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Induced breeding of medicinal fish *Heteropneustes fossilis* by using ovatide

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Abstract

The present study explores the induced breeding of *Heteropneustes fossilis*, a commercially and medicinally valuable freshwater catfish, using the synthetic hormone Ovatide under controlled hatchery conditions. Conducted between July and December 2022 at the Department of Zoology and Applied Aquaculture, Barkatullah University, Bhopal, the breeding trials were carried out during the monsoon season. Healthy, mature brooders were sourced from local markets and departmental ponds, then acclimatized and maintained for two months in treated tanks. Water quality parameters were regularly monitored and optimized for breeding. Brooders were fed a nutritionally rich artificial diet and managed in tanks with aquatic vegetation (Hydrilla and Pistia) to support egg adhesion. Brooders exhibited growth during the management phase, with females reaching 40–60 g and males 30–50 g. Hormonal induction was performed with intramuscular injections of Ovatide at dosages of 1 ml for females and 0.5 ml for males, followed by hand stripping and artificial fertilization. Fertilized eggs were incubated under controlled conditions with continuous water flow and oxygenation to enhance survival and hatching rates. Results showed successful fertilization (80%) and survival (50%), with eggs adhering to aquatic weeds and developing under incubation. The study demonstrated that induced breeding using Ovatide is an effective method for the mass production of *H. fossilis* seed. This technique ensures a reliable and timely supply of quality fish seed, offering a strategic approach for the conservation, commercial cultivation, and sustainable management of this important indigenous species.

Keywords; Induced breeding, Heteropneustes fossilis, ovatide.

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1 Introduction

Heteropneustes fossilis (Bloch, 1794), commonly known as the singhi or stinghi catfish, belongs to the sub-order Siluroidei and family Sacchobranchidae. It is considered a highly suitable species for aquaculture due to its commercial importance. In the Indian subcontinent, this species is highly valued by consumers for its nutritional and medicinal properties, often commanding premium prices. It makes up a significant share of India's air-breathing fish production, which currently relies largely on capture fisheries (Nayak et al., 2018). However, the lack of readily available seed has posed a major challenge for its cultivation. To address this issue, the ICAR-Central Institute of Freshwater Aquaculture has successfully developed a standardized breeding and seed rearing technique for *H. fossilis* to meet increasing demand among fish farmers (Sahoo et al., 2018). The species is typically found in ponds, swamps, ditches, and marshes, and can also survive in muddy river environments. It is an omnivorous fish and can tolerate mildly brackish waters. Singhi generally spawns in confined waters during the monsoon season, but it can also breed in derelict ponds and ditches with sufficient rainwater (Kiran et al., 2016).

Notably, the singhi catfish can inflict a painful sting on humans through a venom gland located on its pectoral fin spine. The species can grow up to 30 cm in length and holds considerable value in local fisheries, aquaculture, and the ornamental fish trade (Rainer & Pauly, 2011). Its rapid growth, adaptability to dense stocking, resilience in oxygen-deficient water, low fat content, high protein and iron levels, and medicinal benefits make it an ideal candidate for aquaculture (Rahmatullah et al., 2016). Farming this species not only supports increased fish production but also contributes to the conservation of its declining wild populations. In Bangladesh, *H. fossilis* is known as shingh and is a widely favored air-breathing catfish. Its popularity among farmers has been growing steadily due to its profitability and hardy nature. However, the degradation of natural habitats has made it increasingly difficult to source fry from the wild. Though capture fisheries were the primary source of fry and fingerlings in the past due to limited hatchery infrastructure, the advancement of induced breeding techniques has significantly improved seed availability in Bangladesh. Despite this progress, hatchery management challenges such as poor practices, genetic depression, and unintended hybridization have affected seed quality.

Induced breeding has become a vital component of aquaculture, especially in composite fish farming systems (Saha, 1995). Hormonal spawning techniques play a critical role by influencing the reproductive process at multiple stages, either enhancing or suppressing it. There are two primary strategies in induced breeding: the manipulation of environmental conditions to replicate natural spawning cues and the administration of reproductive hormones or synthetic analogs through injections or diet. These strategies are often used together for greater effectiveness. Several hormones have been used successfully in aquaculture, but recent research and commercial practices highlight two main approaches as being the most cost-effective and efficient: (i) the use of GnRH analogs combined with dopamine antagonists, and (ii) injections of purified gonadotropins, such as human chorionic gonadotropin (hCG).

Understanding the embryogenesis of *Heteropneustes fossilis* is crucial for improving artificial propagation, growth rates, farming practices, and understanding species-specific biological traits and environmental requirements (Borcato et al., 2004). The early life stages—embryonic and larval—are particularly sensitive to environmental changes and are vital for studying developmental biology (Verreth et al., 1992). Gaining insights into these stages is essential for enhancing larval survival and growth and for optimizing aquaculture systems. Consequently, the present study aimed to explore the detailed embryonic and larval development of *H. fossilis* under controlled hatchery conditions.

A. Methods and Materials

1. Study area

The present study area was conducted in July-December 2022 at the Department of Zoology and Applied Aquaculture, Barkatullah University, Bhopal, M.P., in the hatchery. Breeding trials of *Heteropneustes fossilis* were performed during the monsoon season.

2. Tank preparation and Collection of brooders

Firstly, the tank was collected and wash it with water also wash it with KMnO4 and then washed it again with water for cleaning purpose in other to prevent the germs or any kind of bacteria. It has been collected from Kasturba market and from ponds in the department with the help of hand-net or small basket. From the market it was carried out by handling and kept them in hundies or basket, it is easy process to carried brooders from market to Department because its air breathing catfish they can survive more than 16 hours without water. Also, it was again treated by KMnO4 for 2 minutes per each brooder. So, as to keep them and control from stress or any kind of infection. After that it has been released out into the tank for two months (July – August).

3. Brood collection

The brood fish for the artificial breeding of *H.fossilis* were obtained from the respectively fish market. And a total of 25 brooders were stock in the tank. By which the mature female fishes can be identified through big-jelly like type and swollen abdomen where, as mature male fish is flattened and pointed genital papillae All the brood-stocks were acclimatized before the induced breeding procedures and were kept separately in tank.

4. Brood stock management

In the experiment of the breeding period, brood stock management has been done for 2 months (July-August, 2022)

I. **Water quality:** During the experimental period different physical and chemical parameters (flow rate, temperature, pH, dissolved oxygen, free carbon dioxide, hardness and alkalinity, nitrate, nitrite, total ammonium, phosphate, iron and calcium) of brooders tank water and hatchery water were analyze as per the standard methods. Water is the most deciding crucial parameters which is very important for fish breeding.

i. **Feeding methods:** The brood fishes were fed on supplementary died from rice bran 20%, mustard oil cake 4%, 1% vitamin premix, earthworm, snail, white portion of boil eggs etc. the brooders were also treated with cow dung and soil manure respectively.

ii. **Length and weight measurement:** During the month of July and August the fish weight has been attained 40-60 gram for female and 30-50 grams male, and the length has measured with scale approximately 19-23 cm.

Aquarium preparation: Two aquariums were prepared before, that is breeding tank and control tank.

- **Breeding tank:** from the breeding tank brooders after injected were keep together both males and females so that they can reproduced along with the aquatic weed (ceratophylum & pistia), as eggs of *H. fossilis* is adhesive and cannot seen by naked eyes.
- **Control tank:** From the control tank brooders after they release (eggs and sperm), were collected it in the breeding tank and transfer into the control tank, as *H. fossilis* is a carnivores they can be eaten their own eggs.

iii. **Hormones preparation:** Brooders were injected using the syringe injection made with 0.5 mm Ovatide and 9.5 mm distilled water in a 1 ml syringe, depending on the length and weight size of fishes.

iv. **Injected of brooders:** Brooders were injected in intra-muscular part it is generally given in the muscle below the tip of the dorsal fin, at a 45' angle. At evening time (7:30. 28-aug-2022) with a dosage of 1 ml for females and 0.5 mm for males at the ratio of 3:3 around 10-11 hours, kept with the unwanted plants (aquatic weed like hydrilla, pistia etc). It was injected again using the same dosage of hormones for both males and females around 5 hours, and further proceeded to stripping procedure.

v. **Stripping methods:** The female fishes were stripped using with hand and kept in a tray and male were incised and take out the sperm in a tray. Further, it was mixed both the milt and eggs in a tray with the help of feather and distilled water, washed it with water several times and kept in an aquarium for the incubation period. And then continuous showering water for oxygenation in order to fertilized and again further proceed to survival rate.

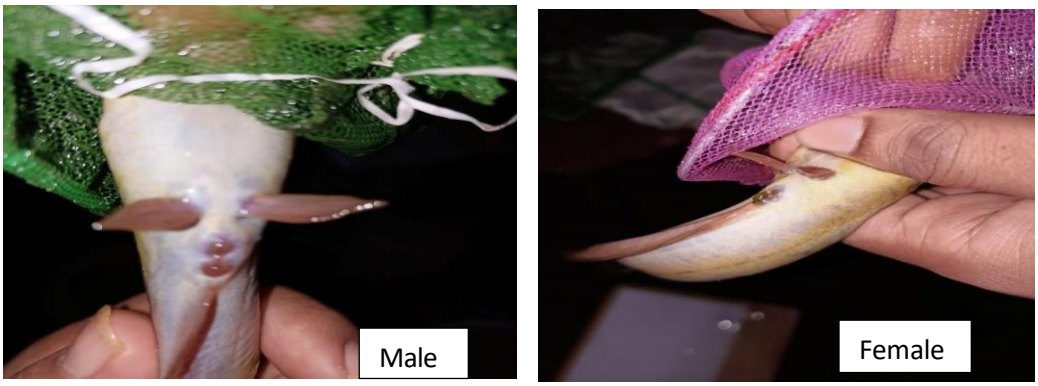

Figure 1 Showing mature Female & Male (*Heteropneustes fossilis*)

2 Results

a) **Water quality parameters:** All importance water quality parameters of different setup during breeding trials were monitored regularly and also kept recorded especially during brood stock management in two months (July-August 2022). These include: pH, Temperature, Alkalinity, Hardness, Carbon dioxide levels etc

Table 1 Physico-chemical parameters recorded of brooders during brood stock management (July-august 2022).

Parameters	Week 1 July	Week 2 July	Week 1 August	Week 3 August
pH	7	6	7	8
Temperature (°C)	30	28	29	28
Alkalinity (mg/L)	156	148	128	126
Hardness (mg/L)	110	126	126	132
Dissolve Oxygen (PPM)	5	5	4	5

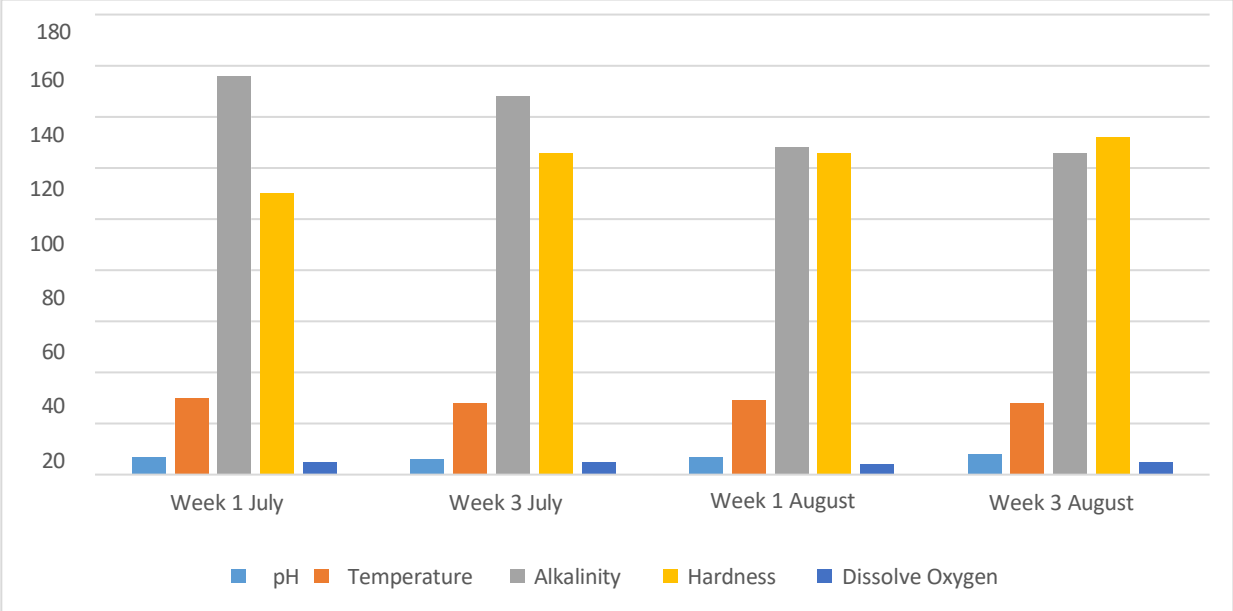

Figure 2

b) Length and Weight of the mature brooders

At the end of the brood stocking management, it was found that the brooders (Males and Females) grew higher than before collection. The length of the *H. fossilis* ranges 16-21 cm (both male and female), whereas, weight of the females is 40-60 grams and males 30-50 grams.

Table 2 Length and Weight of Brooder fish before injected (28-08-2022).

Sex	Length (cm)	Weight (g)
Female	21	57
Male	20	39

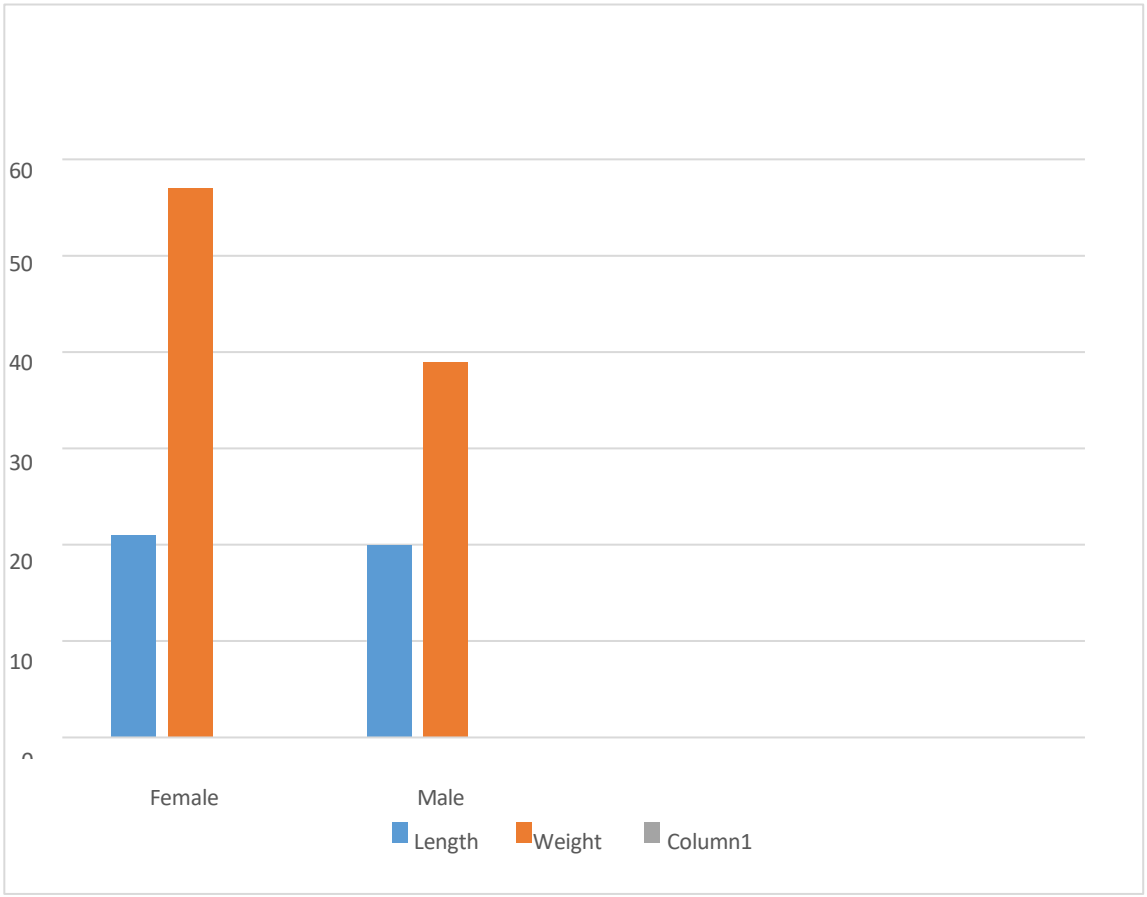

Figure 3: Showing variation of length and weight mature *H. fossilis* (Female and male)

c) Doses for females and males: From the study, ratio 3:3 were mature fish injected by using ovatide hormones. Brood fishes were feed in the brood rearing tank by provide artificial diet for good health and full maturation.

Table 3 Doses of ovatide hormones for female and male.

Species name	Sex	Number of species	1st dose (mm/g body weght)	Intervals	2nd dose (mm/g body weight)	Intervals	Hatching time (hours)
Heteropneustes fossilis	Female	3	1	11	1	5	72-78
-	Male	3	0.5	11	0.5	5	-

d) Fertilization rate and survival rate: From the experiment, the data has been found that

Table 4: Mean of fertilization rate (%) and survival rate (%). Whereas, the fertilization rate

Species	Ovulation rate %	Fertilization rate %	Survival rate %
Heteropneustes fossilis	75%	80%	50%

Survival rate was calculated by using the following formula:

a) Fertilization rate (%) = No. of fertilized eggs

   Total no. of eggs = 80%

b) Survival rate (%) = No. of survival fry

c) Total no. of hatching = 50%

3 Discussion

In the present investigation, the fertilization rate was recorded 80% with the survival rate of 50%. The current result is also supported by the report of Haniffa & Sridar (2002) on *Heteropneustes fossilis* where they recorded fertilization rate of 85% and survival rate of 60% respectively. However, in some earliest studies, there are reports of higher fertilization and hatching rate in *H. fossilis* (Rahman et al 2013). The variation in fertilization and hatching rate is might be due to difference in environmental conditions particularly water temperature and precipitation during the study period. From the study, the breeding pattern of induced breeding in *Heteropneustes fossilis*, it has been observed and learnt that it creates and important part for fish culture, by injection the fish using ovatide hormones. The use of this techniques is growing tremendously as expected in near future that there is every possibility of fish seed quality. Mostly, male brooder used to sacrifice during breeding because it contains very low of milt by incised and take out the sperm and mixed it with eggs, whereas females proceed by stripping. The dependence on artificial breeding of fishes will be felt all the more by apprehend that in future, will be improve more and more and the chance of procuring sufficient quantity of fish seed. It is the fact that the requisite for successful induced breeding is an assured supply of pure quality of fish seed. It has been found that it

has more profitability be utilized in using this technique to breed and obtain seed for fish farming. The experiment studied have been highly encouraging and hence show great promises for further purpose.

4 Conclusion

The use of induced breeding techniques presents new possibilities and advancements in the field of aquaculture. It enables the production of pure spawn for specific fish species under cultivation and allows for multiple breeding cycles within a single year. Unlike natural conditions where seed availability is unpredictable, induced breeding ensures a consistent and timely supply of quality seed, capable of meeting demand at any time. Further improvements in this technique can be achieved by carefully managing key factors such as temperature, water hardness, pH, circulation, and hormone dosage. It is also essential to use healthy broodstock and maintain suitable environmental conditions for both brooders and their offspring. This study contributes significantly to addressing the shortage of fish seed and supports strategic planning for the conservation and sustainable culture of valuable indigenous fish species in the future.

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