A Seminar Paper

On

Induced Breedingof Stinging Catfish*(Heteropneustes fossilis*) : Comparison Among DifferentInducing Agents

Course Title : Seminar

Course Code : GFB 598

Submitted To

Course Instructors	Major Professor
Dr. A. K. M. Aminul Islam Professor Department of Genetics & Plant Breeding BSMRAU **Dr. Satya Ranjan Saha** Professor Department of Agroforestry & Environment BSMRAU **Dr. Shaikh Shamim Hasan** Professor Department of Agricultural Extension & Rural Development BSMRAU **Dr. Dinesh Chandra Shaha** Associate Professor Department of Fisheries Management BSMRAU	**Dr. Md. Shahanoor Alam** Associate Professor Department of Genetics & Fish Breeding BSMRAU

Submitted By:

Fahmida Akter
Reg No:17-05-4321 **(**MS Student)
Dept. of Genetics & Fish Breeding
Submission Date: 30.03.23
Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur-1706

Induced Breeding of Stinging Catfish*(Heteropneustes fossilis)* : Comparison among Different Inducing Agents[1]

by

Fahmida Akter [2]

ABSTRACT

During the past two decades induced breeding by carp pituitary extract has been attempted in obligatory air-breathing fishes. The ever increasing cost of donor pituitary and the cumbersome process obliged experts to test alternative hormones such as human chorionic gonadotropin hormone (HCG), luteinizing hormone releasing hormone and ovaprim. The present study furnishes the comparison on the performance of different inducing agents in the induced breeding of the stinging catfish *Heteropneustes fossilis*. Breeding success was found to be higher in ovaprim treated individuals in all aspects including latency period, ovulation rate, fertilization rate, hatching rate and incubation period compared to that of PGE and HCG induced individuals.In addition, the present investigation also revealed that, ovaprim is more efficient in terms of ovulation, fertilization and hatching rates when using at a rate of 0.5 ml/kg body weight of female fishes than using at a rate of 0.3 ml/kg body weight of female fishes. Results of the present study would help the hatchery managers in managing the induced breeding programs of *H. fossilis* and other catfishes.

Keywords *:* *Heteropneustes fossilis*, induced spawning, pituitary gland extract, ovaprim, ovulation rate, fertilization rate, hatching rate.

[1] Title of the seminar paper as a part of course GFB 598 during Winter' 2022 term

[2] MS Student, Department of Genetics & Fish Breeding, BSMRAU, Gazipur -1706

TABLES OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I
INTRODUCTION

The Asian stinging catfish also known as fossil cat (*Heteropneustes fossilis*) is a air sac species catfish found in Bangladesh, Pakistan, India, Bhutan, Nepal, Thailand, Sri lanka and Malyasia.. It has also been found in the Tigris River Basin in Iran.The stinging catfish *Heteropneutes fossilis* (Bloch, 1974) belongs to the family Heteropneustidae is a commercially important fish species in Bangladesh. This is initially a fish of ponds, ditches, beels, swamps and marshes, but often found in muddy rivers (Jha and Rayamajhi, 2010; Froese and Pauly, 2012). The air-breathing organ of stinging catfish allows it to exist in almost any kind of water. It is also c apable of tolerating slightly brackish water. Usually, during the summer season *H. fossilis* lives in semi-liquid and semi-dry mud, and even when the mud becomes dry they take their bodies to the bottom of fissures and crevices formed by the cracking mud. *H. fossilis* can respire for a long time by gulping in air at various intervals when the oxygen content of water is low (Munshi, 1993). Meanwhile it is heavily utilized for food and for medicine in many parts of its range, and it may be threatened by over exploitation and habitat loss and degradation (especially from pollution anddams) and subsequently, it is considered least concern at present (IUCN, 2012). Because of its fast growth, tolerance to high stocking densities, high market value, ability to survive in oxygen-low waters, low fat, high protein and iron content and medicinal values, *H. fossilis* is considered as an ideal fish species for aquaculture (Haniffa and Sridhar*et al* , 2002; Pauly and Froese, 2013). Also, this species will be helpful not only in increasing the overall production in aquaculture but also in the conservation of this valuable fish species.

Aquaculture of the *Heteropneustes fossilis* in our country is widely spreading. However, constant supply of good quality fingerlings is vital for the culture of any fish species including *H. fossilis*. Although, farmers bought the fry and fingerlings for aquaculture were mainly from the capture fishery due to the limited capacity of the then existing hatchery facilities in the past, nonetheless, induced breeding techniques have continually improving in Bangladesh.

The knowledge of artificial breeding is a key aspect as it permits intensive production of a given species in controlled conditions. Only a reliable induced breeding and fry rearing technique

canensure a steady supply of quality fish seeds (Mollah *et al.,* 2008). Carp Pituitary gland (PG) extract and Human Chorionic Gonadotropic (HCG) hormone both were commonly used as inducing agents. There are some more inducing agents named as Ovaprim, Ovaclean, Flash etc. They can be used for both indigenous and exotic fish species. Subsequently, at present, hatchery produced fry/fingerlings become the major sources of seed for the aquaculture industry in the country. While the production of fish seed from hatchery sources has increased dramatically, the quality has not improved owing to poor hatchery management practices causes negative effects such as inappropriate selection,genetical inbreeding depression, interspecific hybridization etc.

Rather a few studies on the induced breeding of *H. fossilis* are done including effects of carp pituitary gland extract, human chorionic gonadotropin (HCG) and synthetic hormone (ovaprim) doses on induced breeding, maturation and ovulation of *H. fossilis* (Begum *et al*., 2001; Nayak *et al*., 2002; Haniffa *et al*., 2002),. Matter of regret that detailed studies on the induced breeding of *H. fossilis* are not found in Bangladesh. Subsequently, the present study describe and distinguish the comparative performances of different inducing agents on the breeding success of *H. fossilis.*

Objectives of the Study

The specific objectives of this review paper as follows :

1. To estimate the appropriate doses for induced breeding of *Heteropneustes fossilis*
2. To compare and select among inducing agents for the successful induced breeding

CHAPTER II

MATERIALS AND METHODS

This paper is entirely a review paper. So, this paper is mainly based on secondary information. Different published reports and articles are used to prepare this paper. Information has been assembled from various articles published in the various books, journals, proceedings and websites available on the online platform.

Valuable suggestions from my major professor and course instructors helped me to for the betterment of this paper. Personal communication with respective resource personnel helped me to collect valuable information to prepare the paper. After collecting of all the related information, it was compiled and logically presented in the present form.

CHAPTER III

REVIEW OF FINDINGS

In this chapter, findings of different authors have been accumulated and discussed under different headings to ascertain the objectives of the paper.

3.1 Different inducing agents usage (natural and synthetic hormones) in induced breeding of fishes

By introducing hypophysation, differentl methods of both artificial and natural inducement by several hormone extracts were practiced for breeding the fish up to 100% perfection (Panda, 2016). They are as mentioned below:

3.1.1 Pituitary hormone (PG)

Fish breeding by pituitary gland extraction is an effective and dependable way of obtaining pure seed of cultivable fishes.In 1955, by injecting of pituitary gland hormone were initiated in Indian Major Carps at the Central Inland Fisheries Sub-station for inducing, Cuttack. In 1957, it was initiated to breed the major varieties of Indian carps by pituitary hormone injection (Chaudhuri and Alikunhi, 1957). Pituitary hormone injection plays an important role in the development and maturation of gonads and induced spawning in fishes. In general, two doses are given to female at an interval of 4-6 hours. The primary dose level of pituitary gland extract was 2-3 mg/kg and second dose was 6-9 mg/kg in female. For male, only one dose at the time of second dose to female was given as 3-4 mg/kg body weight. The dose depends upon the maturity of fish, age, sex and also the environmental conditions.

3.1.2 Human Chorionic Gonadotropin (HCG)

According to Panda (2016), HCG is a glycoprotein hormone which is produced by the placenta in the pregnant woman. During early pregnancy, the hormone appears in the urine in large quantities. By injecting it to mature fish, the hormone causes maturity and release of gametes. The action of inducing male release sperm and ovulation is a joint action, parallely with the circulating pituitary hormones. When HCG is injected singly, it is not so effective as when it is injected together with pituitary gland extract.

3.1.3. Sumaach and Synahorin

INFAR (India) Ltd. has brought out a product which is cheaper as compared to the pituitary gland extract and has a long shelf life (Panda, 2016). The product is mixed in distilled water (2mg in 0.2ml) and the mixture is centrifuged.

3.1.4 Pimozide and LH RH-A

Pimozide is a dopamine antagonist having ovulatory role of LHRH-A. It is quite effective on Indian major carps. The LHRH (Luteininsing hormone releasing hormone) and its analogue (LHRH –A) are very effective on brakish water fishes (*Mugil* and *Lates).* They are cheap but, at present preparations are short-lived. Their application shall await production of long-lasting preparations.

3.1.5 DOCA (II-Deoxycorticosterone acetate)

DOCA is another effective drug which has been tried on catfish, *Clarias* and *Heteropneustes*. They are a bit different in the sense that they not only cause ovulation but may also bring about maturation of eggs.

3.1.6 Ovaprim

Ovaprim is a preparation of extract collected from Salmon gonadotropin RH and Dopamine antagonist in a stable solution. It is prepared in glycerin and alcohol at particular proportion.In the hypothalamus dopamine neurons have synaptic connections with that of gonadotrophic releasing hormone (GnRH) neurons. Thus, the inhibitory signal from dopamine neurons can be transferred to the GnRH neurons through the synaptic connections.These are introduced into the fish with a dose of 0.3 to 0.5 mg /kg body weight of female and .01 to 0.3 mg / kg body weight of male. Good feedback in India is found in the use of it in Uttar Pradesh, West Bengal, Bihar, Assam, Madhya Pradesh, Andhra Pradesh, Karnataka, Kerala, Odisha and Maharashtra. It has been researche that it works better than pituitary extract. Ovaprim can be stored at room temperature even in the tropics for more than a year.

3.1.7 Ovatide

Ovatide is a common, cost-effective and new hormonal formulation for induced breeding of fishes. The new formulation is having the base of a synthetic peptide which is structurally related

to the naturally occurring hormone, gonadotropin releasing hormone (GnRH). The doses determined for female brooder are 0.20-0.40 ml/kg and males are 0.10-0.20 ml/kg for rohu (Piska and Naik, 2007). Central Institute of Fisheries Education, India (1997-1998) carried out extensive field trails on induced breeding of fishes including carps using ovatide in Madhya Pradesh, Andhra Pradesh, Haryana and Maharashtra and reported encouraging results (CIFE, 1999).

Use of Ovatide represents the most modern and advanced technology for spawning of fish at considerably low cost (Sharad *et al.*,2015). Fishes injected with Ovatide produce increased number of eggs through complete spawning with high fertilization and hatching percentage with low viscosity of Ovatide makes it easily injectable. Application in a signal dose without causing any negative effect on brood fish. Ovatide produce healthy fish seed of good growth rate.

3.1.8 Ovapel

Ovapel was developed by scientist in the university of Godollo in Hungary, compiling with mammalian GnRH analogue and dopamine receptor lactosum, carriers and selected dose 1-2 pellet/kg of fish in catla and Rohu (Sharad *et al.*, 2015) .

The hormone is also available in pellet form. Each pellet contains superactive gonadoptropin releasing hypothalamic hormone analogue with an equal effect which a 3 mg normal acetone-dried dehydrated carp hypophysis gland has.

3.2.1 Spawning season of *Heteropneustes fossilis*

This is a common catfish found in freshwater swamps, ponds and tanks throughout the country. It is also suitable for pond culture. This fish breeds in ponds and hatchery throughout the year, peak season being monsoon.During rainy days, fishes move from wells to shallow inundated areas of paddy fields for breeding. The eggs are orange colour and are usually found attached to weeds.

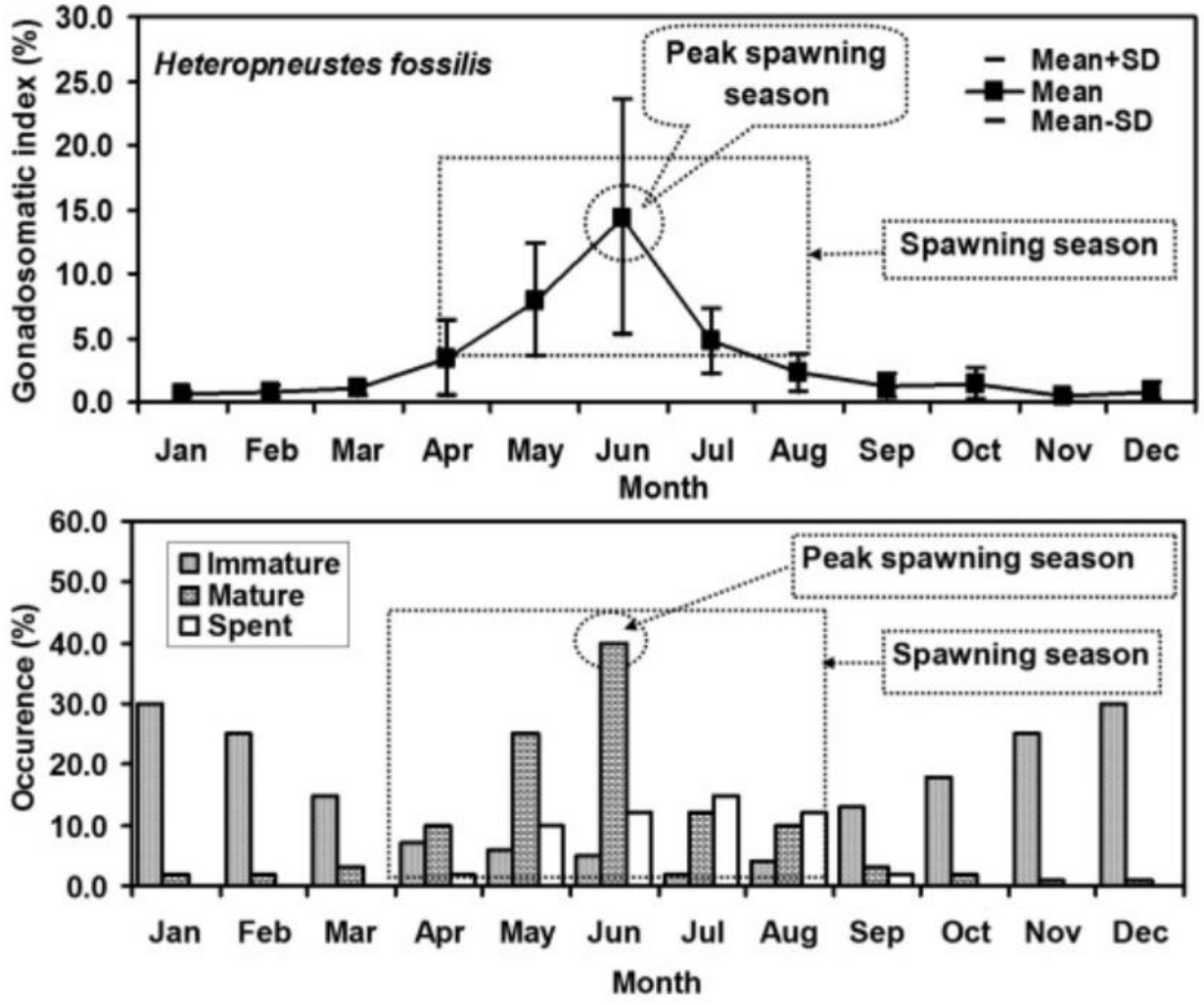
Spawning season was estimated using two methods

(i) monthly changes in gonadosomatic Index (GSI) (Khatun *et al*., 2019) and

(ii) seasonal progression of gonads (maturation stages of gonad) (Zhang *et al.*, 2009).

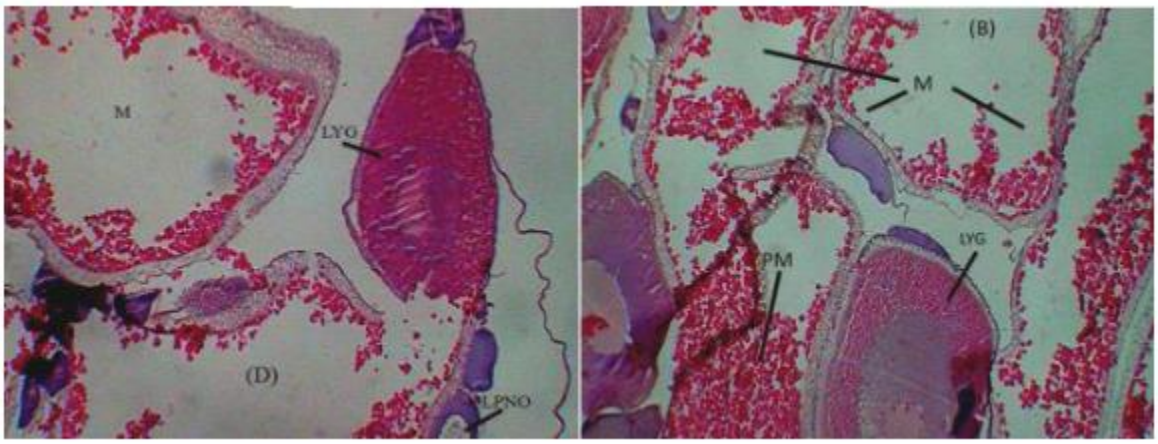
The macroscopic feature explains that the maturation stage was determined by the transparency of the gonads, the durability and vascularization and the final coloring of the gonads (Nath *et al*., 2013)

Monthly variations in GSI values are represented in figure 1. The ovary started to mature in March and continued until August. The highest GSI values were observed in April to August to define the full reproductive period of *H. fossilis*. In addition the highest GSI values were observed in the June that defines the peak season for spawning of *H. fossilis*.



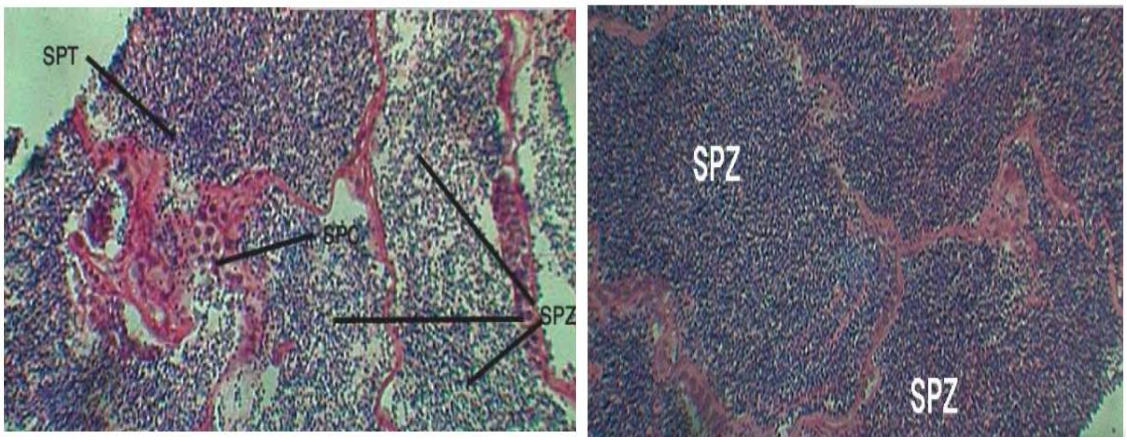
Source : Hasan *et al.*,(2022)

Figure 1 : The monthly variation of Gonado Somatic Index (GSI) and maturation stage based on colour with highest and lowest values of female *Heteropneustes fossilis* in the Gajner *Beel*, Bangladesh.



Source : Saha *et al*., (2014)

Figure 2 : Sections of ovaries sampled in early June



(a) (b)

Source : Saha et al., (2014)

Figure 3 : 3(a) Sections of testes sampled in early May

3(b) Sections of testes sampled in early June

3.2.2 Inducing the stinging catfish brood stock with different inducing agents and doses

The dehydrated carp pituitary gland extracts (PGE) which is commercially available and another synthetic hormone named ovaprim were used. On the contrary dehydrated carp pituitary gland extracts (PGE), synthetic hormone ovaprim and human chorionic gonadotropin (HCG) were also

used in another study. The body weight (g) of each brooder was weighed on an electronic balance (College B204-S, Switzerland) to estimate the required amount of inducing agents.
There were two groupof brooders consisting of three females and five males of *H. fossilis* each in both experiments, and then initialized to hormone treatment.

Table 1 : Brood Stock injection and doses for comparative study

Name of inducing agent	No. of female fish	No. of male fish	Dose For Female (mg/kg body weight)	Dose for Male (mg/kg body weight)
PGE	3	5	6 mg/kg	2 mg/kg
Ovaprim	3	5	0.3 ml/kg	0.1 ml/kg

Source :(Rahman *et al*.,2013)

Table 2 : Brood Stock injection and doses for another study

Name of inducing agent	No. of female fish	No. of male fish	Dose For Female (mg/kg body weight)	Dose for Male (mg/kg body weight)
PGE	3	5	6 mg/kg	2mg/kg
Ovaprim	3	5	0.5 ml/kg	0.1 ml/kg
HCG	3	5	1000 IU/Kg	1000 IU/Kg

Source :(Rahman *et al*., 2013)

3.2.3 Breeding and egg transfer for incubation :

After about 10-15 hours the brooders were ovulated by the action of injection the experiments. Then the brood fishes were transferred from the holding tanks after completing of ovulation. Therefore, in the figure 7 there are showed fertilized eggs taken to mini rectangular hatching tray by continuous aeration with taking precaution. Because any risk can be occurred by damage and fungal or bacterial contamination while collecting the egg. The estimation of number of eggs

released into each tray was estimated using gravimetric methods adapted from Legender (1986) and reviewed by Lagler (1992). Significantly a continuous flow of water was maintained for aeration to guarantee the environmental conditions were optimal for the hatching process.

3.2.4 Determination of growth,ovulation, fertilization and hatching Rate

Growth Rate

Table 3 : Growth rate of *Heteropneustes fossilis* in both study

Study No	Groups of brooders	Body weight of female	Total length of female	Body weight of male	Total length of male
1	2	40 to 150g (90.60±36.56)	17 to 25cm (20.84±2.58)	30 to 70g (55.30±11.58)	15 to 20 cm((17.29±1.95)
2	3	38 to 161g (107.1±39.68)	15 to 26cm (21.82±3.16)	25 to 80g (62.56±15.46)	14 to 23 cm (18.18±2.15)

Source : **(** Rahman *et al*., 2013)

However, Mann-Whitney U-test revealed no significant differences between the two groups of brooders in study. Kruskal-Wallis test revealed no significant differences in length and weight of 3 groups of brooders in study 2.

3.2.5 Ovulation rates

Table 4: Ovulation rate by using different inducing agent in *Heteropneustes fossilis*

Inducing Agents	Experiment 1			Experiment 2		
	LP (hrs)	OR (%)	IP (hrs)	LP (hrs)	OR (%)	IP (hrs)
PG	15	78.67 %	5.0	15	76.51%	5.0
Ovaprim	10	90.00%	3.5	10	93.77%	3.5
HCG	--	--	--	15	82.67 %	5.0

LP, Latency period; OR, Ovulation rate; IP, Incubation period

Source : (Rahman *et al*., 2013)

Nonetheless, the latency period was significantly shorter in ovaprim treated fish in contrasting to PGE and HCG injected brooders. On the contrary, using same dose of Ovaprim, much longer latency period were recorded by Kohil and Goswami (1987). However, it is problematic to evidently interpret the instrumental factors for the observed variances. Furthermore, a group of factors are likely to influence biological experiments particularly those involving hormones thereby leading to deviations in the observed latency periods (Gheyas *et al.*, 2002).

3.2.6 Fertilization rates

Table 5 : Showing details of fertilization rate of induced breeding in Stinging Catfish, *Heteropneustes fossilis* using different inducing agents

Inducing Agents	Experiment 1			Experiment 2		
	LP (hrs)	FR (%)	IP (hrs)	LP (hrs)	FR (%)	IP (hrs)
PG	15	69.23%	5.0	15	70.45%	5.0
Ovaprim	10	86.67%	3.5	10	90.83%	3.5
HCG	--	--	--	15	75.33%	5.0

LP, Latency period; FR, Fertilization rate; IP, Incubation period

Source : (Rahman *et al*., 2013)

However, chi square test exposed no significant differences between the fertilization rates of ovaprim and PGE treated fishes. Finding of this study agrees previous studies indicating the rate of fertilization is generally higher with ovaprim treatments (Nandeesha *et al*., 1990; More *et al*.,

2010). Such deviations in the fertilization rate can be attributed to the huge differences of hormonal doses, size of the brood fish, seasonal variation (Gheyas *et al*., 2002; Haniffa and Sridhar 2002; Nwokoye *et al*., 2007), environmental factors, water quality parameters (alkalinity, DO, pH, hardness) (Khan *et al*., 2006). The quality of the PGE hormone may also have influencing impact on the fertilization rates.

3.2.7 Hatching Rates

Table 6 : Showing details of hatching rate in Stinging Catfish *Heteropneustes fossilis* using different inducing agents

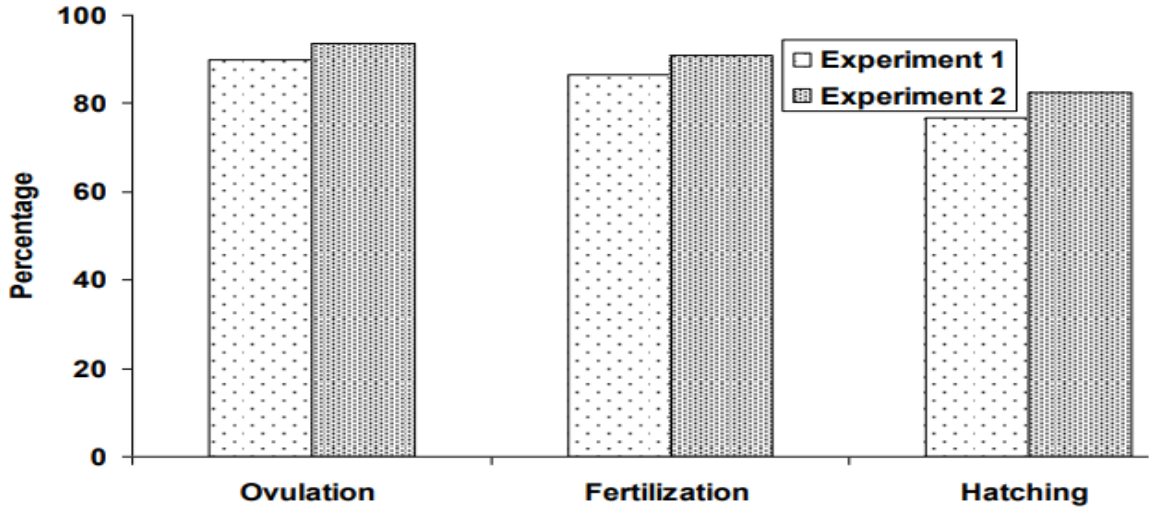
Inducing Agents	Experiment 1			Experiment 2		
	LP (hrs)	HR (%)	IP (hrs)	LP (hrs)	HR (%)	IP (hrs)
PG	15	72.72%	5.0	15	70.25%	5.0
Ovaprim	10	76.92%	3.5	10	82.48%	3.5
HCG	--	--	--	15	66.58%	5.0

LP, Latency period; HR, Hatching rate; IP, Incubation period

Source : (Rahman *et al*., 2013)

However, chi square test showed no significant differences in hatching rates between ovaprim and PGE treated fishes. Moreover, the incubation period for eggs in the PGE treated fish was more than 1.5 h longer than the ovaprim treated fish. . Nonetheless, Nayak *et al*., (2001) reported a hatching period of 10- 12 h in *H. fossilis* treated with ovaprim treatment at 27±1º C and obtained higher hatching rate of 96% using ovaprim at the rate of 0.4 ml/kg body weight. However, in terms of hatchling rate, ovaprim treated fish yielded better results compared the PGE treated fish (Nandeesha *et al*., 1990; More *et al*., 2010). All these studies to some extent support the findings of the present study.

Comparison on the performance of different doses of the synthetic hormone ovaprim in the induced breeding of *Heteropneustes fossilis* are shown in the figure 4.



Source : (Rahman *et al*., 2013)

Figure 4: Comparison on the performance of different doses (Exp. 1: 0.3 ml/kg body wt offemale; (Exp. 2: 0.5 ml/kg body of female) of the synthetic hormone ovaprim in the induced breeding of *Heteropneustes fossilis*.

3.2.8 Ovaprim doses administration

One group was injected with ovaprim hormone with T1 (0.3), T2 (0.4), T3 (0.5), and T4 (control) ml/kg/body weight The dose according to the. Table 1summarized the stimulatory substances and their doses and method of application. Various doses were followed . The induction and administration of the hormone doses were done between 6pm and 7pm. Then the injected fishes were taken to previous tank.

Table 7 : Substances used as ovulation stimulators, their doses

Group	Ovulation stimulator	Doses per 1 kg of female body weight
1	Ovaprim	0.3 ml
2	Ovaprim	0.4 ml
3	Ovaprim	0.5 ml
4	--	control

Source : (Talib *et al*., 2018)

Table 8 : Reproductive performance of *H. fossilis* female treated with T1 (0.3), T2 (0.4), T3 (0.5), and T4 (0) ml/kg/B. wt (means± SE)

Treatments				
Parameters	**Mean±SD**			
	T_1	T_2	T_3	T_4
Body Wt. before injection	1.4667^{a}±0.25	1.6367^{a}±0.60	1.38^{a}±0.15	1.6367^{a}±0.60
Body Wt. after injection	1.4533^{a}±0.24	1.6167^{a}±0.60	1.3467^{a}±0.15	1.6367^{a}±0.60
Egg weight (g)	13.33^{a}±5.77	20.00^{a}±10.00	33.33^{a}±5.77	0.00^{a}±0.00
Egg numbers	8233.33^{c}±1628.015	17200.00^{b}±5216.32	25006.67^{a}±2178.21	0.00^{d}±0.000
Spawning hours	14.67^{c}±1.52	11.00^{b}±1.00	8.00^{a}±1.00	0.00^{d}±0.000
Fertilization rate%	74.33^{c}±5.85	89.33^{b}±2.08	95.00^{a}±1.000	0.00^{d}±0.000
Hatchability rate%	78.33^{c}±9.504	80.33^{b}±2.082	84.33^{a}±4.933	0.00^{b}±0.000
Hatchability hours	37.167^{a}±1.2583	36.333^{a}±1.2583	37.500^{a}±1.3229	0.000^{b}±0.0000
Survival rate%	32.67^{c}±4.726	42.00^{b}±3.000	53.67^{a}±8.145	0.00^{d}±0.000

Means with same superscript letter have no significant differences (P>0.05). SD: Standard deviation, SE: Standard error

Source : (Talib *et al*., 2018)

This study was conducted to determine the artificial breeding with application of optimum dosageofstimulatory Ovaprim hormones. Female treated with T1 (0.3), T2 (0.4), T3 (0.5), and

T4(control) ml/kg/bodyweight. The result showed that stimulated with T3 (0.5) obtained better eggs quantity (25006) followedbyT2 (0.4) (17,200), while the lowest quantity (8233) was in T1 (0.3), but T4(control) was failed. The spawning hours, fertilization and hatchability, was significantly affected by three doses ($P < 0.05$). The hatchability hours was not significantly affected by hormone doses ($P > 0.05$).

Table 9 : Effect of different doses of two hormones on induced spawning in *C. punctatus* and *H.fossilis*. The low, medium and high dosages with different superscripts are significantly different ($P<0.05$).

Hormone	Fish mass (g)	Dosage kg/bw	Latency period (h)	Fertilization rate (%)	Egg output	Hatching rate (%)	Survival rate of hatchlings (%)
C. punctatus							
Ovaprim	65 - 80	0.1 ml	-	-	30 ± 8^{a}	-	-
	65 - 80	0.3 ml	28 - 34	73.5	3276 ± 75^{b}	65.0	30
	75 - 85	0.5 ml	28 - 34	75.0	198 ± 10^{c}	50.0	10
	60 - 70	1000 IU	-	-	102 ± 20^{a}	-	-
HCG	70 - 80	2000 IU	28 - 34	75.5	699 ± 78^{b}	65.5	50
	65 - 85	3000 IU	28 - 34	78.0	1253 ± 126^{c}	70.5	65
H. fossilis							
	100 - 105	0.3 ml	18 - 24	70.0	258 ± 85^{a}	50.5	10
Ovaprim	90 - 105	0.5 ml	18 - 24	75.0	1052 ± 220^{a}	60.0	30
	90 - 100	0.7 ml	18 - 24	70.0	6692 ± 790^{b}	50.0	15
	80 - 105	1000 IU	18 - 24	78.0	6336 ± 800^{a}	75.0	60
HCG	90 - 100	2000 IU	18 - 24	75.0	18376 ± 1020^{b}	60.5	50
	110 - 115	3000 IU	18 - 24	70.0	82922 ± 5432^{c}	60.0	55

Source : (Haniffa *et al.*, 2002)

3.2.9 Limitations

The freshwater fish *Heteropneustes fossilis* (stinging catfish), were induced bred and morphological studies of the larvae were carried out by Teji and John(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. Induced spawning of catfish, *Clarias*

batrachus, was attempted by Yadav *et al*., (2011) using different doses of ovatide and ovaprim at varying latency period (interval between the time of injection and spawning). In both the sGnRH-based drugs, decreased doses with increased latency period gave better results of fertilization and hatching.

CHAPTER IV

CONCLUSIONS

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.We should practice induced breeding as alternative method for quality seed supply and production. Many species of fish will not readily reproduce under certain culture conditions. Others will but not necessarily when the farmer desires. In these cases, induction of spawning can be of great value. Two techniques are commonly used, sometimes in conjunction with one another. The first is manipulation of the culture environment to mimic some important quality in the fish's natural environment. The second is injection of hormones to stimulate spawning.

Ovaprim treated brooders of *H. fossilis* showed better performance during induced breeding.Breeding success was found to be higher in ovaprim treated individuals in all aspects including latency period, ovulation rate, fertilization rate, hatching rate and incubation period compared to that of PGE and HCG induced individuals.However, ovaprim was found to be more efficient in terms of ovulation, fertilization and hatching rates when using at a rate of 0.5 ml/kg body weight than using at a rate of 0.3 ml/kg body weight of female fishes.Results of the present study would be beneficial for apposite management of induced breeding programs of *H. fossilis* and other catfishes.

Many procedures have been developed for inducing fish to undergo the last steps of spawning. Farmers should thoroughly research the procedures that have been developed for their species of fish through experimentation and select those that best suit the circumstances. In addition, once the fish have spawned, there are many techniques involved in incubating and caring for the eggs and caring for the hatched fry. These too must be thoroughly researched.

REFERENCES

Rahman, M. M., Hossain, M. Y., Hossain, M. I., Provhat, S. J., Islam, M. S., & Hossain, M. B. (2013). Induced breeding of the stinging catfish, *Heteropneustes fossilis*: comparison among different inducing agents. *Turkish Journal of Fisheries and Aquatic Sciences*, *13*(3).

Surnar, S. R., Kamble, A. D., Walse, N. S., Sharma, O. P., & Saini, V. P. (2015). Hormone administration with induced spawning of Indian major carp. *International Journal of Fisheries and Aquatic Studies*, *3*(1), 01-04.

Alok, D., Krishnan, T., Talwar, G.P., Garg, L.C. 1993. Induced spawning of catfish, *Heteropneustes fossilis* (Bloch), using D-Lys6 salmon gonadotropin-releasing hormone analog. Aquaculture 115(1-2): 159-167.

Begum, N., Rahaman, M.A., Hussain, M.G. and Mazid, M.A. 2001. "Effects of carp PG doses on induced breeding of shing, *Heteropneustes fossilis* (Bloch)" Bangladesh J. Fish. Res. 5(2): 145-148.

Dehadrai, P.V., Yusuf, K.M. and Das, R.K., 1985. Package of practices for increasing production of air breathing fishes, p. 1-4. In Aquaculture Extension Manual. Information and Extension Division of CIFRI (ICAR), India. New Series. No. 3.

Froese, R. and Pauly, D. (Eds). 2012. Fishbase 2012. World Wide Web electronic publication. Available at: http://www.fishbase.org (Accessed on 23 June 2012).

Gheyas, A.A., Islam, M.S., Mollah, M.F.A., and Hussain, M.G. 2002. A comparative study on the embryonic development of gynogen, triploid, haploid and normal diploid of stinging catfish, *Heteropneustes fossilis*. Bangladesh J. Fish. Res. 6 (2): 107-115.

Haniffa, M.A.K. and Sridhar, S. 2002. Induced spawning of spotted murrel (*Channa punctatus*) and catfish (*Heteropneustes fossilis*) using human chorionic gonadotropin and synthetic hormone (Ovaprim). Vet. Archiv. 72: 51-56.

IUCN, 2012. IUCN Red List of Threatened Species. Version 2012.1. IUCN 2012. IUCN Red List of Threatened Species. Downloaded in June 2012.

Jha, B.R. and Rayamajhi, A. 2010. *Heteropneustes fossilis*. In: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.1. www.iucnredlist.org. (Accessed on 19 May 2013).

Khan, A.M., Shakir, H.A., Ashraf, M. and Ahmad, Z. 2006. Induced spawning of *Labeo rohita* using synthetichormones. Punjab Univ. J. Zool. 21 (1-2): 67-72.

Kohil, M.P.S. and Goswami, U.C. 1987. Spwaning behaviour of a freshwater air breathing Indian catfish *Heteropneustes fossilis* (Bloch). Matsya 12: 180–183.

Lagler, K.F. 1982. Freshwater Fishery Biology. WM. C. Brown Company Publishers. Iowa, 2nd edn, pp.108-109.

Legender, M. 1986. Seasonal changes in sexual maturity and fecundity and HCG induced breeding of the catfish *Heteropneustes longifilis* val. (Flarridae) reared in Ebrie lagoon (Ivory coast). Aquaculture 55: 201–213. doi: 10.1016/0044-8486(86)90115-8

More, P.R., Bhandare, R.Y., Shinde, S.E., Pathan T.S. and Sonawane, D.L. 2010. Comparative Study of Synthetic Hormones Ovaprim and Carp Pituitary Extract Used in Induced Breeding of Indian Major Carps. Libyan Agric. Res. Cen. J. Intl. 1 (5): 288-295.

Munshi, J.S.D. 1993. Structure and function of the air breathing organs of *Heteropneustes fossilis;* in Advances in fish research I (ed.) B R Singh (Delhi: Narendra Publishing House) pp.99-138.

Nandeesha, M.C., Rao, K.G., Jayanna, R., Parker, N.C., Varghese, T.J., Keshavanath, P. and Shetty, H.P.C. 1990. Induced spawning of Indian Major carps through single application of Ovaprim C., pp: 581- 585. In Hirano, R and L. Hanyu (Eds.) The second Asian Fisheries Forum, Asian Fisheries Society, Manila, Philiphines.

Nayak, P.K., Mishra, T.K., Singh, B.N., Pandey A.K. and Das, R.C. 2001. Induced maturation and ovulation in *Heteropneustes fossilis* by using LHRHa, pimozide and Ovaprim for production of quality eggs, and larvae. Indian J. Fish. 48(3): 269-275.

Nwokoye, C.O., Nwuba, L.A. and Eyo, J.E. 2007. Induced propagation of African clariid catfish, *Heterobranchus bidorsalis* (Geoffrey Saint Hillarie, 1809) using synthetic and homoplastic hormones. Afr. J. Biotechnol. 6 (23): 2687-2693.

Vijayakumar, C., Sridhar, S. and Haniffa, M.A. 1998. Low cost breeding and hatching techniques of the catfish (*Heteropneustes fossilis*) for small-scale farmers. Naga 21: 15-17.

Talib, E. A. H. (2018). Effects of Ovaprim Hormone on Induced Breeding of *Clarias garipineus* *Agricultural Extension Journals*, 2(2), 75-77.

Hasan, M. R., Hossain, M. Y., Mawa, Z., & Hossain, M. A. (2022). Reproductive biology of *Heteropneustes fossilis* in a wetland ecosystem (Gajner Beel, Bangladesh) in relation to eco-climatic factors: Suggesting a sustainable policy for aquaculture, management and conservation. *Saudi Journal of Biological Sciences*, 29(2), 1160-1174.

Saha, S. K., Ali, M. H., & Rashid, H. (2014). Gametogenesis in Captive Stinging Catfish (*Heteropneustes fossilis*) During Spawning Season. In *Proceedings of 5th International Conference on Environmental Aspects of Bangladesh* (pp. 126-128).