

Captive Breeding of Singhi, (*Heteropneustes fossilis*) for Mass-scale Seed Production

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ABSTRACT: An experimental study has been carried out to standardize the minimal hormonal dose for *Heteropneustes fossilis* (Bloch, 1794). Collected 20 pairs of fish from Bandhab Hatchery, West Bengal and experimented with six different trials using brooders in a ratio of 2:1 in three tanks where a low hormonal dose of 0.25ml/kg body weight to male and 0.5 ml/kg body weight to female were injected with proper dilution with sterile water. Without the dry stripping method and sacrificing, the technique was more effective for successfully induced breeding results up to 90% spawning success. Using a glass breeding tank (4ft × 2ft × 1ft) with a submersible pump water fountain was created to stimulate breeding performance. The greenish or yellowish eggs hatched out within 20±2 hours at a temperature of 32±1°C. The highest fertilization rate achieved was 85.5 %. The spawn was fed on egg yolk, and infusoria and the fry was fed on tubifex worms, mosquito larvae, and green water two to three times a day. Then the fingerlings were fed on commercial feed. The observed maximum survivability rate was 83.3%. This study aims to provide a standardized minimal hormonal dose that can effectively increase the production of singhi on a large scale.

Keywords: Hormonal dose, Induced Breeding, Seed production, Survivability, Fertilization, Spawning.

INTRODUCTION

Heteropneustes fossilis (Bloch, 1794) also known as singhi, belongs to the family Heteropneustidae. It is highly profitable for its medicinal and economic value. It is often recommended for patients after recovery from malaria due to its therapeutical qualities (Bhuiyan, 1964). This fish is found in mainly ponds, swamps, beels, ditches and muddy rivers in our country. It contains a rich amount of protein and minerals. The substance is highly abundant in protein and minerals. Fish is mostly composed of water (72%), followed by protein (19%), fat (8%), calcium (0.15%), phosphorus (0.25%), and vitamins (0.10%) (A, B, C, and D). The fish muscles have been documented to possess a significantly higher concentration of iron (226 mg/10 g) (Saha and Guha 1939) and a rather substantial amount of calcium in comparison to numerous other freshwater fish species (Ali *et al.*, 2014). It helps the fish to tolerate slightly brackish water and enables them to survive in almost any type of water. *Heteropneustes fossilis* can breathe aerially at various intervals when Halder *et al.*,

the oxygen content in the water is much lower (Dutta *et al.*, 1993). It is a less concerned (Alok *et al.*, 1993). This species is generally stocked at densities 10 times greater than IMC because of its hardiness and air-breathing characteristics which makes them favourable for culture. Stinging catfish has a higher market price than carp. These are sold for \$7-10/kg in India (Ghosh *et al.*, 2020) and around \$20/kg in the USA.

Singhi is a significant contributor to the overall production of air-breathing fish in India, primarily sourced from capture fisheries. The lack of availability of seeds is a major concern for the cultivation of stinging catfish in our nation. This is mostly due to the delayed supply of fry and fingerlings, as well as the difficulty in consistently collecting them from natural sources (Vijaykumar *et al.*, 1998). Furthermore, the farmers' lack of knowledge poses a challenge in the development of stinging catfish. Therefore, it is imperative to provide a continuous supply of seeds, as well as proper management of brooders, implementation of breeding techniques, and effective

larval rearing. Induced breeding strategies have been demonstrated to be effective in achieving seed production. Nevertheless, although this artificial technology has achieved success, it has not yet been accessible to marginal farmers due to its intricate nature. Only a small number of hatcheries have begun to produce seeds through hormonal intervention using the stripping method. However, throughout the process of stripping, female brooders experience a state of physical weakness because of high levels of stress in their bodies. Therefore, undeveloped eggs may be discharged during the process of ovulation. In addition, stress might cause injury to the gonad. In addition, male brooders are intentionally sacrificed to harvest sperm. To overcome these challenges, we standardized the technique of *H. fossilis* to meet the increasing demand for seeds among farmers.

Various breeding techniques have been introduced in recent years. Begum *et al.* (2001), experimented with the induced breeding trials of *H. fossilis* using five doses of carp pituitary gland. Marx *et al.* (2007), injected ovaprim and ovatide, female fish were stripped out to collect eggs and males were sacrificed. The highest rate of fertilization success (96%) was achieved using Ovotide at a dosage of 0.5 ml per kilogram of body weight. Rahman *et al.* (2013), used a dosage of pituitary gland extract (PGE) administered to female fishes was 6 mg/kg body weight, while male fishes received a dosage of 2 mg/kg body weight. Distinctly, female and male subjects were administered ovaprim at a dosage of 0.3 ml/kg and 0.1 ml/kg body weight, respectively. Ali *et al.* (2014), experimented on *H. fossilis* where they injected HCG Hormone for the induced breeding and found out 84% fertilization rate. They used PG hormone also and found out 95% fertilization rate. Mishra *et al.* (2017), studied the induced breeding technique. They injected intramuscularly with hCG hormone (6.95 IU/g dose of body mass). After 12 hours of latency period, they found a 98% fertilization rate and a 98% hatching rate.

MATERIAL AND METHODS

Study Site: The present study was conducted at Fish Breeding Training Centre, Panchpota, Garia, Kolkata-700152 (Latitude- 22.467519, Longitude- 88.419551) from 26th August 2023 to 24th January 2024 (Fig. 1).



Fig. 1. Geographical Location of the Study Site.

Broodstock Management. Brooders were collected from Bandhab Aqua, Diamond Harbour, South 24 Parganas, West Bengal (Latitude 22.228250, Longitude 88.183553) (Fig. 2). A total number of 20 pairs of *Heteropneustes fossilis* with an average weight of 70g, ranging from 60-80g for the experiments were transported in a sealed oxygenated polybag with methylene blue. Then the fish were transported to the Fish Breeding Training Centre, Panchpota, Garia.



Fig. 2. Geographical Location of the Brooder Collection Site.

Experimental Set-up. After the proper procurement of the brooders, they were separated into male and female, whereas the number of males was 30 and females was 10. We set up a small, improvised glass breeding chamber (4ft × 2ft × 1ft) for the experiment (Fig. 3), kept the selected healthy brooders inside the brood cage, and then used an artificial shower for better acclimation. The fish were kept under a natural photoperiod during the experiments.



Fig. 3. Improvised Glass Breeding Chamber.

Induced Breeding in Captive Conditions. First made a breeding cage that not only helps to separate the brooders but also increases the egg efficiency. Used a submersible pump for an artificial shower so that eggs could be easily separated from the breeding cage. After the setup was done, healthy and mature male and female brooders in a 2:1 ratio were selected and used the synthetic hormone HatchMe for induced breeding. Injected HatchMe hormone by diluting it with 80-90% sterile water (Fig. 4). Later experimented 6 trials of different doses chronologically (Trial 1: Female-0.25ml/kg, Male-0.5ml/kg), (Trial 2: Female-0.25ml/kg, Male-0.5ml/kg), (Trial 3: Female-1.0ml/kg, Male-2.0ml/kg), (Trial 4: Female-0.75ml/kg, Male-1.45ml/kg), (Trial 5: Female-0.5ml/kg, Male-1.0ml/kg), (Trial 6: Female-0.25ml/kg, Male-0.5ml/kg). Therefore, standardized the perfect minimal dose with the best performance. After that, the injected brooders in the

breeding setup checked the water parameters and later recorded the observations. After 10-12 hours of spawning time, greenish or yellowish hatched eggs (Fig. 5) were separated with proper precautions to avoid damage in the hatching tank (4 ft × 2 ft × 1 ft).



Fig. 4. Intramuscular hormonal injection.



Fig. 5. Hatched eggs.

Use of Experimental Feed. On the DPH (Day Post Hatch) 4, the yolk sac disappeared and was absorbed. Then started feeding boiled egg yolk emulsion with water in 1/4 proportion also used twice a day at 8 am and 4 pm with infusoria (Fig. 6), mosquito larvae and dry tubifex from DPH 10. After 20 DPH, artificial feed for rearing them from fry to early fingerlings.



Fig. 6. Feeding of infusoria.

Growth Performance. The growth performance of experimental fish (*Heteropneustes fossilis*) was analysed at the initial and final time during 60 days of the experiment.

RESULTS AND DISCUSSION

The result of the present experimental study shows that induced breeding of *Heteropneustes fossilis* without sacrificing or dry stripping the brooders by using the minimal hormonal dose where 0.25ml/kg body weight for males and 0.5ml/kg body weight for females with 90% spawning success after 10 hours of spawning period (Table 1). Synthetic hormones are used as 1.0-1.5 ml per kg body weight for Singhi brooders as Sahoo and Ferosekhan (2023) described.

Fertilized eggs were greenish or yellowish whereas we found 85.5% (Trial 1), 74.5% (Trial 2), 55% (Trial 4), 45% (Trial 5) and 75% (Trial 6) fertilization rate consecutively. During the third trial, we observed the highest mortality rate as a result of excessive water exchange and extreme temperatures. Hatching (%) found in trial 1, trial 2, trial 4, trial 5 and trial 6 is 86%, 85%, 85%, 88%, 80% consecutively (Table 1). During the present experiment, we found a maximum survivability rate in the tanks where 83.3% is the highest and 50% is the lowest (Table 3). The highest survival rate 83.33% and the lowest survival rate 63.33% were reported by Hossain *et al.* (2016).

In the first 21-22 hours, no feed was provided until the yolk sac was absorbed properly (Ghosh *et al.*, 2022). Then, boiled egg yolk was fed to spawns twice a day. After that, infusoria, dry tubifex, and green water were fed to fry twice a day. Lastly, artificial feed (36% protein) was fed to the fingerlings (Table 2).

The average length of singhi fingerling was 1.0 cm and the average weight was 0.80 g of 15 DPH singhi fingerlings. Average length and average weight were measured at 1.0 cm and 0.80 g respectively of 30 DPH fingerlings. After that, the average length and average weight were measured at 11 cm and 11 g respectively of 60 DPH singhi fingerlings (Table 5).

Table 1: Breeding Observation of *Heteropneustes fossilis*.

Total body weight of brooders (g)		No. of Brooders		Dose of injection (ml/kg)		Spawning (h)	Fertilization (%)	Incubation (h)	Hatching (%)
M	F	M	F	M	F				
110	194	2	1	0.25	0.5	10	85.5	20	86
300	280	6	3	0.25	0.5	12	74.5	19	85
180	180	4	2	1.0	2.0	—	—	—	—
250	201	4	2	0.75	1.45	10	55	18	85
280	250	6	3	0.5	1.0	12	45	20	88
200	220	4	2	0.25	0.5	10	75	22	80

Abbreviation: h- hour, M- male, F- Female, AT- Air Temperature, WT- Water Temperature, DO- Dissolved Oxygen

Table 2: Feeding in different stages of larval rearing of *Heteropneustes fossilis*.

Sr. No.	Stage	Age	Feed type	Remarks
1	Fertilized eggs	0 DPH	-	No feed
2	Hatchling	21-22 hrs	-	No feed
3	Spawns	42-44 hrs	Boiled egg yolk	2 times per day
4	Spawn to fry	3 DPH	Boiled egg yolk, infusoria	2 times per day
		4-8 DPH	Dry tubifex. Infusoria, green water	2 times per day
		9-15 DPH	Dry tubifex. Infusoria,	2 times per day
		16-20 DPH	Dry tubifex. Infusoria, mosquito larvae	2 times per day
5	Fry to advanced fry	21-40 DPH	Dry tubifex. Infusoria, Mosquito larvae	3 times per day
6	Advanced fry to early fingerlings	41-60 DPH	Artificial fish feed	3 times per day

Abbreviations: DPH- Day Post Hatch

Table 3: Larval rearing methodology of *Heteropneustes fossilis*.

Parameters	HT	NT	RT-1	RT-2
Size of aquarium (cm)	4ft × 2ft × 1ft	4ft × 2ft × 1ft	4ft × 2ft × 1ft	6ft × 5ft × 2ft
Stage of fish	Hatchling to spawn	Spawn to fry	Fry to advance fry	Advance fry to fingerling
Age (from fertilized eggs)	70-90 h	20 days	40 days	60 days
Days of Rearing	3	17	20	20
Nos. of fish stock	1000	800	400	300
No. of fish harvested	800	400	300	250
Survivability rate (%)	80%	50%	75%	83.3%

Abbreviations: HT = Hatching tank, NT = Nursery tank, RT-1 = Rearing tank-1, RT-2 = Rearing tank-2

Table 4: Water Quality Parameters.

S. No.	Parameters in different experiments	Duration			
		15 days	30 days	45 days	60 days
1	Temperature (°C)	28-29 Avg 27.66	30-31 Avg 30.83	27-28 Avg 27.77	27-30 Avg 27.81
2	pH	7.5	8	8	8.5
3	Dissolved Oxygen (ppm)	7	7	8	7
4	Hardness (ppm)	340-345	335-350	340-345	320-335
5	Ammonia (ppm)	0.02-0.03	0.01-0.02	0.01	0.01-0.02
6	Nitrite (ppm)	0.05-1	0.05-1	0.03-1	0.05-1
7	Nitrate (ppm)	5-10	5-10	5-10	5-10

Abbreviations: °C= degree Celsius, pH = potential of hydrogen, ppm = Parts Per Million

Table 5: Length-Weight of *H. fossilis*.

Duration	15 DPH	30 DPH	60 DPH
Length (Average)	1.0 cm	2.5 cm	11cm
Weight (Average)	0.80 g	2 g	11 g

Abbreviations: DPH- Day Post Hatch

CONCLUSIONS

H. fossilis have a relatively high market price compared to carp. Nevertheless, the native population of these indigenous catfish species continues to decline because of excessive exploitation and the destruction of their breeding habitats caused by the use of pesticides in crops and the introduction of non-native fish species. The advancement of captive breeding technologies, while preserving the male, will encourage seed producers to adopt the species for the commercial production of young fish. Presently, farmers choose to cultivate carp due to their ease of breeding in captivity and the ready availability of seeds for cultivation. Additionally, the present study simplifies the breeding of singhi which will encourage the marginal farmers to promote the species for mass-scale seed production. This will help replenish the natural population in its natural habitat and meet domestic demands.

FUTURE SCOPE

This present study of captive breeding opens up new scopes and opportunities in Aquaculture. We developed a simplified improvised breeding technique that can uplift the socio-economic situation of the marginal farmers in our country. Further improvements may be made by controlling physio-chemical factors like temperature, hardness, pH, and ammonia. Farmers should prepare appropriate environmental conditions for better production of brooders, spawns, and fingerlings. By using our advanced technique, aquaculturists can produce seeds for commercial purposes in a small place. Even though many researchers have been working on the sustainable production of Singhi, there are still obstacles that prevent the marginal farmers from producing seeds at mass scale. Policymakers and institutes should look into the overall production and

social upliftment of the farmers for the betterment of the Aquaculture sector.

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