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Short Communication

Effect of Body Weight on Absolute and Relative Fecundity of *Hypophthalmichthys molitrix* with Intramuscular Injection of Ovaprim–C

MUHAMMAD NAEEM[1], ABDUS SALAM, NOOR ELAHI†, MUZAFFAR ALI, ABIR ISHTIAQ AND ANNA ANDLEEB
Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan 60800, Pakistan
†*Department of Fisheries, Fish Hatchery Satyana Road Faisalabad. 38000, Pakistan*
[1]Corresponding author's e-mail: dr_naeembzu@yahoo.com

ABSTRACT

The aim of the present study was conducted to evaluate effectiveness on induced spawning of silver carp (*Hypophthalmicthys molitrix*), fecundity, fertilization rate and hatchling rate by using single intramuscular injection of Ovaprim-C during July, 2007 in private Himalaya Hatchery. After fertilization hatching occurred within 18-24 h. In the present study, ovulation, fertilization and hatchling rate values were estimated to 100, 50.99 and 73.92%, respectively. Regression analysis was also used to assess the body weight dependence on absolute and relative fecundity. Absolute fecundity was positively correlated with wet body weight ($r = 0.667^{***}$) ($p < 0.001$), while relative fecundity remains fairly constant ($r = 0.275^{n.s}$) ($p > 0.05$) with increasing wet body weight. Wet body weight and absolute or relative fecundity can also be described by linear equation: $Y = a + bX$, which can be used to estimate the absolute fecundity with a fair amount of accuracy. © 2011 Friends Science Publishers

Key Words: Induced spawning; Fecundity; Ovaprim-C; LHRH; Gonadotropin; Fishes; *Hypophthelmicthes molitix*

INTRODUCTION

Hypophthalmichthys molitrix (Valenciennes, 1844) matures within three years and spawns preferably during mid May to June (Naeem *et al.*, 2005a & b). It is fresh water, omnivorous fish (Williamson & Garvey, 2005). It was introduced in Pakistan from China and Nepal in 1982-1983 (Froese & Pauly, 2010). Induced breeding of captive fish may be approached in two ways, hormonal and environmental (Marte, 1989). The concept about induced breeding on the basis of administration of pituitary extract was first successfully attempted in Brazil in 1934. The success of induced breeding operation depends upon proper selection of brood fish particularly female (Shri *et al.*, 2008).

Fish seed is the critical and basic input for successful fish culture operations. However, the major problem in the carp culture is the non-availability of quality fish seed (Naeem *et al.*, 2005a & b; Rokade *et al.*, 2006) and timely seed supply is one of the major constraint (Radheyshyam, 2010). A breakthrough achieved during the 50's in induced breeding through hypophysation gave the thrust to mass production of quality spawn in a controlled environment thereby reducing the dependence on natural seed collection. A number of studies conducted in breeding various species of cultured fish in China with LH-RH analogues led to the development of "Linpe method" (Peter *et al.*, 1988), wherein the releasing hormone is combined with a dopamine antagonist for successful spawning (Das, 2004). Fish seeds were collected from river coasts by cloth happas, but this technique was unsafe as with the collection of carp seed, some seeds of predatory fishes were also collected accidentally. Chaudhary and Alikunhi (1957) for the first time successfully carried out a study on the spawning of Indian major carps with induced breeding by pituitary extracts. This technique was then used all over India but the potency and the quality of the pituitaries were used for preparing the extract became undependable and because of this problem there is failure in spawning and results of pituitary extracts in many farms. Human chorionic gonadotropin (HCG) was then used as a substitute for pituitary gland but it could not get the success. The search for a suitable substitute was going on and then after Ovaprim-C was introduced in the market as a substitute for pituitary gland. All the fish breeders readily showed acceptance for this drug (Nandeesha *et al.*, 1990a).

Ovaprim-C utilizes the fish's own hormonal control mechanism to safely induce maturation and coordinate spawning dates. Ovaprim-C contains analogue of Salmon gonadotropin releasing hormone analogue (sGnRH; D-Arg^6, Pro^9, Net) and dopamine inhibitor required for culturable species (Naeem *et al.*, 2005a; Rokade *et al*., 2006; Jeffrey *et*

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al., 2009). Use of Ovaprim-C have certain advantages, reduces handling due to single injection, can be used in any size of fish, induces maturation of gametes immediately after injection for fast result (Naeem *et al.,* 2005a).

The aim of the present study is to determine economics of seed production, effect of topographical changes on *Hypophthalmichthys molitrix,* scientific development in the process of induced breeding and seed production rate, fecundity and hatching rate of *H. molitrix* by using single intramuscular injection of Ovaprim-C.

MATERIALS AND METHODS

The experiments were conducted at Himalaya Hatchery situated 33 km distance from Lahore at G.T. road near Gujranwala (Lat: 32° 9' 0" N, Long: 74° 11' 0" E), Pakistan during the month of July 2007. Circular tanks with 2 m in diameter with optimum water quality variables were used in induced spawning for 20 specimens having weight range about 2.2–6.5 kg of *Hypophthalmichthys molitrix* at 26.0–26.5°C. Specimens were anesthetized with 2- phenoxy ethanol at the dose rate of 100–200 ppm. Female with soft and bulging abdomen and slight swollen with pinkish and protruding genital opening are selected for breeding purpose (Naeem *et al.,* 2005a & b; Metwally & Fouad, 2008; Shri *et al.,* 2008).

Hormonal injection: Ovaprim-C is injected intramuscularly into the dorsolateral region of both male 0.2 mL/kg and females 0.6 mL/kg in a single dose (Haniffa *et al*., 2002) by using hypodermic syringe after cleaning the area with cotton swab soaked in alcohol. Ovaprim-C was also used for induced spawning of silver carp by Das (2004). Since, intramuscular hormone administration to the carp brooder is a well-accepted method and is being practiced by most breeders (Das, 2004).

Estrus: After 9-10 h spawning, their behavior was observed by using single dose of Ovaprim-C. This was indicated by the intermittent splashing on the water surface as males chased the females (Naeem *et al.,* 2005b). The activity lasted for 30-60 min after that fish were netted out for stripping. In their natural range *H. molitrix* spawn at temperature 18°C, during rising water, in larger rivers with high velocities (>70 cm/s) (Krista, 2007).

Stripping: Females were stripped, eggs were collected in dry plastic bowl and males were stripped simultaneously with a female to fertilize eggs following the semi dry fertilization method (Naeem *et al.,* 2005b).

Fertilization: Milt was mixed with the eggs using a bird feather for 15-30 sec prior to washing, this washing procedure will decrease the distance for the sperm to the micropyle of the egg. The fertilization rate was calculated by examining a minimum of three, one gram samples.

Assessment of hatching: Hatching occur after 18-22 h at water temperature 20.0-24.5°C and hatchling were kept in circular spawning tanks with bolting cloth for three days until yolk was absorbed. According to Naeem *et al*. (2005b), these relationships of *Hypophthalmichthys molitrix* can also be described efficiently by a linear equation:

$$Y = a + b X$$

Here Y is variable dependant, a and b are constant, X is wet body weight. When values are expressed in logarithm then equation becomes:

$$\text{Log } Y = a + b \log X$$

Descriptive statistical analysis including regression analysis and calculation of correlation coefficients were performed using a computer package (Lotus 1-2-3) following Zar (1996).

RESULTS

In the experiments conducted on 20 specimen of *Hypophthalmichthys molitrix,* which shows 100% ovulation, 50.99% fertilization rate and 73.92% hatchling rate (Table I) when they are treated with single intramuscular injection of Ovaprim-C, with dose rate 0.6 mL/kg for females and 0.2 mL/kg for males (Table II). In the present study, it was found as 4.22 million for total no. of eggs, 2.1519 million for total no. of fertilized eggs, 32141 eggs/kg for number of fertilized, 23762 for number of hatchling/kg and 63032 for relative fecundity (Table I). With an increase in wet body weight, total no. of eggs also increased but total No. of eggs/kg fairly remained constant with increasing in wet body weight. It was observed that wet body weight has a positive influence on absolute fecundity.

Regression analysis was also applied on the wet body weight and dependent variables. Significantly positive correlation between wet body weight and total no. of eggs was found (r = 0.667***). Also, the correlation between wet body weight and relative fecundity was non-significant (r = $0.275^{n.s}$) as given in Table III.

When the total values of absolute and relative fecundity of *H. molitrix* were transformed into log-log scale showing wet body weight as a positive influence (r = 734***) on absolute fecundity and no influence (r = $0.297^{n.s}$) on relative fecundity (Table-III).

Table I: Effect of Ovaprim-C on spawning of *Hypophthalmichthys molitrix*

Parameters	Ovaprim-C treatment
No. of females treated	20
Total weight of females	66.95
Total no. of eggs	4220000
Total no. of fertilized eggs	2151900
Total no. of hatchlings	1590880
Overall fertilization percentage	50.99%
Overall hatching percentage	73.92%
Average no. of eggs / kg	63032
Average no. of fertilized eggs / kg	32141
Average no. of hatchling / kg	23762

Table II: Spawning response of female *H. molitrix*

Month	Water temperature °C	No. of females	Total weight of females (kg)	Dose of ovaprim-C (mL/kg)	No. of eggs (million)	Fertilization rate (million)	No. of hatchling (million)
July,07	26.0	10	33.7	0.6	2.02	1.011	0.8088
July,07	26.0	4	14.05	0.6	1.24	0.6744	0.47208
July,07	26.5	4	13.7	0.6	0.685	0.274	0.137
July,07	26.0	2	5.5	0.6	0.275	0.1925	0.173

Table III: Statistical parameters of body weight versus total no. of eggs and No. of eggs/kg of *H. molitrix*

Relationships	r	a	b	S.E (b)
Wet body weight, (x) Total no. of eggs, (y)	0.667***	-0.067	0.081	0.021
Wet body weight, (x) Total no. of eggs/kg, (y)	$0.275^{n.s.}$	0.036	0.008	0.006
Log wet body weight, (x) Log total no. of eggs , (y)	0.734***	-1.430	1.402	0.305
Log wet body weight, (x) Log total no. of eggs/kg, (y)	$0.297^{n.s}$	-1.430	0.402	0.305

r = Correlation Coefficient; a = Intercept; b = slope; S.E= Standard Error; ***: P<0.001; $^{n.s.}$ = P > 0.05

DISCUSSION

Results of the present study showed wet body weight had positive influence on the absolute fecundity and relative fecundity remained fairly constant to wet body weight of *H. molitrix*. When single intramuscular injection of Ovaprim-C was applied, ovulation, fertilization and hatchling values were found 100%, 50.99% and 73.92%, respectively. However, in a previous study conducted at fish hatchery Islamabad, Pakistan by Naeem *et al.* (2005b), they reported corresponding values as 100%, 72.56% and 71.09% for *Hypophthalmichthys molitrix*, respectively. This variation of results was due to change in topographical condition. The present study showed lower relative fecundity than that of Naeem *et al.* (2005b) and also lower than previous reported by Ling (1980) and Fermin *et al.* (1989), this variation may also be due to change in climatic condition and low nutritional status of brood fish (Ume-e-Kalsoom et al., 2009).

In general, the response of fish to Ovaprim-C was well found by considering the percentage of spawning success, number of eggs released, percentages of fertilization and hatching. The hormone, Ovaprim-C has solved many problems on carp seed production. Its use not only saves considerable amount of time but also helps in reducing post-spawning mortality, since the brood fish is required to be handled only once (Nandeesha *et al.*, 1990b; Das, 2004; Naeem *et al.*, 2005a & b). In the trials conducted on the Ovaprim-C under the present study it was indicated that this hormone had no adverse effect on brood fish or spawns. The several investigators have published on fecundity and successful induction of spawning of carp by Ovaprim in Pakistan on different species; *Labeo rohita* and *Cirrhinus mirigala* by Khan *et al*. (1992); *Aristichthys nobilis* by Naeem and Salam (2005); *Catla catla* by Naeem *et al.* (2005a); *Hypophthalmichthys molitrix* by Naeem *et al.* (2005b). Further studies are required to evaluate the growth performance of spawn produced with Ovaprim-C at different geological conditions.

In conclusion, the dose of Ovaprim-C for female brood fish is 0.6 and males respond at 0.2 mL/kg. Ovaprim-C does not require refrigerated storage and reduce post spawning mortality. The equations obtained from the present paper can provide reliable estimates of absolute and relative fecundity with a fair amount of accuracy.

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