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A Review on Induced Breeding of Fresh water Cat fish, *Clarias batrachus* in India

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Abstract

Induced breeding of Asian cat fish *Clarias batrachus* of India by various hormonal analogues is reviewed based on published data. The breeding period of cat fishes in India is variable. Environmental factors play an important role in regulating reproduction in fishes. Pitutary gland extract, HCG and synthetic hormones viz., ovaprim and ovatide are successfully being tested for the induced breeding of fishes by various researchers in different parts of the country, under different climatic conditions, with varying degree of success. Even though natural spawning is the favorite method for breeding of cultivated fresh water fishes, induced breeding is necessary to control timing and synchrony of egg production.

Key Words: Cat fish, *Clarias batrachus*, Induced breeding, Synthetic hormones.

Introduction

Fishes form an important element in the economy of many nations as they have long been a stable item in the diet of many people. They constitute slightly more than one-half of total number of approximately 54,711 recognized living vertebrate species; there are descriptions of an estimated 27,977 valid species of fishes (Nelson, 2006).

Clarias batrachus is a species of freshwater air breathing catfish native to Southeast Asia, but also introduced outside its native range where it is considered an invasive species. It is named for its ability to "walk" across dry land, to find food or suitable environments. While it does not truly walk as most bipeds or quadrupeds do, it has the ability to use its pectoral fins to keep it upright as it makes a sort of wiggling motion with snakelike movements (Figure 1)

("Catfish 'walk' down street". Metro.co.uk. *2008-07-18*. Retrieved 2008-07-18; http://en.wikipedia.org).

Aquaculture has assumed the status of fast expanding industry in India. India is basically being a carp country and the indigenous and exotic carps account for bulk of production. But now the culture of catfishes also received increased interest in recent years due to their high market price and hardy nature. Among the catfishes the air breathing species *Clarias batrachus* is a popular culturable fish in Asian countries. It has many advantages over other species. The hardy nature and tolerance to adverse ecological condition enable its high density culture with a high production per unit area (Sharma et al., 2010).

The basic requirement of the controlled fish culture industry is the fish seed but now 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 and production (Sharma et al., 2010).

Among several inducing agents used in fish breeding, salmon gonadotropin releasing hormone (sGnRH) or luteinising hormone releasing hormone (LHRH) analogues in combination with dopamine antagonists was found to be effective in fish breeding (Lin and Peter 1996).

Catfishes due to their great demand and high market value and non-availability of stocking material largely hinder the organized culture of these fishes in our country. The small catfish species are noteworthy for their size, taste and market value. They are distributed in lentic and lotic water bodies and breed naturally in perennial rivers during monsoon. Though breeding and larval rearing of certain catfishes has been done successfully for their commercial production is yet to be achieved. Hence, maintenance of catfishes plays a key role in achieving successful induced breeding.

CLARIAS BATRACHUS BREEDING TECHNIQUES

Table 1 depicts the comparative study on induced breeding of *Clarias batrachus* by carp pituitary Extract (CPE) & various synthetic hormones by various authors.

The Asian cat fish, *Clarias batrachus* locally known as Magur fish, is an important air-breathing cat fish with good markets in North-Eastern parts of India where it fetches a higher price than the major carps. The scarcity of marketable fish as well as seed

from the natural ground has been felt in this catfish. The potential to obtain magur seed from natural sources has become low due to the increasing use of pesticides in the paddy fields which are the main breeding grounds of this fish. The breeding performance is an important parameter to evaluate the breeding success in captive condition which depends on the type of hormone used and its potency, dose of hormone and maturity status of the fish Human chorionic gonadotropin (HCG) at 14-23 h latency in combination with 3000-4000 IU HCG dose is one among them and is reported successful in catfish during induced ovulation. Appropriate combinations of the proper dose of inducing agent and stripping time always yield maximum egg output during induced breeding. A single injection of 0.6ml/kg body weight of ovaprim was the most effective. The males were given a single dose of 0.1-0.2 ml/kg body weight. Again administration of fish PG of dosage varied from 12 to 30 mg/kg weight of fish given in two doses, a provocative dose of 5-10 mg /kg and a final dose of 8-20 mg/kg, 5-6 hours interval was found successful in spawning. In hapa nursing of magur, we achieved an average survival of 51% while feeding with rice bran and mustard oil cake mixture at 1:1 ratio and termite twice daily (Datta,2016).

Before the female are stripped male fish with gravid testis are to be sacrificed and testes are taken out and macerated in normal saline (0.9% NaCl). The spermatozoa become inactive in this medium and this extract can be maintained for few hours in refrigerator. After 16 hours of latency period female fish is stripped and ova are collected in to dry enamel tray. Before fertilization milt (spermatozoa) extract medium is activated by addition of fresh water. Sperms become active and motility of sperms can be confirmed in microscope. Sperm preparation thus obtained will be sufficient to fertilize the ova stripped from 2 females. Sperm extract is sprinkled over the ova and gametes are mixed gently with bird feathers and allowed to 2 to 3 minutes for fertilization. After repeated washing with fresh water fertilized eggs are transferred to hatching trays for incubation (Datta, 2016).

Nayak et al. (2000) reported the plasma steroid profiles during oocyte maturation and LHRHa pimozide induced ovulation in the Asian catfish, *C. batrachus* and opined that the levels of estradiol-17ß and estrone rapidly increased reaching a peak during the vitellogenic phase, while a decline was observed during the spawning phase.

Sahoo *et al*. (2004) and Rath *et al*. (1995) reported that induced breeding of *Clarias batrachus* and carps during pre-monsoon season might cause ovulation of immature eggs and might lead to the abnormal development of the larvae. But Teji and John Thomas (2006) reported the percentage of malformed embryos was high during monsoon period, especially in detritivorous, bottom living, soft-bodied catfishes. Normal ovum maturation and ovulation is controlled by episodic release of gonadotrophins. The surge of gonadotrophic hormones that follows the ovaprim injection might be one of the reasons for the observed deformities in fish larvae.

Mahapatra (2004) used two types of inducing agent, carp pituitary gland extract (CPE) and Ovaprim for induced breeding of *Clarias batrachus* . The ideal dose of CPE is 20mg / kg body weight for males and 35mg / kg body weight for females. The ideal dose of Ovaprim is 0.75ml and 2ml / kg body weight for males and females respectively. The fishes were injected near the base of the pectoral fin. After injection, they were kept in a cement cistern with aeration. The females were kept under observation to detect the most appropriate time for stripping. Usually female fish attain the free flowing condition of eggs around 17 hours after injection. Generally, fertilization rates of 80 % and 70% were obtained with CPE and Ovaprim respectively (Mahapatra,2004).

Sahoo et al. (2005) conducted experiments 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 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 percentage of deformed larvae. More normal larvae were produced from the females when injected with 1 ml dose. One ml of Ovatide per kg body weight was found optimum for best breeding performance and egg quality in *C. batrachus*.

Table 1: Comparative study on induced breeding of cat fish *Clarias batrachus* by carp pituitary Extract (CPE) & synthetic hormones in India by various authors

Sl. No.	Hormone	Dose	Fertilization	Hatching percentage	References
1	Ovaprim	1.0-2.0ml	70.6-72.8	60.7-55.3	Srivastava etal., 2012
2	Ovatide	1.0ml	83	71	Sahoo et al., 2005
3	Ovaprim	1.0-1.5ml	-	-	Sahoo et al., 2007
4	Ovatide	1.0ml	82.33	55.35	Sharma et al 2010
5	SGnRHa +domperidone	20-30µg 10-15µg	High	High	Sahoo et al., 2005
6	Pitutary Gland Extract	50 mg/kg –Male 120 mg/kg-Female	80	71	Kishore Dhara & Nimal Chandra Saha, 2013
7	Ovaprim	0.4-1.0 ml/kg- Male 0.8-2.0 ml/kg -Female	77	65	Kishore Dhara & Nimal Chandra Saha, 2013
8	Ovaprim	0.5-1.0 ml/kg –Male 1.0-2.0 ml/kg-Female	70.6-72.8	55.3-60.7	Srivastava et al., 2012
9	Ovatide	0.5 Male 1.0- Female	80±2.1	75±2.5	Jagtap & Kulkarni, 2015
10	Ovaprim	0.6	93.16	55.10	Das ,2002
11	HCG	3000-4000 IU	-	-	Sahoo et al., 2008
12	Ovaprim	2 ml/kg	91.06	-	Bordoloi,2014
13	Ovaprim	2.0 ml/kg	80	60	Basu et al., 2000
14	Ovaprim	2.0-2.5 ml/kg	60-65	-	Mohapatra et al., 2000

Sahoo et al. (2005) demonstrated that *C. batrachus* could be successfully induced to spawn with an injection of SGnRHa in combination with domperidone.

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Administration of 20–30 µg SGnRHa kg^{-1} body weight of female and stripping at 14 and 17 hr post injection resulted in the highest rate of fertilization, hatching and normal larval production. This information would be of value for commercial catfish hatcheries, in order to ensure collection of the maximum quantity and optimum quality of eggs.

Figure 1: *Clarias batrachus* fish (Source: en.wikipedia.org)

Ovaprim has been successfully employed for induced spawning of fishes in a number of commercially important food as well as ornamental and threatened species (Lakara et al., 1996; Pandey et al., 1998, 1999, Sridhar et al., 1998; Nayak et al., 2001; Sarkar et al., 2006; Rath et al., 2007; Hill et al., 2009). The product has been reported to be an efficient inducing agent for oocyte maturation and ovulation in *C. batrachus*. The latency period of 16-18 hours after the injection of Ovaprim (dose 1-2 ml Kg-1 bw to female and 0.5-1.0 ml Kg-1 bw to male) to subjected fishes was found suitable for the ovulation of this species. Similar findings were also reported by Sahoo et al. (2005) in the same species while using sGnRHa in combination with domperidone (14 to 23 hours) (Srivastava et al., 2012).

The results of Sharma et al.(2010) indicated that the total weight of stripped eggs and spawning fecundity were the highest ($p < 0.05$) when females *C.batrachus* 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 0.6 ml Ovatide per kg BW of female brood fish. At the 1 ml dose, the percentages of total fertilized egg and hatching were 82.33 and 55.35% respectively, which were the highest ($p < 0.05$) among all treatments. The net survival of fry was found to be 98.52% at 1 ml Ovatide per kg BW. They recommended that one ml of Ovatide per kg BW of female brood fish was found optimum among the three experimental doses for best breeding performance and egg quality in *Clarias batrachus*.

Yadav et al (2011) attempted induced spawning of *Clarias batrachus*, by using different doses of ovatide and ovaprim (Figure 2) at varying latency period (interval between the time of injection and spawning).preliminary dose of ovaprim (male 0.1 ml/kg; female 0.5 ml/kg) administered intramuscularly 45 days prior

to spawning for gonadal maturity resulted in higher rate of fertilization and hatching success. Optimum doses of ovaprim and ovatide were found to be 0.8-1.0 and 0.6-1.0 ml/kg body weight with latency period between 14-16 h.

Srivastava et al (2012) observed the breeding and larval rearing of Asian Catfish, *Clarias batrachus* fed with live and/or artificial feed for 21 days in an indoor hatchery. They reported that fishes were successfully induced bred using ovaprim @ 1.0–2.0 ml/kg body weight (bw) to females and 0.5–1.0 ml/kg bw to males. Fertilization, hatching and survival percentages at spawn stage were respectively recorded 70.6 - 72.8, 60.7 - 55.3 and 54.3 - 56.2.

Dhara and Saha (2013) conducted breeding experiments on *Clarias batrachus* with pituitary gland extracts (40 and 120 mg/kg. body weight for female and 25 and 50 mg/kg. body weight for male) and Ovaprim (0.8 and 2.0 ml/kg body weight for female and 0.4 and 1.0 ml/kg body weight for male) at 26º, 28º and 30ºC. The highest rates of fertilization (80%) and hatching (71%) of eggs were recorded in *Clarias batrachus* injected with carp pituitary gland extracts @ 50 mg/kg body weight of male and 120 mg/kg body weight of female at 28ºC with a latency period of 15 hours. The fertilization and hatching rates were 77% and 65% respectively at 28°C at the higher doses of Ovaprim.

Jagtap and Kulkarni (2015) experiments to evaluate the breeding performance of *Clarias batrachus*, at different doses of ovaprim in laboratory condition. Different doses of ovaprim were tried on male and female fish, the optimum response was showed at dose 1 ml/kg body weight of female and 0.5 ml/kg body weight of male. The brooders weight range was 250-450 g. Breeding response studied under different parameters and showed latency period (h), fecundity, fertilization and hatching (%), 12±1.2, 4500±150, 80±2.1 and 75 ± 2.5 respectively.

Figure 2: Ovaprim and Ovatide used for Induced breeding of fishes
(Source: kingsaquaculture.com)

Conservation and protection of Fish Breeders

Several factors may contributed to the decline of freshwater catfish *Clarias batrachus* population. These consist of

habitat degradation, water pollution, siltation, increased predation by and competition with exotic species. Strict enforcement of fishing regulations to prevent fishing with explosives, poisoning, etc. Prevention of killing of brood fish and juveniles and replenishment of stock by artificial propagation (Ogale, 2002).

Conclusion

The production of good larvae of *Clarias batrachus* was obtained by using CPE, HCG , ovaprim and ovatide . Sustainable conservation of the genetic resources of *C. batrachus* for food and aquaculture is to be achieved. Non-availability of fish seeds and also scarcity of matured brood fishes which is the major limitation in the culture of this species. To overcome this problem seed production and culture as well in aquatic environment through induced breeding. The use of synthetic inducing hormones for successful ovulation followed by stripping is a common practice and has been studied by several researchers in India.

According to FAO estimates the demand for catfishes throughout the world is increasing & *Clarias batrachus* with its several beneficial aspects remain as a hit among the Asians in particular. Besides in order to protect the genetic resources of this species from unwanted hybridization, which the species is very much vulnerable, the fish genetists & the government bodies should work together (Surajit Debnath, 2011).

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