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Full Length Research Paper

Breeding performance of sustainable fish *Ctenopharyngodon idella* through single intramuscular injection of Ovaprim-C at Bahawalpur, Pakistan

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Effect of intramuscular injection of Ovaprim–C on the number of eggs/kg, fertilization rate and hatching percentage were studied at a private Fish Hatchery at Bahawalpur, Pakistan, during April to June 2008, on *Ctenopharyngodon idella* (**Grass carp). Studied fish specimens were spawned successfully following a single dose of injection of Ovaprim-C (LH-RH analogue) with 0.6 ml kg^{-1} for female and 0.2 ml kg^{-1} for male brooders. Ova and milt were stripped simultaneously and mixture was stirred for 15 to 30 s during which fertilization occurred. Hatching occurred within 18 to 30 h after fertilization. The experiment was conducted in circular spawning tank with 2 m diameter. It was observed that body weight has positive influence on absolute fecundity (r = 0.967), while relative fecundity remained constant with increasing body weight. If it is impossible to determine the absolute and relative fecundity then these parameters can be determined from the body weight.**

Key words: Induced spawning, Ovaprim-C, fecundity, *Ctenopharyngodon idella*.

INTRODUCTION

Aquaculture in Pakistan is a recent development and in many parts of the country, the management of this sector is still poor with culture practices varying across the different provinces. Virtually all aquaculture currently carried out in Pakistan is pond culture of various carp species. Pakistan has not yet begun any coastal aquaculture operations although there is good potential all along Pakistan's 1100 km coastline. In Pakistan, the fish fauna is rich but only seven warm water species and two cold water species are cultivated on a commercial scale (Akhtar, 2005). Grass carp was imported in Pakistan from China for the first time in 1964 (Froese and Pauly, 2011). The purpose of its introduction, in addition to culture, was the biological aquatic weed control in natural waterways, rivers and man-made lakes (Khan et al., 2004). Some species of fish will not readily breed in captivity solution. The single most important downside of large-scale commercial culture of several fish species is the deficiency of quality seed of uniform size, free of diseases, parasites, and pests at the time of stocking in culture ponds. These strict basics are seldom fulfilled where the seed is obtained from the natural water bodies (Mylonas and Zohar, 2001). For this reason, hormonal treatment has been attempted for stimulating the gametes maturation and has been successfully used to spawn many commercially important fish species. The injection of different inducing agent in fish breeding is adopted for successful ovulation and collection of eggs in different cultivable fish species (Adebayo and Fagbenro, 2004). The breeding performance in captive condition depends on the type of hormone used and its potency, dose of hormone and maturity status of the fish (Legendre and Oteme, 1995). Moreover, the knowledge of fish fecundity is needed in establishing its production

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Table 1. Effect of Ovaprim-C on spawning of *Ctenopharyngodon idella*.

Parameter	Ovaprim-C treatment
Number of females treated	27
Total weight of females (kg)	102.9
Total number of eggs	6030000
Total number of Fertilized eggs	4610000
Total number of hatchling	3658000
Overall fertilization (%)	76.45
Overall hatching (%)	79.35
Average number of eggs/kg	58601
Average number of fertilized eggs/kg	44801
Average number of hatching eggs/kg	35549

potential and consequently its exploitation and management rationale (Fawole and Arawomo, 2000).

The aim of this work was to determine the breeding performance of *Ctenopharyngodon idella* by using single intramuscular injection of Ovaprim-C and to estimate the absolute and relative fecundity in relation to wet body weight.

MATERIALS AND METHODS

These experiments were conducted on brood fish stocked at a Fish Hatchery Bahawalpur, Pakistan during the month April to June 2008. 27 females of *C. idella* weighing from 2.0 to 5.0 kg were used to assess their reproductive performance. Females with soft belly and pink-red genital papilla, and males, which release milt when subjected to gentle pressure on the abdomen, were selected. Fish were transferred into cemented holding tanks of hatchery.

Hormonal injection

The studied fish were injected Ovaprim-C intramuscularly in a single dose to both female and male brooders to induce breeding following protocol of Nandeesha (1991). The dose for female spawner was 0.6 ml/kg body weight and for male, it was 0.2 ml/kg body weight. The injected males and females were transferred to a circular tank with slow flowing water.

Stripping

Spawning started generally 6 to 12 h after single dose of injection. A ripe female, which upon slight pressure on the abdomen releases some eggs from the urinogenital pore when fish was held ventrally, were stripped gently to receive ova in a clean and dry plastic bowl. Milt from healthy males was gently mixed by feather with ova for 15 to 30 s prior to washing for dry fertilization. For three times, 1 g egg samples were weighed out and counted. The total number of eggs spawned was calculated by multiplying the average number of eggs with total weight of eggs sampled. Eggs were repeatedly washed with clean water several times.

Egg incubation

Fertilized eggs were then incubated for hatching in circular tanks. The circular tanks were covered with a canvas screen. The rate of water flow in incubation tanks was maintained at 38 L/min. The dimensions of circular tank were 32 inch radius and 26 inch depth. Fertilization rate expressed as the ratio of fertilized eggs to total eggs counted (n = 100) from each of three sampling of known volume (eggs + water) was determined 6 to 7 h post incubation period. Hatching occurred after 72 h. During experiments, temperature of the circular tank where the injected fishes and eggs after fertilization were kept ranged from 26.0 to 31.0°C, with optimum water quality variables, while the temperature of pond where fry were shifted after three days of hatching was between 31.8 and 33°C. Hatching rate was then determined. Newly hatched larvae remained in the circular tank for three days until yolk was absorbed. Fry of grass carp were fed with boiled egg of hen. After 72 h, all the fry were shifted to a pond of one acre area.

Data analysis

Influence of body weight on absolute and relative fecundity was studied, following Naeem et al. (2005a, b), described by a linear equation:

$$Y = a + bX$$

Where 'a' and 'b' are constant, X is the body weight and Y is dependent variable.

When total values of absolute fecundity and relative fecundity of *C. idella* were transformed into log-log scale, a linear relationship of the following form was obtained:

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

Correlation coefficient was calculated to check the significance level of relationship between the given variables. Results were interpreted following Zar's (1996) method.

RESULTS

In the conducted experiments, successful spawning of *C. idella* was observed with 100% ovulation, 76.45% fertilization and 79.35% hatching (Table 1) when 27 females were treated with a single intramuscular injection of synthetic hormone Ovaprim-C with dose rate 0.6 ml/kg

Table 2. Spawning response of female *C. idella*.

Month	Water temperature (°C)	Number of female hatching	Total weight of females (kg)	Dose of Ovaprim-C (ml kg^{-1})	Number of eggs	Fertilized eggs	Number of hatchlings
April 2008	26	3	14.5	0.6	870000	690000	550000
April 2008	27	3	14.0	0.6	840000	580000	470000
April 2008	27	4	14.0	0.6	870000	690000	550000
April 2008	27	4	15.0	0.6	900000	720000	570000
April 2008	27	3	12.0	0.6	670000	530000	430000
May 2008	28	4	14.0	0.6	840000	670000	530000
May 2008	28	4	15.5	0.6	860000	600000	480000
June 2008	31	2	3.9	0.6	180000	130000	78000

for females (Table 2) and 0.2 ml/kg for males. In the study, total numbers of obtained eggs were found to be 60.3 $\times 10^5$, 46.1 $\times$ 10^5 for fertilized eggs and number of hatchlings was 36.58 $\times$ 10^5. Relative fecundity (eggs/kg) was found to be 58601, number of fertilized was 44801 and number of hatchling/kg was 35549 (Table 1). Highly significant relationship ($P<0.001$; $r = 0.967$) was found between wet body weight and absolute fecundity, whereas relative fecundity remained constant with wet body weight of the fish. When total values of absolute fecundity and relative fecundity of *C. idella* were transformed into log-log scale, a linear relationship was obtained showing a high degree of correlation ($P<0.001$; $r = 0.961$) between log transformed wet body weight of the fish and absolute fecundity (Table 3).

DISCUSSION

Absolute and relative fecundity was found to be related to body weight in *C. idella*. Regression analysis was applied to assess the body weight dependence of these variables. Body weight had a positive influence on absolute fecundity, but no influence on relative fecundity of *C. idella*. These results are in general agreement with Naeem et al. (2011).

Results of this study show that ovulation, fertilization and hatchling values were found to be 100, 76.45 and 79.35%, when single intra-muscular injection of Ovaprim-C was applied. However, in previous studies, Naeem et al. (2005a) reported corresponding values as 100, 72.56 and 71.09% for *Hypophthalmichthys molitrix* while Naeem et al. (2011) reported 100, 50.99 and 73.92%, natural productivity of the reservoir and were not given artificial feed throughout the rearing period.

The results of the hormonal stimulation in this work are similar to the effectiveness and usefulness by using Ovaprim - C (Jamroz et al., 2008). Certain hormones used individually like different analogues of LH-RH without pituitary gland, results in failure of spawning, which clearly indicates that dopamine blocks the action of LH-RH on the secretion of gonadotropin (Naeem and Salam, 2005). However use of dopamine antagonists like Pimozide or Domperidone, potentiate the action of LH- RH, resulting in successful spawning (Chang and Peter, 1983). But speed and gentleness during fish capture and handling are of utmost important (Basaran and Samsun, 2004). Major breakthrough 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 in Pakistan (Khan et al., 1992; Naeem et al., 2005a, b; Naeem and Salam, 2005; Naeem et al., 2011) revealed the superiority of Ovaprim in induced spawning. Dose of Ovaprim-C used in *C. idella* in this experiment was 0.6 ml/kg, while experiments conducted by Peter et al. (1986) and Nandeesha et al. (1990) reported the dose rate as 0.7 ml/kg.

Conclusion

This study therefore reveals that Ovaprim-C use is

Table 3. Regression analysis and statistical parameters of body weight versus total no. of eggs and no. of eggs/kg of *C. idella*.

Relationship	r	a	b	S. E. (b)
Wet body weight (x) and Total no. of eggs (y)	0.967***	-2900.29	80079.54	3733.98
Wet body weight (x) and no. of eggs / kg (y)	0.010 ^{ns}	78933.83	69.80	1205.26
Log wet body weight (x) and Log total no. of eggs (y)	0.961***	4.8981	0.9989	0.0506
Log wet body weight (x) and Log total no. of eggs/Kg (y)	0.004^{ns}	4.8981	-0.0011	0.0506

r, Correlation coefficient; a, intercept; b, regression coefficient; S.E (b), standard error of b; probabilities (P), ***P < 0.001, $^{n.s}$P > 0.05; n = 27 in each case.

more economical in commercial carp seed production, as it saves a considerable amount of time and avoids the excessive handling of brood fish. It also has advantages than commercial pituitary e.g. reduced handling of brood fish due to the single dose, which not only decrease the post spawning mortality but also increase spawning response. In addition, adverse effects on the health and growth of hatchlings are absent and it is very easy to use by unskilled farmers. Further studies are needed to determine the minimum effective dose of Ovaprim-C that could be used to spawn a brood fish under captive condition.

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