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A COMPARATIVE STUDY ON HORMONE OPTIMIZATION TECHNIQUES EMPLOYED IN CAPTIVE CONDITIONS FOR ARTIFICIAL BREEDING AND SEED PRODUCTION OF MAGUR (CLARIAS BATRACHUS)

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ABSTRACT

This review was meant to foster a brood supply of Magur (Clarias batrachus), counterfeit rearing, and seed creation under hostage conditions. Fifteen sets of brood C. batrachus were used in the trial. The farm raised the broodfish until they were mature enough to spawn by feeding them artificial food for four to five months prior to the beginning of the breeding season. In this analysis, the broodfish were actuated with WOVA-FH (1 ml.kg-1 body weight) and HCG (2272 IU.kg-1 body weight) hormones. The broods were given a single infusion of all the medicines. The review was directed at the initiated production of magur (Clarias batrachus) at Sanjeev Agrawal Global Educational (SAGE) University, Bhopal, from March to August 2022. Physically full-growth, sound, healthy male and female fish were collected from the brood stock lakes for prompt reproduction. The fish were then moved to clean, microbe-free molding tanks for 7-8 hours. During molding, a consistent glimmer of water was given by keeping the male and female isolated. The fish were then independently gauged and infused intramuscularly with WOVA-FH and HCG hormones. Only females were infused at a 45° point in the caudal locale. Approximately 23–24 hours postinfusion, the testicles from the male fish were removed and blended with stripping eggs for treatment. The brooding time frame for the prepared eggs was 23–30 hours for each of the medicines. The fertilization rates were 73.22±11.32 and 84.22±6.24%, and the hatching rates were 78.53±12.12 and 86.79±6.94% for the WOVA-FH and HCG treatments respectively.

INTRODUCTION

Catfish are found worldwide and are classified into four families: the Ictaluridae, Claridae, Pangasidae, and Siluridae. The catfish tradition of Southeast and South Asia have long been known. In some regions, catfish are highly valued, while in others, they are regarded as medium- and lower-value market species. Catfish are naturally hardy; they can go through extended periods of time without drinking and have a secondary respiratory system. In certain regions of Eastern India, there exists a specialized market for live fish commerce.

The Asian catfish, or *Clarias batrachus*, is the most significant species of catfish used in aquaculture. The Claridae family includes this group. *Clarias macrocephalus*, another significant species, is highly favor because of its appearance. Nevertheless, this topic has received the least attention because of poor growth performance and lack of seeds.

Typically, freshwater and brackish waters with reduced dissolved oxygen contents are home to claria species. Even under unfavorable environmental conditions, these bacteria can grow. While in Eastern India they are produced in somewhat renovated marshes, they are raised to market size in clay ponds. Because of its therapeutic benefits, *Clarias batrachus*, also known as magur in India, is highly valued and in high demand there.

The freshwater species known as magur are becoming less common these days. Magur catfish grow far more quickly than any other catfish. India is a highly populated country, and to meet the growing demand for protein, it is necessary to produce magur seeds through artificial propagation. Therefore, scientists studying fisheries devised a method for inducing breeding to produce high-quality fish seeds.

Numerous induced and natural occurrences in aquatic ecosystems have resulted in significant degradation of the natural breeding and feeding grounds of major floodplain and riverine fishes, as well as their habitats. The viability of open-water capture fisheries is threatened by factors such as shifting aquatic ecosystems, soil erosion, siltation, building drainage and flood control facilities, dumping of agrochemicals, and industrial pollutants.

Therefore, the most important elements in extending the practice of culture for this species are appropriate methods of induced breeding and larval rearing for large-scale production of fry. Despite some earlier research on its induction of breeding, the methods currently in use have not been standardized for farmers. Some attempts have been made to concentrate on these features in the current work. Air-breathing catfish, or *Clarias batrachus*, which belong to the Clariidae family, typically spawn between April and August, reaching a maximum length of 35 cm and a maximum weight of 250 g (1).

MATERIALS AND METHODS

Study location and duration

At my home, Contai, Purba Medinipur, Pin-721401, West Bengal were the sites of experiments for PhD purposes from March to August 2022.

Brood stock Management

To produce healthy fry and increase the survival rate, it is crucial to have a robust brood stock, particularly when kept in captivity. The first year is when *C. batrachus* reaches maturity. The earthen pond where the broodfish are raised has a stocking density of 2-3/m2. The broodfish are moved to a cement tank with a dirt base (2–3 cm) before the spawning season begins, to be conditioned, usually within two to three months. The broodfish in the commercial hatchery were placed in hapa in the same ponds so that they could be trained. A mixture of rice bran and groundnut oil cake, equal to three to five percent of the fish's body weight, was fed to the broodfish. To improve breeding performance, the fish were fed a combination of groundnut oilcake, soybean meal, rice bran or vitamin and mineral mixture at 32% protein concentration during gonadal development.

To keep the water in the culture system of high quality, 20 to 30% of the water was removed. Furthermore, it is best to prevent inbreeding as it lowers fry survival, growth rate, and deformities. By adding broodfish from the natural habitat or trading broodfish among farmers, this can be avoided. Secondary sexual traits can be used to distinguish between sexes. The female has a round, protruding abdomen and anal papillae that resemble buttons. The anal papilla of the male was pointed. Typically, magur breed from June to August.

While fish greater than 100 g are preferred, fish between 100 and 150 g are advised for optimal breeding results. A 150 g female broodfish should lay between 5000 and 6000 eggs.

Collection and selection of Fish for Reproduction

In the late morning, the broodfish were removed from the broodstock ponds and placed in plastic buckets filled with water. The fish were then allowed to breed. Mature male and female broods can be easily distinguished from one another based on secondary sexual traits.

- The monsoon breeder magur reaches sexual maturity at one year of age and typically breeds from June to August.
- The recommended size for broodfish used in induced breeding operations between June and August is 100–150 g.
- The female has a spherical and button-shaped genital papilla near the anus, whereas the male has an elongated, pointed papilla near the anus. When the female is fully mature, her abdomen bulges and her vent turns crimson, signaling that she is ready to spawn.
- A delicate, flexible catheter can be carefully inserted into the vent to assess the stages of female development. When the size of the eggs is between 1.2 and 1.4 mm, the female is ready to breed.
- For the purpose of induced breeding, the chosen brooders were housed in indoor tanks and divided according to their sex.

A **B**

Fig. 1. A) Male Brooder B) Female Brooder

Broodfish conditioning

The earthen pond where the broodfish are raised has a stocking density of 2-3/m2. The broodfish are moved to a cement tank with a dirt base (2–3 cm) before the spawning season begins, to be conditioned, usually within two to three months. The broodfish in the commercial hatchery were placed in hapa in the same ponds so that they could be trained.

Hormone preparation

All hormone (WOVA-FH and HCG) samples were collected from the local market and stored at room temperature. The production of hormones allowed easy handling of the brood fish. The fish were kept on foam during the injection, and a moist, soft cloth was placed around the fish's head. The females were then intramuscularly administered HCG (2272 IU/kg body weight) and WOVA-FH (1 ml/kg body weight) at a 45° angle to the caudal region. The females were then moved to the breeding tank following the injection.

Spawning, Fertilization and Hatching

The fish were artificially allowed to spawn in this experiment. To be stripped, the females were removed from the breeding tank. The mature eggs were removed from the bowl. Male testes were removed, sliced into pitches, and mixed with mature eggs to facilitate fertilization. Using the direct counting approach, the total number of eggs and the rate of fertilization were determined. A total of three 1.50-liter trays were employed for this objective. To calculate the rate of fertilization, approximately 300 eggs were placed on each tray. The fertilized eggs were then counted after being examined under a magnifying glass. Transparent eggs were regarded as fertilized after 12 to 15 hours of fertilization, but opaque eggs were regarded as unfertilized. A thin pipe was used for puncturing, and water was allowed through the pipe to keep the eggs under water during incubation and hatching. After they had been incubated for 22–24 hours, the eggs hatched, and hatchlings emerged. The hatchlings were counted via visual observation. Initially, the hatchling was removed with a syphon and placed in a white enamel bowl.

The fertilization rate was calculated by the following formula.

$$\text{Fertilization rate } (\%) = \frac{\text{Number of fertilized eggs}}{\text{Total number of eggs (Fertilized and unfertilized)}} \times 100$$

The following formula was used to determine the rate of hatching based on the number of hatchlings:

$$\text{Hatching rate } (\%) = \frac{\text{Number of hatchlings}}{\text{Total number of fertilized eggs}} \times 100$$

Analysis of Data

The gathered information was entered into a Microsoft Excel 2007 spreadsheet and is presented as the mean±standard deviation (SD) for each therapy. Tables and figures show the quantity of eggs released, the percentage of fertilized eggs, and the percentage of hatched eggs for *C. batrachus* under various treatments. Student's t test was used to examine how the treatments differed in terms of hatching rate, fertilization rate, and number of released eggs.

Table 1. Number of fertilized eggs, hatchlings and unfertilized eggs after hormone treatment.

Tray No.	Total eggs	Fertilized eggs		Hatchling		Unfertilized eggs	
		WOVA-FH	HCG	WOVA-FH	HCG	WOVA-FH	HCG
1st	300	208	246	160	211	92	54
2nd	300	235	251	189	219	65	49
3rd	300	216	261	169	228	84	39

Fertilization rate :- (Using WOVA-FH) Fertilization rate :- (Using HCG)

1st tray = (208 × 100)/300 = 69.33% 1st tray = (246 × 100)/300 = 82.00%

2nd tray = (235 × 100)/300 = 78.33% 2nd tray = (251 × 100)/300 = 83.67%

3rd tray = (216 × 100)/300 = 72.00% 3rd tray = (261 × 100)/300 = 87.00%

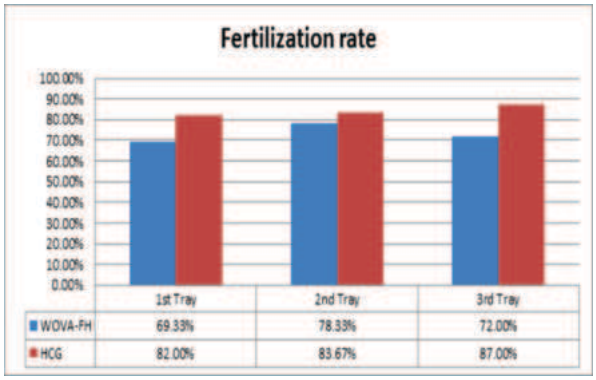

Fig. 2. Rate of fertilization after treatment with two hormones.

Hatching rate :- (Using WOVA-FH) Hatching rate :- (Using HCG)

1st tray = (160 × 208)/100 = 76.92% 1st tray = (211 × 246)/100 = 85.77%

2nd tray = (189 × 235)/100 = 80.43% 2nd tray = (219 × 251)/100 = 87.85%

3rd tray = (169 × 216)/100 = 78.24% 3rd tray = (228 × 261)/100 = 87.36%

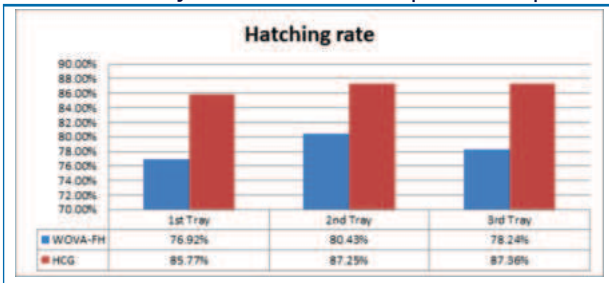

Fig. 3. Rate of hatching after treatment with two hormones.

Standard deviation of the fertilization rate when using WOVA-FH:
SD = √(384.67/3) = √128.22 = 11.32
The Standard deviation of the hatching rate was calculated using WOVA-FH:
SD = √(440.67/3) = √146.89 = 11.32
Standard deviation of the fertilization rate when using HCG:
SD = √(116.67/3) = √38.89 = 6.24
The Standard deviation of the hatching rate when using HCG, was calculated as
SD = √(144.67/3) = √48.22 = 6.94

Hypothesis analysis for fertilization rate:
Null hypothesis: There is no significant effect of WOVA-FH or HCG on the fertilization rate.

Alternative hypothesis: WOVA-FH and HCG have a significant effect on the fertilization rate.

WOVA-FH X1)	HCG (X2)	X1– X2 = D	D2
208	246	-38	1444
235	251	-16	256
216	261	-45	2025
		∑D= -99	$\sum D^2$=3725

$$\bar{D} = \frac{-99}{3} = -33$$

$$SD = \sqrt{(3725 - 3267)/2} = \sqrt{\frac{458}{2}} = \sqrt{229} = 15.13$$

$$SE = \frac{15.13}{\sqrt{3}} = \frac{15.13}{1.73} = 8.75$$

$$ItI = \frac{-33}{8.75} = 3.77$$

df= 3-1=2

The critical value at 0.05 when df=2 is 2.920 which is less than the calculated value, i.e., 3.77. This means that an alternative hypothesis is accepted.

Hypothesis analysis for hatching rate:
Null Hypothesis: There is no significant effect of WOVA-FH or HCG on the hatching rate.

Alternative Hypothesis: WOVA-FH and HCG have a significant effect on the hatching rate.

WOVA-FH (X1)	HCG (X2)	X1– X2 = D	D^2
160	211	-51	2601
189	219	-30	900
169	228	-59	3481
		∑D= -140	$\sum D^2$=6982

$$\bar{D} = \frac{-140}{3} = -46.67$$

$$SD = \sqrt{(6982 - 6533.33)/2} = \sqrt{\frac{448.67}{2}} = \sqrt{224.34} = 14.98$$

$$SE = \frac{14.98}{\sqrt{3}} = \frac{14.98}{1.73} = 8.66$$

$$ItI = \frac{-46.67}{8.66} = 5.39$$

df= 3-1=2

The critical value at 0.05 when df= 2 is 2.920 which is less than the calculated value, i.e., 5.39. This means that an alternative hypothesis is accepted.

RESULTS

Breeding behavior

Table 1 provides an overview of the length and weight of the broodfish utilized in the breeding studies, as well as information on egg production, fertilization, and hatching rate in response to various hormonal treatments. After hormone delivery for 6–10 hours, the *C. batrachus* brooders in each of the two treatment groups exhibit different coupling behaviors. Every female was partnered with a male, and vigorous courtship was used to advance the breeding process. As the female swam around the breeding tank, the male followed her. Females frequently swam up near the water surface prior to spawning. After hormone injection, the female was stripped of her eggs using a process called stripping, the males were dissected and their testes were removed from their bodies and mixed with 0.9% saline water to collect milt.

Breeding Efficiency

Table 1 shows the brooder size, number of released eggs, rate of fertilization, hatching rate and length of incubation for each treatment. T2 produced a considerably greater total number of eggs (6213) than did T1 (4235). Similarly, Table 1 shows that T2 had a greater fertilization rate (84.22±6.24) than did T1 (73.22±11.32). As a result, T2>T1 was the breeding performance of the various experimental treatments.

Treatment	Brooder Size		NER	FR(%)	HR (%)	LP (Hr)
	Male	Female				
T1(WOVA-FH)	190.62	203.12	4235	73.22±11.32	78.53±12.12	30
T2 (HCG)	197.34	210.41	6213	84.22±6.24	86.79±6.94	27

Note: NER= Number of eggs released, FR= Fertilization rate, HR= Hatching rate, LP= Length of incubation.

Table 2: Breeding performance of *Clarias batrachus* under two different treatments.

Figure 4 shows the number of eggs released/gm of body weight in relation to the hormonal treatments. T2 was found to release the most eggs/gm of body weight (29.53), followed by T1 (20.85), indicating that T2 was superior to T1.

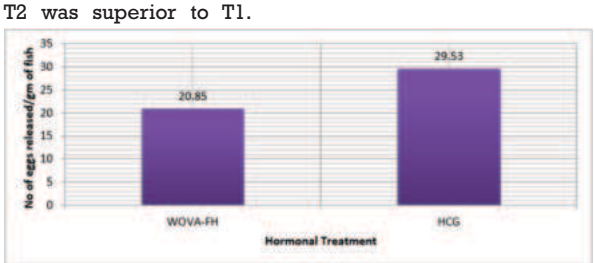

Fig. 4. The quantity of eggs released by *C. batrachus* in response to various hormones.

Figure 5 shows the *C. batrachus* hatching rate under the various hormone treatments. After WOVA-FH and HCG therapy, the average hatching rates were 78.53±12.12%, and 86.79±6.94%, respectively. T2 had the greatest hatching rate (86.79%) compared to T1 (78.53%) which was a considerable difference. As a result, the hatching rates for the various treatments were T2>T1.

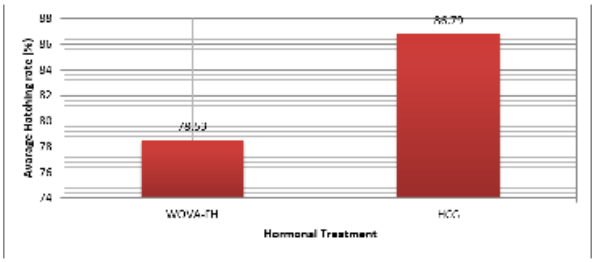

Fig. 5. The rate of hatching of *C. batrachus* in response to various hormones.

DECISION

In India, Bangladesh and in many other regions one of the most significant catfish species is *Clarias batrachus*. Because of their high market value, lucrative culture, and resilient character, fish farmers in Bangladesh and India are becoming increasingly interested (2). The artificial breeding of this species has become essential to the fry production process of hatcheries to produce high-quality fry. The goal of the current work on *C. batrachus* induced by breeding with WOVA-FH and HCG is to create an effective artificial breeding method for this species that will aid in the production of high-quality fry. To ensure the generation of high-quality eggs, fry, and fingerlings, proper care of the brood stock is essential (3). Before the breeding season began, the broods utilized in this experiment were meticulously cared for on a farm that provided artificial food.

The majority of the fish in this study reached adulthood between April and May; however, some fish reached adulthood earlier. Seeing the fish and eggs being handled and the breeding processes taking place at night is inconvenient for an individual. In other words, fish were injected between 5.30 and 6 p.m. throughout the current experiment. At 9:30 a.m., fourteen to fifteen hours later, the eggs were fertilized. The stripping of the fish began 20 hours after injection. In this experiment, fertilized eggs were incubated for 23–24 hours at a water temperature of 27–28°C following fertilization.

According to Khanom (2010) (4), the hatching time of *C. batrachus* eggs is between 23 and 24 hours at 27 to 28°C. Tomas PC et al. reported that the incubation duration was shorter than that in the present study, ranging from 16 to 19 hours at 28 to 30°C (5). A higher temperature could also be the cause of this. In the experiment, three replications of each trial were conducted after *C. batrachus* was triggered with the hormones WOVA-FH and HCG.

Numerous studies have been carried out to standardize the PG dose required for successful ovulation; nonetheless, there is still uncertainty surrounding the doses reported by different researchers (6). Regarding the dose optimization of the inducing substances, there are numerous references available in Bangladesh and other parts of the world concerning the induced breeding of *C. batrachus*. In light of this, the current study was carried out with the goal of inducing several hormones through which an evaluation of the seed production method could be performed. Inducing ovulation in *C. batrachus* was also reported to be successful at doses of 6 and 9 mg PG/kg (7). In *C. batrachus*, Hossain et al. (2006) reported that 10.0 mg PG/kg body weight at the first dose and 45.0 mg at the second dose successfully induced ovulation (8). However, as reported by Rao JB et al., 30–60 mg/kg body weight improved the breeding response of 140–260 g catfish (9). Using HCG at a rate of 4000 IU/kg of body weight during breeding also resulted in an experiment by Nase MN et al. (10).

In the present study, the rates of fertilization and hatching after WOVA-FH and HCG treatment were 73.22% and 84.22%, respectively, and 78.53% and 86.79%, respectively. In *C. batrachus*, 1.0-2.0 ml/kg of ovaprim was reported to result in a fertilization rate of 70.6-72.8%, which was lower than that in the present study (11). Like in the current study, Sahoo et al. (2005) reported an 83% fertilization rate for *C. batrachus* using 1.0 ml/kg of ovatide (12). The present study indicated that the calculated value of 't' was always greater than the tabulated value of 't' for both the fertilization rate and hatching rate when using the WOVA-FH and HCG hormones. Therefore, the null hypothesis is rejected, and the alternative hypothesis is accepted, which means that in this study, we used two different hormones, i.e., WOVA-FH and HCG, which have significant effects on magur breeding. HCG has a greater effect than WOVA-FH. The aforementioned explanation leads to the conclusion that the results of this study were broadly consistent with the results of the other experiments. The current study provided some information on the biology of the species *C. batrachus*, the substances and dosages used, and the breeding methods used. There is room for additional research to determine whether additional inducing chemicals work well enough to induce this species to proliferate.

CONCLUSION

For hundreds of years, people have been fishing for catfish for food throughout Africa, Asia, Europe, and North America. Eaters in Bangladesh and India consider catfish to be a favorite food. Overexploitation, other climate conditions, and habitat degradation all contribute to the progressive decline in *Clarias batrachus* abundance. The study's goal was to create an artificial breeding method using an inducing substance to address this issue. Of the three hormones, flash hormone produced the greatest results at water temperatures between 27 and 28°C in terms of fertilization (93.09%) and hatching (75.77%). Although the greatest percentage of hatching and fertilization rates attained in the present study may be regarded as satisfactory, there is still room for process improvement. Higher percentages of fertilized and hatched plants were obtained from a relatively lower dose of hormone in the current study on fish induction breeding. Given that cost and hormone quality are two of the most crucial considerations for a hatchery manager, this approach can be considered an efficient method of producing commercial seeds in hatcheries.

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Conflicts Of Interest

The authors have stated that there are no conflicting interests.

Author Contributions

Each author actively participated in the ideation, planning, data analysis, writing, and editing of the manuscript. The final manuscript was read and approved by all writers.

REFERENCES

1. Areerat S. Clarias culture in Thailand. Aquaculture. 1987; 63:355–362.
2. Saha MR, Mollah MFA, Roy PK. Growth and Survival of C. batrachus (Linn.) 324 Larvae fed on Formulated Diets. Bangladesh Journal of Fisheries Research. 1998; 2(2):151-158.
3. Robert RJ. A handbook of diseases of cultured Clarias (pladuk) in Bangkok, Thailand. National Inland Fisheries Institute. 1982; 37.
4. Khanom T. Study on Seed Production Technique of Indigenous Magur (C. batrachus), Shing (H. fossilis) and Pabda (Ompok pabda) Through Induced Breeding.
5. Thomas PC, Rath SC, Mohapatra KD, Pillay TVR. Breeding and seed production of finfish and shellfish. Daya Publishing House, Delhi. 2003; 121-122.
6. Thakur NK and Das P. Length weight relationship of H. fossilis (Bloch). Journal of Inland Fisheries Society of India. 1974; 6:95-96.
7. Nwadukwe FO, Ayinla OA, Abbey-Kalio NJ. Effects of various doses of acetone-dried powder carp pituitary extract and several changes of fecundity of *Heterobranchus longifilis*. In: JG Tobor and BIO Ezenwa (Editors). Proceedings of a seminar, 10th December, NIOMR, Lagos, Nigeria. 1991; 40-45.
8. Hossain Q, Hossain MA, Parween S. Artificial breeding and nursery practices of *C. batrachus* (Linn). Scientific World. 2006; 4:32-37.
9. Rao JB and SJ Karamchandani. On the spawning biology of *Ompok bimaculatus* from Kulgarhi reservoir of Modhya Pradesh. Journal of Inland Fisheries Society of India. 1986; 18(2):40-47.
10. Naser MN, Shafi M, Shah MS, Barua G. Development of a new methodology on the artificial propagation of catfish *C. batrachus* (L) by influencing some physio-chemical parameters of the water. Bangladesh Journal of Zoology. 1990; 18(1):23-31.
11. Srivastava PP, Raizada S, Dayal R, Chowdhury S, Lakhra WS, Yadav AK, 353 Sharma P, Gupta J. Breeding and larval rearing of Asian catfish, *C. batrachus* (Linnaeus,1758) on live and artificial Feed. Journal of Aquaculture Research and Development. 2012; 3(4):1-4.
12. Sahoo SK, Giri SS, Sahu AK. Breeding performance and egg quality of *Clarias batrachus* to evaluate ovatide doses (0.5, 1.0, 1.5 and 2.0 ml/kg body weight of female). Advanced Applied Scientific Research. 2005; 4(4):310-323.