity (r = 0.926). Equations were developed to describe these relationships. If it is impossible to determine the absolute and relative fecundity, then these parameters can be determined from the body weight. The equations of these parameters are highly significant (P < 0.001) and can be used to estimate the absolute and relative fecundity with a fair amount of accuracy.

Key words: Fish, induced spawning, fecundity, ovaprim-C, *Ctenopharyngodon idella*.

INTRODUCTION

Grass carp (*Ctenopharyngodon idella*) was imported in Pakistan from China for the first time in 1964. The purpose of its introduction, in addition to culture, was for biological aquatic weed control in natural waterways, rivers and man-made lakes (Khan et al., 2004). Grass carp (*C. idella*) matures at the age of 2 years and above; generally, males mature little earlier than female in cultivable carps (Rath, 1993).

Spawning is the release of sexual products (ova in the case of female and milt in the case of male) to the exterior of the body (Basaran et al., 2008). Induced breeding is a method in which exogenous hormones are injected into the body of mature parent fish for induction of breeding (Heggberget, 1996). During the past two decade, pituitary extract was used for induced breeding. The ever increasing cost of donor pituitary and cumbersome process obliged expert to test alternative hormones such as HCG (human chorionic gonadotropin), LHRH (leutinizing hormone releasing hormone) and

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ovaprim-C (Haniffa and Sridhar, 2002). Ovaprim is a product that contains salmon gonadotropin releasing hormone analogue (sGnRH;D-Arg^{6},Pro^{9},Net) at a concentration of 20 ug/ml and dompridone, a dopamine antagonist at 10 mg/ml (Hill et al., 2009). Dopamine antagonists are used for ceasation of dopamine activity which acts as an inhibitory factor for the synthesis of gonadotropin (Naeem et al., 2005a). Ovaprim is used to induce ovulation and spermiation in fishes mostly by intramuscular or intraperitonial route. Reported adverse effects are also observed which includes bruising, redness, wounds, ulceration, darkened body coloration (hyperpigmentation) and loss of body colour (hypopigmentation) at the site of injection and overall post spawning mortality occurred (about 1.3%) (Hill et al., 2009). In Pakistan, major break through was achieved when Chaudhary and Alikunhi (1957) succeeded in induced breeding of Indian major carp using pituitary extract (Alikunhi et al., 1962; Naeem et al., 2005b). Carp culture is rapidly expanding but the major constraint in the development of this industry is the non availability of quality fish seed.

The gonadotropin releasing hormones (GnRH) in teleosts fishes is also influenced by gonadotropin inhibitory factor (GRIF) from the hypothalamus, which is later identified as dopamine and demonstrated to have inhibitory activity (Peter et al., 1988). Several commercial synthetic successful spawning agents in ready made form containing GnRH and dopamine antagonist like ovaprim-C, ovopel, daginand and aquaspawn are available, and used in different fish species (Peter et al., 1988; Cheah and Lee, 2000; Das, 2004; Brzuska, 2006; Marimuthu et al., 2009). This will be helpful in obtaining reliable amounts of eggs by using exogenous hormones treatment to stimulate ovulation under artificial conditions. In the absence of optimum conditions, brood fish encounter stress, resulting in spawning failure or perhaps, mortality (Basaran et al., 2008). Many factors that inhibit reproductive process in teleosts, results in gonadal regression example, endogenous rhythms, photoperiod, temperature, stress, nutrition and contents of food, water quality, pollutants, salinity, food and water current, weather cycle, lack of spawning substrate and disease and parasites (Singh et al., 2010).

The aim of this study was to investigate the induction of spawning, fertilization rate and hatching rate of the Grass carp (*C. idella)* in culture conditions at Fish Hatchery, Satyana Road, Faisalabad.

MATERIALS AND METHODS

The experiment was conducted at Fish Hatchery, Satyana Road, Faisalabad, Pakistan during the month of April to June 2008 with the aim of accessing the relative fecundity, that is, number of eggs/kg of body weight, by artificial spawning in Grass carp (*C. idella)*.

Twenty two specimens were used for this investigation, their weight ranged from 2.8 to 6.5 kg; they were free of all diseases and sexually ripened. Tests were conducted in circular spawning tank 2 m in diameter and 1 m in depth with optimum water quality variables and fish was acclimatized for one day before the commencement of experiment. Ovaprim-C was injected in a single dose because trials by Nandeesha et al. (1990), has found the effectiveness of a single and simultaneous injection. So, risk was not taken to try varied doses due to shortage of ovaprim-C and brooders. Good quality and well matured male and female brood fish are prerequisite for successful artificial propagation (Muir and Robert, 1985), so, most suitable size of spawners taken was 4 to 6 kg, to avoid difficulty in handling large size and requirement of large doses of expensive hormones. Brood fishes were transferred into cemented holding tanks of hatchery and anesthetized with 100 to 200 ppm 2-phenoxy ethanol in 1000 L one ton capacity fiberglass tank half filled with tap water. Sex ratio of one female to two males was used in induced spawning for achievement of best results. Brood fishes were weighed and dosage of ovaprim-C solution was calculated according to Nandeesha (1991):

Quantity to be injected (ml) = weight of brood fish (kg) x dosage of ovaprim-C

Male brooders were injected 0.2 ml/kg and females 0.6 ml/kg by intramuscular route into the dorsolateral region of the fish in a single dose (Haniffa and Sridhar, 2002) by using hypodermic syringe after cleaning the area with cotton swab soaked in alcohol. Ripeness can be accessed by several indicators example, in female, the abdomen was round, soft and genital opening was swollen, protruding and pinkish red, anus was also swollen and reddish. In male, secondary sexual characteristics were evident (Metwally et al., 2008).

Required amount of ovaprim-C was withdrawn from the bottle by keeping the needle upward and air was removed from syringe. During injection, brood fish was placed in cloth bag, lying laterally in water and upper half of fish was held above the water surface. At the inner side of basal part of the pectoral fin where it was scaleless, needle was inserted gently towards the head at an angle of 45° to the body's longitudinal axis to a depth of about 1.5 cm and injected slowly. Immediately after administering the hormones, the breeding sets (two males and one female) were released into cemented tanks (capacity 500 L) containing dechlorinated tap water, flowing at the rate of 12 L/min (Haniffa and Sridhar, 2002).

Fish were observed for behavioral signs after 8.30 h of dose administration. Estrus is restlessness of female and its abdomen and tail become extremely constricted, which lasts for 30 to 60 min after which fish were netted out for stripping. Sex product was stripped from the spawners by gentle massage and pressure on the abdomen (Jamroz et al., 2008) into a dry plastic bowl. Following the semi dry fertilization method by Chaudhary et al., (1984), milt was mixed with the eggs using a bird feather for two minutes, this will decrease the distance from the sperm to the micropyle of the egg.

Eggs were washed with water for 10 min; they absorb water and attain the size of 1 to 1.4 mm in diameter. The number of eggs released was calculated following the gravimetric method (Haniffa and Sridhar, 2002) in which 1 g of egg sample was weighed three times and mean value was multiplied with the total weight of egg sample:

No. of eggs kg^{-1} = total no. of eggs / total weight of fish

After 3 to 4 h of fertilization, division of cell was so regular that the size and shape of blastomeres was equal in fertilized egg, and distinguishable from unfertilized eggs. So, the fertilized eggs were calculated according to Muir and Robert (1985):

Fertilization rate (%) = no. of fertilized eggs / total no. of eggs x 100.

Table 1. Effect of ovaprim-C on spawning of Grass carp (*C. idella)*.

Parameter	Ovaprim treatment
No. of females treated .	22
Total weight of females	115.3 kg
Total no. of eggs	7210000
Total no. of fertilized eggs	5794000
Total no. of hatchling	4606000
Overall fertilization percentage	80.36%
Overall hatching percentage	79.49%
Average no. of eggs/Kg	62532
Average no. of fertilized eggs/kg	50251
Average no. of hatching/kg	39947

Hatching occur after 18 to 22 h at water temperature of 20.0 to 24.5°C and hatchling were kept in circular spawning tanks with bolting cloth for three days until yolk was absorbed, and percentage hatchling calculated by:

Percentage of hatchling = total no. of hatchlings / total no. of fertilized eggs x 100

RESULTS

Fish given single injection of ovaprim-C were successfully induced to spawn during the month of May, 2008. 22 females were injected with ovaprim-C, ovulation of fish in these treatments were 100%, total number of obtained eggs were 62532 kg^{-1}, while overall fertilization and hatching rate was 80.36 and 79.49%, respectively (Table 1). Absolute and relative fecundity was found to be related to body weight in Grass carp *(C. idella*). Regression analysis was applied to assess the body weight dependence of these variables. Body weight had a positive influence on absolute fecundity, while there was no influence on relative fecundity (Table 3) with increasing body weight. Each of these relationships was statistically significant ($P < 0.001$) and well described by a linear equation:

$$Y = a + bX$$

Where, 'a' and 'b' are constant; X is the body weight and Y is the dependent variable. When total values of absolute fecundity and relative fecundity of Grass carp *(C. idella)* were transformed into log-log scale, a linear relationship of the following form was obtained showing a high degree of correlation (Table 4):

$$Log\ Y = a + b\ log\ X$$

Statistical analysis, including regression analysis and calculation of correlation was carried out by using a computer package EXCEL following Zar (1996).

DISCUSSION

In the present study, a single intramuscular injection of synthetic hormone, ovaprim-C resulted in successful spawning of Grass carp (*C. idella*). The results of the hormonal stimulation in the current work are similar to the effectiveness and usefulness by using ovaprim-C (Jamroz et al., 2008). But speed and gentleness during fish capture and handling are of utmost important (Basaran et al., 2008).

Certain hormones are used individually like different analogues of LH-RH, without pituitary gland, which results in failure of spawning and clearly indicates that dopamine blocks the action of LH-RH on the secretion of gonadotropin (Naeem et al., 2005a). However, use of dopamine antagonists like pimozide or doperidon, potentiate the action of LH-RH, resulting in successful spawning (Chang et al., 1983). Major break through in the history of aquaculture happened when extensive research on Chinese carp (Peter et al., 1988) and a new Linpe method was introduced in which LH-RH analogue is combined with a dopamine antagonist. Then Canada introduced the ovaprim-C containing the analogue of salmon gonadotropin releasing hormone (D-Arg^6, Pro^9, Net) and dopamine antagonist, and studies conducted in India (Nandeesha et al., 1990) and Pakistan (Khan et al., 1992; Naeem et al., 2005a, b, c) revealed the superiority of ovaprim-C in induced spawning. Dose of ovaprim-C used in Grass carp (*C. idella*) in the present experiment is 0.6 ml/kg, while experiments conducted by Nandeesha et al. (1990) and Peter et al. (1986) reported the dose rate of 0.7 ml/kg. So, the present study reveals that ovaprim-C use is more economical in commercial carp seed production, as it saves a considerable amount of time and avoids the excessive handling of brood fish. Dosage of ovaprim-C for carps at different locations is given in Table 5.

Grass carp (*C. idella*) preferably spawn during mid April to June (Table 2). Fertilization rate is 80.36% and hatchling percentage is 63.88% (Table 1) at the favorable

Table 2. Spawning response of female Grass carp (*C. idella*).

Month	Temperature of air/water (°C)	Number of female	Total weight of female	Dose of ovaprim-C (ml/kg)	Number of egg (Lac)	Fertilization rate (Lac)	Number of hatchling (Lac)
March, 2008	29/26	3	18	0.6	10.8	8.64	6.9
April, 2008	29/27	3	18	0.6	12.9	10.3	8.2
April, 2008	30/27	2	12.5	0.6	7.5	6.0	4.8
April, 2008	30/27	3	13	0.6	7.8	6.2	5.0
May, 2008	30/28	4	20	0.6	12	9.6	7.6
May, 2008	30/28	3	16	0.6	10.2	8.7	6.9
May, 2008	30/29	3	15	0.6	9.6	7.6	6.1
June, 2008	34/30	1	2.8	0.6	1.3	0.9	0.56

Table 3. Statistical parameters of body weight versus total number of eggs and number of eggs/kg of Grass carp (*C. idella)*.

Relationship	r	a	b	S. E. (b)
Wet body weight (X) Total no. of eggs (Y)	0.901***	-19854	66320	7123
Wet body weight (X) No. of eggs / kg (Y)	0.244 $^{n.s}$	53736	1628.9	1448

r = Correlation coefficient; a = intercept; b = slope; S.E= standard error; *** = P < 0.001; $^{n.s.}$ = P > 0.05; n = 22 in each case.

Table 4. Statistical parameters of log body weight versus log total no. of eggs and log no. of eggs / kg of Grass carp (*C. idella)*.

Relationship	r	a	b	S. E. (b)
Log wet body weight (X) Log total No. of eggs (Y)	0.926***	4.6649	1.1787	0.107
Log wet body weight (X) Log total No. of eggs/kg (Y)	0.348 $^{n.s}$	4.6649	0.1787	0.107

r = Correlation coefficient; a = intercept; b = slope; S.E= standard error; *** = P < 0.001; $^{n.s.}$ = P > 0.05; n = 22 in each case.

temperature of 26 to 30°C as shown in Table 2. Generally, the number of eggs spawned by Grass carp (*C. idella*) in the present study was lower than previous report (Ling et al., 1980; Chaudhary et al., 1984; Armando et al., 1989). Statistical parameters reveals that body weight had a positive influence on absolute fecundity (Table 3), while there was no influence on relative fecundity (Table 4). This may be due to low nutritional status of the brood fish as brood stock depended completely on the natural productivity of the reservoir and were not given artificial feed throughout the rearing period. Further studies are needed to determine minimum effective dose of ovaprim-C that could be used to spawn a brood fish under captive condition.

Conclusion

The present study shows the advantages of ovaprim-C over commercial pituitary (example reduced handling of brood fish due to the single dose, which not only decrease the post spawning mortality, but also increase

Table 5. Dosage of ovaprim-C for carps at different locations.

Fish Species	Dose of ovaprim for (♀)	Reference
Aristichthys nobilis	0.4 – 0.5	Nandeesha (1990)
Catla catla	0.4 – 0.5	Nandeesha (1990)
Cirrhina mrigala	0.25 – 0.3	Nandeesha (1990)
Cirrhina mrigala	0.4	Nandeesha et al. (1990)
Ctenopharyngodon idella	0.4 – 0.8	Nandeesha (1990)
Hypophthalmichthys molitrix	0.4 – 0.7	Nandeesha (1990)
Labeo rohita	0.3 – 0.4	Nandeesha (1990)
Labeo rohita	0.4	Nandeesha et al. (1990)
Catla catla	0.7	Khan et al. (1992)
Aristichthys nobilis	0.6	Naeem and Salam (2005)
Hypophthalmichthys molitrix	0.6	Naeem et al. (2005b)
Ctenopharyngodon idella	0.6	Present study

spawning response, adverse effects on the health and growth), and are very easy to use by unskilled farmers.

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