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Effect on Breeding Performance and Egg Quality of *Clarias batrachus* (Linn.) at Various Doses of Ovatide During Spawning Induction

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Abstract

An experiment was conducted to evaluate Ovatide doses (0.5, 1.0, 1.5 and 2.0 ml·kg body weight of female) on breeding performance and egg quality of *Clarias batrachus*. The breeding performance was judged on the basis of the total weight of stripped eggs, spawning fecundity and stripping response. To judge the egg quality, the per cent fertilization, hatching, deformity and normal larvae were considered. The results indicated that the total weight of stripped eggs and spawning fecundity were the highest ($p < 0.05$) when females were injected 1 ml of Ovatide per kg body weight (BW) compared to those injected with other dose levels. The lowest stripping response was observed with injection of 0.5 ml Ovatide per kg BW. There was difficulty in stripping at 0.5, 1.5 and 2.0 ml doses, but at 1 ml dose, it was smooth. At the 1 ml dose, the percentages of fertilization and hatching were 83 and 71 % respectively, which were the highest ($p < 0.05$) among all treatments. Increasing Ovatide doses above 1 ml led to over ripening of ova, which resulted in increased per cent deformed larvae. More normal larvae were produced from the females when injected the 1 ml dose. One ml of Ovatide per kg body weight was found optimum for best breeding performance and egg quality in *C. batrachus*.

Introduction

Clarias batrachus is a popular culturable fish in Asian countries. The hardy nature and tolerance to adverse ecological condition enable its high

density culture with a high production per unit area (Sitasit 1968). The production of up to 100 t·ha has also been reported (Areerat 1987). The spontaneous captive breeding, short supply of quality seed and dependency on wild seeds, which is unreliable, time consuming and uneconomical are major constraints for culturing this fish. To overcome such problems, induced spawning is thought to be the only alternative method for quality seed supply/production. Among several inducing agents used in fish breeding, salmon gonadotropin releasing hormone (sGnRH) or leutinising hormone releasing hormone (LHRH) analogues in combination with dopamine antagonists was found to be effective in fish breeding (Lin and Peter 1996). The use of synthetic inducing agents for successful ovulation followed by stripping in catfish is a common practice and has been studied at several occasions (De Leeuw et al. 1985, Manickam and Joy 1989, Tan-Fermin et al. 1997, Sahoo et al. 2003). There are associated problems in using these hormones, such as weighing such low quantity, preparation of these analogues and storage of these prepared solutions. On account of these difficulties, breeders are reluctant to use them in field conditions. However, the commercially available synthetic inducing hormones in ready made form containing GnRHa and dopamine blocker receptor (Ovaprim, Ovopel, Dagin and Aquaspawn) are becoming very popular and found to be efficient in successful spawning of fishes (Peter et al. 1988, Nandeesha et al. 1990, Cheah and Lee 2000, Brzuska 2001). Ovatide, an injectable inducing hormone consisting of GnRH analogue in combination with dopamine antagonist, is also efficient in induced spawning (Gupta et al. 2002, Sahoo et al. 2004b). The objective of the present experiment was to test the effectiveness of different doses of Ovatide in induced ovulation of *C. batrachus*. The study further investigated the effect of doses on spawning fecundity, stripping response and, per cent fertilization, hatching and normal larval production in this catfish.

Materials and Methods

C. batrachus were reared in 0.01ha earthen ponds at Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar, India. The fish were fed at 2% body weight daily in the evening hour (17.00h) with laboratory prepared pelleted feed containing 30% crude protein and 350 kcal gross energy per 100 g feed. During the breeding season (July-August), both males and females were collected from the brood stock pond for breeding operation. The males were selected on the basis of pointed and reddish genital papilla, while females by a round and reddish papilla, softness of abdomen and uniform size of intra-ovarian oocytes (Sahoo et al. 2004a). The female broods of 120-140 g weight range were selected

for induced breeding. Four doses of Ovatide viz. 0.5, 1.0, 1.5 and 2.0 ml per kg body weight were adopted with. Ten females were tried for each dose. The breeding operation was conducted in eight attempts and five females were considered for one dose level of Ovatide in each attempt. The males and females were kept separately. Before injection, individual female body weight was recorded and marked with coloured chips tied to their dorsal fin to study individual breeding performance and egg quality. The testes were removed from three male fish, incised and squeezed to get sperm. The sperms were pooled and diluted with 3 ml of physiological saline to prepare a sperm suspension. A drop of sperm suspension was checked under the microscope for motility. Before stripping, individual weight of females was recorded. At the end of the desired latency period of 17 h, the females were stripped individually into dry and preweighed petriplates to record the stripped egg weight. The stripped females were killed and stripped ovaries were removed to record their weight. Three sub-samples of 100-150 mg eggs were weighed from the total stripped eggs of each female and the number was counted. Thus the total number of eggs (spawning fecundity) was calculated for an individual female in one Ovatide dose.

Three subsamples of each 250-300 mg egg were weighed and mixed with 4-5 drops of sperm suspension. After thorough washing with water, they were released into the round plastic tray of 5 l capacity provided with flow-through water system (0.2 l·min). After three hours of incubation, the fertilised eggs were counted. The translucent eggs containing embryonic eyes were considered fertilised. The mean fertilised eggs in triplicate trays were recorded and expressed as per cent fertilization per female. The pooled mean fertilization of all ten females was considered as per cent fertilization for a particular hormone dose. In a similar way, hatching, deformed larvae and normal larvae per cent were calculated. The stripping response and gonado-somatic index (GSI) were calculated as described by Zonneveld et al. (1988).

Hatchery water temperature, pH, dissolved oxygen and total alkalinity were 27-28.5· C, 6.8-7.5, 5.8-6.7 ppm and 120-128 ppm, respectively.

Data were analysed by variance component analysis (Snedecor and Cochran 1967) and difference between the means was examined using Duncan's multiple range tests.

Results and Discussion

The ovulatory performance and egg quality of *C. batrachus* at different Ovatide doses is presented in table 1. The total weight of stripped eggs was significantly highest ($p < 0.05$) when females were injected

with 1 ml Ovatide per kg body weight. Injection of 0.5 ml dose of Ovatide per kg body weight resulted in lowest stripped egg yield. It was observed that stripping was difficult in females while injected with 0.5 ml Ovatide. Unovulated eggs was observed in the fishes at the 2.0 ml dose, and with higher pressure during stripping brought out blood tinged eggs as well as egg bunches. The result indicated that the suboptimal dose of 0.5 ml Ovatide was not suitable for complete ovulation for which stripping was not easy. This might be due to insufficient release of gonadotropin, agreeing to earlier studies (Van der Kraak et al. 1983, Billard et al. 1984). The lower weight of stripped eggs and spawning fecundity at higher dose (1.5-2.0 ml) could be due to the blocking of the genital aperture by disintegrated ovarian tissue and egg bunches. The nonsignificance of GSI between different treatments was possibly due to similar body weight of females. The stripping response at 1 ml dose treatments was highest ($p<0.05$), followed by 1.5, 2.0 and 0.5 ml Ovatide dose. The higher response in 1 ml dose level might be due to proper ovulation of eggs. The lower responses at 0.5 and 1.5-2.0 ml dose might be due to ovulation failure or blocking of ovipore by disintegrated ovarian tissue and egg bunches. Also Zonneveld et al. (1988) was of the opinion that the stripping response decreased at lower dose of pituitary in *C. batrachus*. A significant decrease ($p<0.05$) in fertilisation and hatching was observed above 1 ml Ovatide doses. Deterioration of egg quality was also observed at 0.5 ml dose leading to the lowest fertilisation and hatching per cent compared to the other three dose levels. There are possibilities of egg plugging at higher doses and higher stripping pressure resulted to blood tinged eggs. The blood on the stripped egg and protein from ruptured eggs coagulate and clog the micropile, which leads to poor fertilization (Piper et al. 1982). Other authors have also reported a deterioration of egg quality with increasing doses of gonadotropin (Pickford and

Table 1. Effectiveness of various doses of Ovatide on spawning fecundity, stripping response, fertilization, hatching and larval production of *C. batrachus*. The observations are based on n=3 of ten replicates

Parameters	Ovatide doses (ml·kg)			
	0.5	1.0	1.5	2.0
Weight of females (g) (NS)	131.50±1.97	129.30±1.77	130.00±1.91	130.70±2.04
Weight of stripped eggs (g)	4.70±0.32^{d}	15.38±0.34^{a}	10.79±0.23^{b}	9.39±0.31^{c}
Spawning fecundity	2224±150 ^{d}	7613±166^{a}	5237±112 ^{b}	4539±150 ^{c}
Stripping response	25.54±1.68 ^{d}	74.90±1.11^{a}	55.66±1.13 ^{b}	47.46±1.47 ^{c}
Gonadosomatic index(GSI) (NS)	14.10±0.28	15.80±0.34	14.96±0.29	15.10±0.39
Fertilization (%)	31.91±1.29 ^{d}	83.56±0.57^{a}	76.86±1.77 ^{b}	64.26±1.75 ^{c}
Hatching (%)	18.18±1.27 ^{d}	71.92±1.07^{a}	59.38±2.78 ^{b}	39.14±2.22 ^{c}
Deformed larvae (%)	5.36±0.23 ^{a}	2.30±0.14 ^{c}	3.38±0.14 ^{b}	5.60±0.71 ^{a}
Normal larvae (%)	12.90±1.23 ^{d}	69.62±1.01^{a}	56.93±2.41 ^{b}	33.49±2.35 ^{c}

Mean values bearing different superscripts in the row differ significantly ($p<0.05$).
NS: Non Significant

Atz 1957, Billard and Marcel 1980, Rowland 1983). It is usual that higher dose resulted in early ovulation and the ovulated eggs remained in the ovarian lumen in a hypoxic condition for longer time lead to over-ripeness. These eggs are characterised by increased translucency and an aggregation of cytoplasm at the animal pole and reduced fertilization and hatching (Nomura et al. 1974, Hirose et al. 1977, Lam et al. 1978). The lowest fertilization at 0.5 ml dose might be due to asynchrony between maturation and ovulation, lead to low hatching and this was in agreement with the report of Rowland (1988). The good quality eggs were obtained when 1.0 ml Ovatide per kg was injected to this species. A significantly higher ($p < 0.05$) deformed larvae observed at three doses compared to 1 ml dose level. More deformity in larvae at lower or higher dose may be attributed to the fertilization of unripe or over ripe ova. Lam et al. (1978) noted that overripe eggs did not form a perivitelline space when placed into fresh water, suggesting that there had been a change in the permeability of the chorion. Consequently reduced permiability of the chorion to water may adversely affect utilization of the yolk, leading to retarded (Smith 1957) or abnormal embryonic development in the overripe eggs. Rao and Ram (1991) and Goswami and Sarma (1997) have reported similar higher deformed larval production in *C. batrachus* at lower and higher dose of pituitary respectively during induced breeding. The highest production of good larvae was obtained while injecting 1 ml Ovatide per kg to fish followed by 1.5, 2.0 and 0.5 ml doses. This could be due to higher numbers of good eggs, higher fertilization and hatching, and less deformed larvae.

Conclusion

The present investigation demonstrated that *C. batrachus* can successfully be induced to ovulate at 1ml Ovatide per kg body weight ensuring high quality eggs and more normal larvae. Higher or lower doses affected the egg quality, led to spawning failure or low output of hatchlings. This information is very important for commercial hatcheries for optimum collection of good quality eggs leading to higher larval production.

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