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A REVIEW ON INDUCED BREEDING OF STINGING CAT FISH, HETEROPNEUSTES FOSSILIS IN INDIA

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Abstract:

Induced breeding of Asian stinging cat fish Heteropneustes fossilis of India by various hormonal analogues is reviewed based on published information. The breeding period of cat fishes in India is variable. Environmental factors play an important role in regulating reproduction in fishes. HCG, Wova-FH and synthetic hormones viz., ovaprim and ovatide are successfully being tested for the induced breeding of fishes by various researchers in India, under different agro-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: Stinging Cat Fish, *Heteropneustes Fossilis*, Induced Breeding, HCG & Synthetic Hormones.

Introduction:

Heteropneustes fossilis is found mainly in ponds, ditches, swamps, and marshes, but sometimes occurs in muddy rivers. It can tolerate slightly brackish water. It is omnivorous. This species breeds in confined waters during the monsoon months, but can breed in ponds, derelict ponds, and ditches when sufficient rain water accumulates. It is in great demand due to its medicinal value (Froese, Rainer and Pauly, Daniel, 2011; https://en.wikipedia.org/wiki/Heteropneustes_fossilis).

The stinging catfish is able to deliver a painful sting to humans. Poison from a gland on its pectoral fin spine has been known to be extremely painful. This species grows to a length of 30 cm (12 in) TL and is an important component of local commercial fisheries. It is also farmed and found in the aquarium trade (Froese, Rainer and Pauly, Daniel, 2011; https://en.wikipedia.org/wiki/Heteropneustes_fossilis).

Fish reproduction is a periodic phenomenon and is controlled by environmental (exogenous) as well as internal (endogenous) regulatory mechanism. The acts of breeding occur under optimal environmental conditions that are favourable to the survival of the young ones. Environmental stimuli are detected by sensory organs, relayed to brain-that triggers endogenous mechanism into action. Endogenous mechanism is mediated through cascade of various neurotransmitters and hormones secreted by tissues of brain-hypothalamus-pituitary-gonadal axis. The secretion of above axis is regulated through positive and negative feedback mechanisms involving specifically sensitive hormone receptors. The most important reproductive neuro-hormones are hypothalamic gonadotropin-releasing hormones (GnRH) and gonadotropin-release-inhibiting factors (hormones) (GRIF or GnRIH) that regulate secretions of pituitary gonadotropin hormones (GtH) which in turn, regulate the synthesis of gonadal steroids responsible for final maturation of gametes. An

appropriate environmental stimulus may signal the arrival of optimal conditions for the fry, triggering spawning i.e. spermiation and ovulation. Fish in captivity may not always reproduce at the most favourable time. In this situation, hormones play a critical role in the reproductive processes (Ram Singh and Akhilesh Kumar Gupta, 2011).

Hormone-induced spawning techniques influence this sequential mechanism at several steps, either by promoting or inhibiting the process. Induced reproduction in fish includes two main strategies. The first is the manipulation of culture environment to mimic important characteristics of natural spawning environment of that particular fish. The second strategy is the administration of one or more naturally occurring reproductive hormone or their synthetic analogs in brood fish through injection or dietary methods. Both these strategies are commonly used, sometimes in conjunction with one another. Numerous hormones have been used to induce reproduction. However, recent researches and commercial aquaculture practices suggest the emergence of two lines of hormone-induced spawning as the best for successful breeding at the least expense. These are i) injection or oral administration of GnRH analog (LHRH analog) with dopamine antagonist, and ii) injection of purified gonadotropin (e.g. human chorionic gonadotropin-HCG) either alone or mixed with common carp pituitary extract to improve its potency. In spite of these, application of steroids, pheromones and prostaglandins were also used, as a new emerging and less studied field and required more research before its commercial application to achieve captive spawning in majority of cultured fishes (Ram Singh and Akhilesh Kumar Gupta, 2011).

Figure 1: A view of *Heteropneustes fossilis* (Source: https://en.wikipedia.org)

History of Induced Breeding:

The present day concept of induced breeding of fish can be traced back from the work of Houssay (1930) of Argentina who attempted the application of pituitary hormones for spawning of fish. However, Brazil was the first country to develop the technique of hypophysation (Von Ihering, 1935). In India, Chaudhuri (1955) successfully induced spawning, for the first time, in an Indian major carp species using pituitary gland extract. He also bred Pseudotropius atherinoides by administering pituitary extract from *Cirrhinus reba*. Ramaswami and Sundararaj (1956, 1957) reported successful breeding of the catfishes, *Heteropneustes fossilis* and *Clarias batracus,* by hormone injection. In 1957, Chaudhuri and Alikunhi (1957), for the first time, succeeded in inducing breeding of IMCs, rohu and mrigal and minor carps by injecting them with carp pituitary extract. Since then, the application of this technique has spread widely and now, with modifications, forms a regular part of fish culture programmes all over the country. Induced breeding of Chinese carps was successful in 1962 by employing similar technique (Alikunhi, Sukumaran and Parameshwaran,

1963a, b). Chondar (1970, 1984) described in detail the induced breeding technique for the difficult-to-breed IMCs and Chinese carps. By judicious management of broodfish, he was able to make the specimens of several species of carps breed three times in the same season. Varghese et al. (1975) successfully bred carps with pituitary gland of marine catfishes, *Tachysurus thalassima* and *T. jolla* (Basavaraja, 2007).

Fish breeding in India is no longer a complicated technique. Several hormones are being used for induced breeding of fish on commercial scale using Chinese type hatchery mostly at the private sector. A new spawning agent, Ovopel was evaluated for spawning of Indian carps in Assam, India. The study conducted by Das (2004) demonstrated the effectiveness of the new spawning agent, ovopel in inducing complete spawning in most of the species tested thus far. A dose of 1 to 1.5 ovopel pellet/kg brood fish was found to be sufficient to achieve 100% complete spawning. Ovopel induced 100% complete spawning in majority of carp species tested under the study with a response time varying between 4 hrs 50 minutes to 9 hrs.

Induced Breeding of Stinging Cat Fish, *Heteropneustes Fossilis:*

Table 1 depicted comparative study on induced breeding of cat fish *H.fossilis* by various synthetic hormones in India by various workers. Table 2 gives comparative hatching time in different agro-climatic conditions as given by different researchers in India.

Though induced breeding method in H. *fossilis* was reported by many authors (Sundararaj and Goswami 1966, Khan and Mukhopadhyay 1975, Sarkar et al. 1979, Saha 1996, Marimuthu et al. 2000, Nayak et al. 2001), this species is not spawned artificially on a commercial scale. The non-availability of adequate seeds is a major problem faced by the farmers to take up this species for aquaculture.

The first success in induced breeding of *Heteropneustes fossilis* was achicvcd by using homoplastic pituitary glands (Ramaswami and Sundararaj, 1956). Hctcroplastic pituitary glands of Indian major carp were successfully used by Khan (1972). The Al1 India Co-ordinated Research Project on Air-breathing Fish Culture recommended a dose of 80-120 mg.kg ' of female *H. fossi1is* (Kohli and Goswamii, 1987). There is a growing interest in the seed production of this species for aquaculture.

Ramaswami and Lakshman (1959) conducted a study on effect of mammalian hormones on the spawning of *Heteropneustes fossilis*. Using chorionic gonadotropin they observed that *Heteropneustes fossilis* needs 2500 1U/kg for ripening of eggs. Thakur and Das (1974) administered carp pituitary at a dose of about 150 mgPG/kg body weight for induced breeding purpose.

Table 1: Comparative study on induced breeding of cat fish *H.fossilis* by HCG & various synthetic hormones in India by various workers

S.No	Hormone	Dose	Fertilization Rate (%)	Hatching Rate (%)	References
1	Ovaprim	0.5 ml/kg	-	80	Vijaykumar et al., 1998
2	Ovaprim	0.5 ml/kg	75	60	Haniffa & Sridhar, 2002
3	HCG	1000IU	78	75	Haniffa & Sridhar, 2002
4	Ovaprim	0.5 ml/kg	92.33	94.67	Karl Marx & Ramanuj Chakraborty, 2007
5	Ovatide	0.5 ml/kg	96	90.33	Karl Marx & Ramanuj Chakraborty, 2007

6	Wova-FH	0.5 ml/kg	87.33	77.33	Karl Marx & Ramanuj Chakraborty, 2007
7	Ovaprim	0.3-0.5 ml/kg	98.18	-	Christopher et al., 2009

Table 2: Hatching time of *Heteropneustes fossilis* as observed by various researchers in India (Source: Singh Kohli & Vidyarthi, 1990)

Sl.No	Hatching time (hr)	Temperature (°C)	Source/Reference
1.	Within 24	25	Sundararaj (1969)
2.	20-24	27-30	Chaudhuri (1971)
3.	18-20	26-30	Khan (1972)
4.	24 18-20	24-26 26-29	Thakur et al (1974)
5.	18-20 24 40	26-29 23-26 21-23	Kohli (1984)
6.	21-24	24-31	Roy & Pal (1986)
7.	16-18	26	Singh Kohli & Vidyarthi (1990)

Nirmal K. Thakur, and NasarManju Sheel (1977) studied spawning behavior of *H. fossilis* by breeding the fish in laboratory through hypophysation using pituitary glands of the Indian major carps such as *Catla catla* and *Labeo rohita* at a dose of about 15 mg/100 g body weight of the recipient. The spawning activity started about 6 to 10 hr after the administration of pituitary injection. The activity lasted 2 to 6 hr in various experiments. During this period, intermittent mating acts were observed, the average rate of mating being once every 2 to 3 min in the initial stages and 5 to 10 min or even longer in the later ones.

Roy and Pal (1986) was studied induced breeding of *Heteropneustes fossilis* (Bloch.) in laboratory aquaria. Among the pairs examined, 83% spawned successfully. Mating behaviour started between 6 and 15 h after administration of pituitary hormones. The number of enfoldings varied from 25 to 350. Over 93% of the released eggs hatched within 21–24 h at temperature 24–31°C.

Singh Kohli and Vidyarthi (1990) carried out induced breeding, embryonic and larval development in *Heteropneustes fossilis* (Bloch) in the agroclimatic conditions of Maharashtra, India. The embryonic development was completed within 16-18 h after fertilisation. Head and tail ends were distinguishable after 3 hand 11-12 somites were visible after 6u 7 h. The eggs started hatching after 14 h of incubation. Average hatching time was 16-18 hat 26°C. In 1st day old pro-larva, notochord was deflected upwards, eyes were darkly pigmented and alimentary canal appeared. In 4th day old post-larva intestinal coiling could be seen and yolk was absorbed. Aerial respiration started by 8th day. The 10 day old post-larva was free swimming and fed voraciously attaining a length of 20mm in 30 days

Keerikkattil P. Joy & Binu Tharakan (1999) worked on induced spawning in mature female Indian catfish, *Heteropneustes fossilis* to spawn in spawning season (July-August) by various ovulation-inducing agents, and the frequency of ovulation was checked after a latency of 12-14 hours of the treatment. Egg mass and fertilization rate were taken as end-points of spawning performance. Their results showed that the Indian catfish, *Heteropneustes fossilis* can be induced to spawn by super active GnRH analogue alone in a dosage range of 0.15-0.2 Ng/g body weight or in combination with DA2 antagonists, such as pimozide or domperidone, in a very low dosage of 0.05 Ng/g.

Alok et al(1999) study demonstrated a substantial 100 fold increase in the potency of sGnRH agonists to induce spawning in the Indian catfish, when a D - amino acid residue was substituted in conjunction with a modification at the C-terminal with ethylamide. The study suggests the use of cGnRH-II to develop potent agonists for the development of catfish spawning induction therapies and possibly for other related species of fish.

Nayak et al. (2001) injected 17α-hydroxy progesterone (17α-P) in the female stinging catfish (*H. fossilis*) were with or without priming with PG homogenate to induce oocyte maturation and ovulation. This was achieved using dosages of 8 µg/g body weights, however, administration of 8 mg/kg body weight PG homogenate with 5 µg /g body weight of 17α-P gave better egg production. Eggs released after 14-18 h after the administration and most of the eggs could be fertilized and hatched normally.

Haniffa and Sivasubbu Sridhar (2002) administered *H. fossilis* a single intramuscular injection of HCG at a rate of 1000, 2000 and 3000 IU/kg body weight which resulted in successful spawning. Haniffa et al. (2007) works on the development of a breeding technology for large- scale production of seeds of the native Indian catfish, *H. fossilis* (Bloch). The use of induced breeding and artificial fertilization for seed production and larviculture of singhi was discussed. Utilization of different types of breeding system such as cylindrical fibre tanks, cement tanks, fibre bath tubes and plastic troughs for induced breeding experiments were also studied. They, however, did not mention about results of fertilization and hatching.

Mani and Pandey (2007) studied on the effect of HCG administration on ovarian maturation and spawning of *H. fossilis* (Bloch). The broodstock (2+ years) of *H. fossilis* (Bloch) were given intramuscular injection of HCG (two doses: 25 IU and 50 IU/kg body weight) at weekly intervals for 28 days during June in order to record the effects of maturity in the catfish, the response of 50 IU HCG was more pronounced as most of the oocytes exhibited germinal vesicle migration (GVM) towards the periphery. The eggs of drug administered catfish were greenish-brown, well-developed, separated and released after a gentle pressure on the abdomen whereas the eggs of control catfish were released in lumps only after a heavy pressure on the abdomen and were bluishbrown in colour. Fertilization percentage and hatching success were better in HCG treated catfish as compared to control.

Improved egg production was observed by Nayak et at. (2000) by using PG at a dose of 8 mg/kg body weight. Successful spawning was observed at 1000, 2000 and 3000 IU/kg body weight administered a single intramuscular injection of HCG to *H. fossilis* by Haniffa and Sridar (2002).

The effect of luteinizing hormone-releasing hormone analogue (LHRHa), pimozide (PIM) and ovaprim on oocyte maturation, ovulation and spawning *of Heteropneustes fossilis* has been evaluated by Nayak et al., (2001). Both LHRHa and pimozide when tested alone, failed to evoke any ovulation response, although both drugs resulted in the advancement of oocyte maturation in catfish as evidenced from germinal vesicle migration. On the other hand LHRHa + PIM (0.05 ug + 5 ug/g/ body weight respectively) when administered in combination intraperitoneally, caused high rate of ovulation and produced an average of 10±2.3 g eggs with high rate of hatching (93.5 ± 1.42%) and high yield of normal larvae (87.3±3.3 %). A single dose (0.6 - 0.8 ml/kg) of ovaprim injected intramuscularly resulted in an average production of 13.75 ±2.9 g eggs having 96.3± 1.7% hatchability and yielded 92.5 ± 1.5% of normal larvae. The latency period (interval between the time of injection and spawning) ranged between 15-18 hrs in LHRHa + PIM and 10-12 hrs in ovaprim treated fish. The eggs

produced after induced spawning were viable and fertilized *in vitro* using homogenized testes and produced higher yield of normal larvae, minimizing deformed and crawled larvae.

Three freshwater fishes, namely *Heteropneustes fossilis* (stinging catfish), *Anabas testudineus* (climbing perch), *Mystus vitattus* (striped dwarf catfish) were induced bred and morphological studies of the larvae were carried out by Teji and John Thomas (2006). Morphological and behavioral abnormalities were noticed among larvae produced through induced breeding techniques in all the three species. Morphological abnormalities were seen in head, trunk and tail region of the larvae. Under-developed head, deformed trunk, enlarged yolk sac, underdeveloped barbel, curved tail and vertebral abnormalities were observed. Tunicate larvae (larvae with undetermined growth) were common in these species.

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

Karal Marx and Ramanuj Chakraborty (2007) used three inducing hormones viz, Ovaprim, Ovatide and WOVA-FH were injected at the rate of 0.5, 1.0, 1.5 and 2.0 ml/kg body weight of Heteropneustes *fossilis* in order to induce oocyte maturation and ovulation. After 10-13 h of injection at a water temperature of 27±.-0.5°C, stripping of eggs and in vitro fertilization was done. Ovaprim gave maximum (94.67%) hatching rate followed by Ovatide (90.33%) and WOVA-FH (77.33%).

Christopher et al (2009) reported that the minimum number of sperm cells required for optimal fertilization success in *Heteropneustes fossilis* was determined. Fertilization success of 78 to 93% was recorded at 8x103 to 8x107 sperm per egg. The highest fertilization success of 98.18% was recorded at 8x107 spermatozoa per egg. They concluded that the sperm cell concentration can be easily assessed spectrophotometrically at 420 nm in 1:500 dilution with HBSS. For optimum fertilization success, a minimum of 8,000 spermatozoa egg-1 is sufficient. Thus the sperm cells can be utilized to the maximum in Heteropneustes *fossilis*.

Puvaneswari et al (2009) investigated the embryonic and larval development of *Heteropneustes fossilis* from fertilization until metamorphosis. The fully matured eggs and sperm were obtained by artificial insemination. The average diameter of the fertilized eggs ranged from 1.30 to 1.50 mm. The incubation period was from 23-24 h at an average temperature of 29±1°C. The newly hatched larvae were 2.5±0.2 mm in length. The yolk absorption was completed within 3 day after hatching. The aerial breathing behaviour of the larvae was observed 10 d after hatching. The larva resembled the adult in its external features and was metamorphosed to young juveniles within 20 d post-hatching.

The induction of final maturation and ovulation was stimulated in *Heteropneustes fossilis* , using a single intramuscular injection of ovaprim, at the rate of 0.3 and 0.5 mg.kg−1 body weight for males and females (Godwin Christopher et al.,2010). Godwin Christopher et al (2010) reported that the minimum number of sperm required for optimal fertilization success in *Heteropneustes fossilis*. An average fertilization success of 78–93% was recorded at 8×10^{3} to 8×10^{7} sperm per egg. The optimum contact duration of gametes was 5 minutes. The highest fertilization rate of 98.18% was recorded at 8×10^{7} spermatozoa.egg−1.

Induced breeding experiments conducted by Roopesh Mishra (2014) in *Heteropneustes fossilis* employing different inducing agents like ovaprim, ovatide and

wova-FH. The catfish responded well to ovaprim (0.40-0.55 ml/kg), ovatide (0.45-0.55 mg/kg) and wova-FH. (0.50-0.60 mg/kg). However, ovaprim gave better results in terms of fertilization and hatching success on low dose and latency period was also less as compared to ovatide and wova-FH. The overall performance of ovaprim was better when compared with the other two drugs. Among ovatide and wova-FH, the latter appeared to be better in spawning performance in comparison to the former.

Chaturvedi et al (2015) conducted induced breeding and larval rearing of stinging catfish, *Heteropneustes fossilis* (bloch), under controlled conditions in Raipur, Chhattisgarh (India). During their experimental trials, 10 female and 20 male brooders were injected with ovaprim @ 0.05 ml/100 gm body weight for male and 0.08 ml/100 gm body weight for female. The latency period for breeding varied between 6-8 hours and successful spawning occurred in all the sets without sacrificing the males as well as stripping of both the sexes. The results were encouraging with 90% survival from eggs to spawn while 70% from fry to fingerling stage resulting in the production of 21,168 fingerlings from 35,600 fertilized eggs and 30,240 fry. The larvae grow to 10-20 mm in 12-15 days of rearing in cement cistern. After yolk-sac absorption, they are fed with plankton, eggs custard and Artemia naupli thrice daily. Post-spawning mortality was not observed in the brooders.

Olufeagba et al (2015) studied induced breeding, embryogenic chronology and larva development of *Heteropneustes fossilis*. One sexually matured female was injected intramuscularly with dry carp pituitary gland suspension at 4 mg/ kg- of body weight. After a latency period of eight hours, the female was stripped while the male was sacrificed to remove the testes which were macerated to fertilize the eggs. Incubation was carried out at 27°C and the different stages of embryo were photographed. Hatching started 23.53 hrs after fertilization and the percentage hatchability was 97.5%. The mean percentage survival was 75%.

Immunological Changes:

Kalpana Dash et al (2000) conducted Haemagglutination assay (HA), humoral-mediated specific immune response and alternative pathway of complement-mediated haemolysis (APCH50) to evaluate the immunological responses of the synthetic hormone ovaprim in *Heteropneustes fossilis*. It was found that ovaprim suppresses the haemagglutinin as well as the specific antibody titres of the catfish sera against rabbit erythrocytes (RaRBCs) and bovine serum albumin (BSA) antigen while combination with |3-glucan restored its normal activity indicating its immunomodulatory effect. Furthermore, APCH50 unit of fish sera was remarkably decreased by ovaprim treatment. Anti-ovaprim antibody could not be detected in serum of the treated catfish.

Conclusion:

Studies on induced breeding of *H. fossilis* were carried out by various researchers in India. The study was conducted to optimize the dose of synthetic hormone for induced breeding of *H.fossilis* and to determine the hatching rate and fertilization rate. The ovaprim and ovatide treated *H. fossilis* fish yielded better results compared to HCG, Wova-FH treated fish in terms of fertilization and hatching rates during the present review. These results would be useful for appropriate management of induced breeding in *H. fossilis* or any catfish.

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