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Enhancing breeding performance of *Osteobrama belangeri* (Valenciennes, 1844): Quantitative evaluation of synthetic hormone HATCHME® dosage and water quality parameters

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Abstract

The present study focused on enhancing the breeding performance of Pengba (*Osteobrama belangeri*) employing the synthetic hormone HATCHME®. Across all trials, twenty-seven brooders were equally allocated and configured at a male-to-female ratio of 2:1. Separation occurred randomly, placing the designated individuals into three distinct groups. Every group was given different dose of the inducing agent. The results demonstrated variations in reproductive performance under the varying experimental conditions. Notably, the best outcome was seen in Treatment 3, where females received 0.6 ml kg^{-1} body weight and males received 0.3 ml kg^{-1} body weight. Treatment 1 was ranked ahead of treatment 2, reflecting differences in reproductive outcomes linked to varying hormone doses. Detailed statistical analysis revealed strong correlations between improved breeding success and a combination of factors *viz*., fish size, hormone doses and key water quality parameters, notably temperature, pH, and dissolved oxygen levels. These important findings provide valuable insights for fish culturists seeking practical and sustainable solutions for successful induced breeding of *O. belangeri*, simultaneously promoting species conservation and leveraging its lucrative market potential after attaining marketable size.

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Introduction

Osteobrama belangeri (Valenciennes, 1844), commonly known as Belanger's barb, and locally referred to as 'Pengba' in Manipur, North-east India, is a medium-sized carp species. This species inhabits both fast flowing rivers and stagnant lakes in Manipur, with its distribution extending to Myanmar and China where it plays a crucial role both ecologically and economically (Basudha and Bishwanath, 1999). Pengba exhibits great promise for freshwater aquaculture diversification in India (Chanu *et al.,* 2018). Pengba gained prominence as it was declared as the state fish of Manipur in 2004 and is celebrated annually. As an integral part of local fisheries and aquaculture, conserving this species is vital for preserving biodiversity and supporting the regional economy (Devi *et al.,* 2009). However, breeding of *O. belangeri* is difficult due to specific reproductive requirements, making effective breeding and conservation a challenge.

Native to the Chindwin River of Myanmar, Pengba migrates for breeding purposes yet confronts numerous threats and classified as "Extinct in the wild" (CAMP, 1999); "Threatened" (Reddy, 2000); "Near Threatened" (Vishwanath, 2010) and "Endangered" (Suresh, 2000; Menon, 2004). Significantly, its once-substantial Loktak Lake fishery suffered a dramatic decline over 45 years because of dam construction obstructing migratory access between the Imphal River in Manipur and the Chindwin River in Myanmar (Angel *et al.,* 2015). Contemporary challenges include the restricted availability of quality fish seeds hindering the successful promotion of marine and freshwater species (Basavaraja, 1994).

Asiatic carp species are open-water breeders and cannot breed naturally in confined environments. Due to this limitation, large-scale aquaculture expansion is not possible, but technological advancement in induced breeding may be helpful for large-scale aquaculture production (Devi *et al.,* 2009). Recent advances in hormonal induction have transformed fish breeding practices, offering more reliable and efficient methods compared to traditional techniques. Historically, pituitary gland extracts were employed for inducing spawning; however, these methods face several limitations, including product variability, handling difficulties, and stress to broodstock (Khan and Ali, 2021). These challenges have prompted the development of synthetic alternatives that provide more controlled and less stressful breeding solutions. Among these innovations is HATCHME®, a synthetic Gonadotropin Releasing Hormone Analogue (SGnRH) developed by Finray Biotech Inc., Kolkata, India.

The efficacy of synthetic hormones in enhancing breeding outcomes has been demonstrated across various fish species. For instance, synthetic agents like Ovaprim and Ovatide have markedly improved spawning efficiency in Indian major carps and koi carp (Das, 2004; Ghosh, 2012; Gorre and Chari, 2023). Furthermore, comprehensive reviews highlight the potential of synthetic hormones to boost reproductive success and sustainability in aquaculture (Zamri, 2022; Chaube, 2023). Despite these advancements, the specific application of HATCHME® for *O. belangeri* has not been extensively studied, particularly in terms of optimal dosage and interaction with environmental conditions. Traditional breeding methods using pituitary extracts present challenges, including variability and stress. Recent advancements in synthetic hormones, such as HATCHME®, offer promising alternatives, yet its application in fish breeding remains underexplored. Unlike established synthetic hormones, HATCHME®'s effects on fish reproductive biology require further investigation to validate its consistency and efficacy. This study aims to address this gap by evaluating HATCHME® in optimising breeding efficiency for *O. belangeri*.

Understanding how hormonal dosages and environmental factors interplay is crucial for optimising breeding practices. Water quality parameters such as pH, dissolved oxygen (DO), alkalinity and hardness are known to significantly impact reproductive success and the health of broodstock (Tiwana and Raman, 2012; Surnar, 2015). Thus, examining how HATCHME® influences reproductive metrics like egg output, fertilisation rates and hatching rates with water quality is essential. *O. belangeri* naturally breeds in riverine environments during the south-western monsoons, primarily between June and August. Reproducing this process in controlled environments presents considerable challenges, underscoring the need for effective hormone-based induction methods (Behera *et al.,* 2010). The success of reproduction is highly dependent on maintaining specific environmental conditions, including water temperature, pH, and DO levels, which are critical for the survival of eggs and larvae hence, mastering captive or induced seed generation technologies becomes imperative for sustaining fragile species stocks, alongside fine-tuning breeding procedures geared towards mass seed output (Angel *et al.,* 2015). Consequently, mastering induced breeding techniques and optimising water quality management are crucial for sustaining this endangered species (Gupta *et al.,* 2008).

Our research aims to fill the gaps in current knowledge by evaluating the impact of various dosages of HATCHME® on the breeding efficiency of *O. belangeri*. The study aimed to assess how body weight and different hormone concentrations affect reproductive success, including fertilisation and hatching rates, while also exploring the influence of water quality parameters on these outcomes. We also aimed to evaluate the ability of this mountain stream fish to artificially breed and release eggs in captivity, while consistently controlling water quality parameters. This study will contribute valuable insights into synthetic hormone applications in fish breeding and offer practical recommendations for the conservation and sustainable aquaculture of *O. belangeri*. By improving our understanding of hormonal induction and environmental interactions, we seek to enhance breeding practices and support the effective management of this ecologically and economically important species.

Materials and methods

Study area and selected species

The present study on induced breeding of *O. belangeri* was conducted during 2020-2022 in the freshwater fish farm at West Bengal Comprehensive Area Development Corporation (WBCADC) in Tamluk, Purba Medinipur, West Bengal. The significant reason behind the selection of *O. belangeri* is that this species is endangered or near threatened and also the species has high food value and has a ready demand in the market (Basudha and Singh, 2022), along with finding out suitable doses of inducing agents on reproductive success by induced breeding technology.

Collection of mature fish and maintenance

Pengba is raised with other carps in earthen ponds and gains maturity after 2 years. Mature Pengba were collected from the earthen ponds of WBCADC using drag net in August and kept in the brooder tank (20,000 l capacity cemented tank with size of 4.2 × 3.3 × 2.3 m), but the volume of water during maintenance was 10000 l. The tank was covered with a net and aeration was fixed to a constant water flow.

The mature Pengba were fed twice a day with pelleted feed having 35% crude protein (Growel Feeds Pvt Ltd.) at 5% body weight, and conditions of the brooder were monitored regularly (Das *et al.,* 2016). Broodstock care improved ova quality, gonadal development and fertility. After two weeks of maintenance, the brooders were ready for spawning and control over broodstock maintenance is critical for commercial aquaculture seed production.

Selection of brooders

Females of *O. belangeri* are larger than males and and can be distinguished by rubbing the pectoral fin, females feel smooth while males feel rough (Angel *et al.,* 2015). The males and females were selected at a ratio 2:1 in each treatment (Table 1).

Table 1. Summary of the results of reproductive parameters of *O. belangeri* in different treatments

Treatments	Sex	Length (cm)	Weight (g)	Doses (ml kg^{-1} body weight)	Latency period (h)	Incubation period (h)
T1	F	24.4±0.09	222±1.15	0.4	8	21.2±0.12
	M	17.8±0.09	141±1.32	0.2		
T2	F	23±0.30	212.7±2.91	0.5	9	22.3±0.18
	M	17.5±0.14	137±1.41	0.3		
T3	F	25.2±0.09	230.7±1.76	0.6	7	20.2±0.2
	M	18.5±0.13	149±1.53	0.3		
p value	F	0.0005	0.003	-	-	0.0004
	M	0.0001	0.0001	-	-	

Values are expressed as mean ±SE, n=27
*Values bearing different superscripts within columns differ significantly (p<0.05)

Hormone administration

After brooder selection, the SGnRH-based synthetic hormone, namely HATCHME®, was selected as an inducing agent for the induced breeding of Pengba. The hormone comprises, Synthetic Gonadotropin Releasing Hormone Analogue (SGnRH): 0.002%, Domperidone: 0.998% and Propylene Glycol: 99%. FRP-made circular tank (500 lcapacity with the size of 2 ft × 1.6 ft) was used for each treatment. The synthetic hormone was prepared by diluting with distilled water and administered intramuscularly to avoid stress due to injection (40 units = 1 ml) near dorsal fin above the lateral line in each treatment at the sunset time (17.30 hrs).

Analysis of water quality parameters

The physico-chemical parameters such as temperature, pH and salinity were assessed using a multi- parameter analyser, while DO, alkalinity and hardness were examined in the laboratory following APHA (2005) methods.

Latency period

The latency period is described as the time after the injection up to the laying of eggs. Latency period = Injection time - Egg release time (Chakrabarty *et al.,* 2016). Spawning time varies depending on the doses of injection. After the release of eggs, males and females are removed by a hand net from each treatment and released into the pond.

Fertilisation and hatching

To estimate fertilisation rates, the total number of eggs laid by females was first determined. This was achieved by collecting 3 to 4 water samples from the breeding tank using a standard mug. The number of eggs in each sample was counted and the mean egg count per mug was calculated. This average was then multiplied by the total volume of water in the breeding tank to estimate the total number of eggs laid. The same procedure was employed to estimate the number of fertilised eggs.

The fertilisation rate and hatching rate were subsequently calculated using the formula described by Islam *et al.* (2016):

$$\text{Fertilisation rate (\%)} = \frac{\text{No.of fertilised eggs}}{\text{Total no. of eggs}} \times 100$$

After 20-22 h, the fertilised eggs hatched and the hatching percentage was calculated and recorded by the same sampling method as described for fertilised eggs:

$$\text{Hatching rate (\%)} = \frac{\text{No. of hatchlings}}{\text{Total no. of fertilised eggs}} \times 100$$

Data analysis

The data from different treatments were analysed by using one-way ANOVA in IBM SPSS version 22.0 for Windows to find out the significant differences (p<0.05) between the treatments and the correlation coefficient was analysed using Python software (version 3.12 for Windows). The experimental data are presented as mean±standard error (SE).

Results

In the present experiment, various reproductive parameters and behavioural responses were observed following the administration of synthetic hormones. Table 1 presents the comparative data on length (cm), weight (g), doses (ml kg^{-1}), latency period (h) and incubation period (h) across treatment groups.

Breeding behaviour

It was noted that the mating behaviour of the brooders did not start immediately after hormonal induction. Instead, it commenced roughly 2-3 h following the hormonal injection, featuring males actively pursuing females while engaging in pair formation.

Egg output, fertilised eggs and hatched larvae

The latency period for ovulation varied among the treatment groups, with egg release occurring at 8 h in T1, 9 h in T2 and 7 h in T3 after hormone administration (Table 1). The mean number of eggs released by the females was found higher in T3 (50931±747), followed by T1 (46716±1061) and T2 (41900±578), respectively (Fig. 1). Similarly, the mean number of fertilised eggs was found higher in T3 (42216±1246), followed by T1 (36613±1246) and T2 (31857±923), respectively (Fig. 1). The trend continued with hatching, where T3 produced the highest number of larvae (33,851±1,216), followed by T1 (27,854±1,370) and T2 (23,489±1,059), respectively (Fig. 1). Statistical analysis revealed significant differences ($p<0.05$) in egg output, number of fertilised eggs and number of hatched larvae among the treatment groups.

Fertilisation and hatching rate

The mean fertilisation rate of the eggs was found higher in T3 (82.7±0.88%), followed by T1 (78.3±0.89%) and T2 (76±1.15%), respectively (Fig. 2). The duration of incubation period was shortest in T3 (20.2±0.2 h), followed by T1 (21.2±0.12 h) and T2 (22.3±0.18 h), respectively. Similarly, the mean hatching rate was significantly higher in T3 (80.3±0.82%), followed by T1 (76.0±1.15%). Statistical analysis revealed significant differences in both fertilisation and hatching rates among the treatment groups ($p<0.05$).

Water quality parameters

A small degree of variance in water quality parameters was observed in all treatments. Table 2 shows that no statistically significant difference was found in water temperature across treatments ($p>0.05$). However, significant difference ($p<0.05$) was shown in pH, dissolved oxygen (DO), salinity, alkalinity and hardness between treatment groups (Table 2). The mean values of temperature, pH, DO, alkalinity and hardness were slightly higher in T3, followed by T1 and T2, except salinity, which was highest in T2 (Table 2). That could be the reason why the reproduction success rate was higher in T3 than in the remaining two treatments. To ensure the effects of water quality parameters on reproductive success, a correlation analysis was performed. A summary of the physico-chemical parameters measured in each treatment group is presented in Table 2.

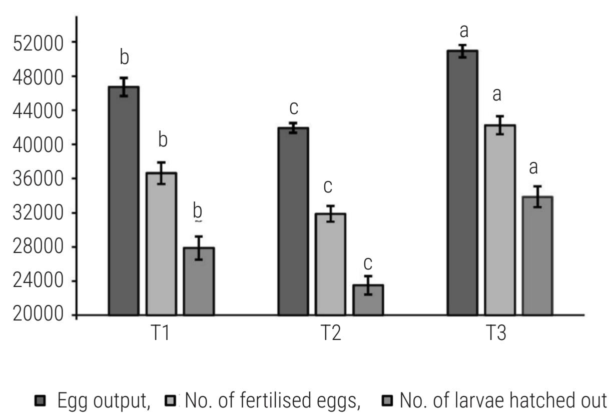

Fig. 1. Egg output, no. of fertilised eggs and no. of larvae hatched out in treatments

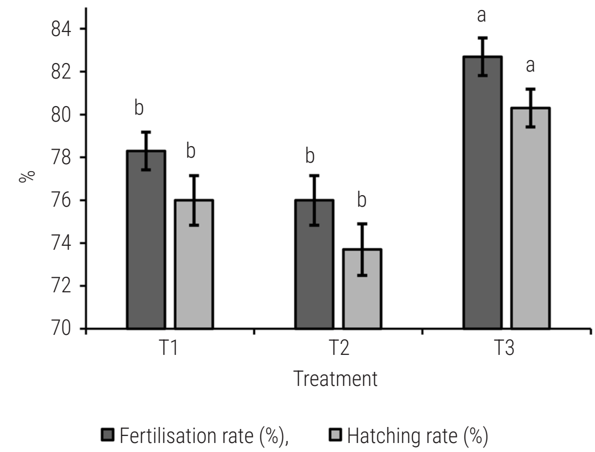

Fig. 2. Fertilisation and hatching rate in treatments

Correlation analysis of brooder weight, hormonal dose and water quality parameters with reproductive parameters

The statistical correlation analysis (Fig. 3) revealed several significant relationships between the studied parameters. Brooder weight showed a very strong positive correlation with egg output (r =0.86), no. of fertilised eggs (r = 0.84) and no. of larvae hatched (r = 0.82). It also had a very strong negative correlation with latency period (r = -0.93) and incubation period (r = -0.88). Hormonal dosage exhibited a moderate positive correlation with egg output (r = 0.44), no. of fertilised eggs (r = 0.50) and no. of larvae hatched (r = 0.53), while showing a moderate negative correlation with latency period (r=-0.50) and incubation period (r = -0.47). Temperature showed a moderate negative correlation with latency (r= -0.54) and incubation (r = -0.51) periods, while a moderate positive correlation with egg output (r = 0.59), no. of eggs fertilised (r = 0.58) and no. of larvae hatched (r=0.59). pH demonstrated a very strong negative correlation with latency period (r = -0.83) and a strong negative correlation with incubation period (r = -0.72) while a strong positive correlation with egg output (r = 0.75), no. of fertilised eggs (r = 0.73) and no. of larvae hatched (r = 0.71). DO showed a very strong negative correlation with both latency (r = -0.98) and incubation (r = -0.98) period and very strong positive correlation with egg output (r = 0.97), no. of fertilised eggs (r = 0.96) and no. of larvae hatched (r = 0.95). Alkalinity also had a very strong negative correlation with latency (r = -0.85) and incubation (r = -0.89) period along with

Table 2. Water quality parameters recorded during the experimental period. Values are expressed as mean±SE (n=6)

Parameters	T1	T2	T3	P value
Water temperature (°C)	27.9±0.18	27.8±0.28	28.0±0.17	0.68
pH	$7.91±0.01^b$	$7.82±0.01^c$	$7.96±0.02^a$	0.0002
DO (mg l^{-1})	$7.4±0.03^b$	$7.2±0.06^c$	$7.5±0.05^a$	0.00002
Salinity (PPT)	$0.15±0.005^b$	$0.16±0.007^a$	$0.13±0.005^c$	0.0006
Alkalinity (mg l^{-1})	$127±1.11^b$	$124±0.82^b$	$131±1.29^a$	0.004
Hardness (mg l^{-1})	$140±1.75^b$	$136±0.96^b$	$148±1.71^a$	0.001

*Values bearing different superscripts within rows differs significantly ($p<0.05$) except water temperature.

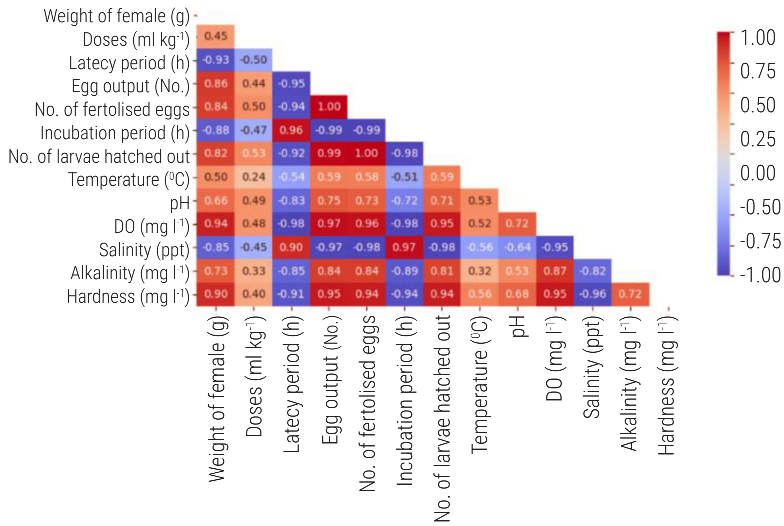

Fig. 3. Correlation analysis of brooder weight, hormonal dose and water quality parameters with reproductive parameters

very strong Positive correlation with egg output (r=0.84), no. of fertilised eggs (r = 0.84) and no. of larvae hatched (r = 0.81). Water hardness showed a very strong negative correlation with latency (r = -0.91) and incubation (r = -0.94) periods and a very strong positive correlation with egg output (r= 0.95), no. of fertilised eggs (r = 0.94) and no. of larvae hatched (r = 0.94). These findings underscore the critical influence of brooder size, hormone dosage, and water quality parameters on the reproductive success of *O. belangeri*.

Discussion

The use of the synthetic hormone (HATCHME®) to enhance the breeding efficiency of *O. belangeri* (Valenciennes, 1844) has gained significant attention for its potential benefits in both conservation and aquaculture. Our current study provides a comprehensive analysis of how body weight, varying hormone dosages and changes in water quality parameters affect reproductive success in induced breeding. Our findings reveal a strong correlation between body weight, hormone dosage and reproductive outcomes. Among various treatments, treatment 3 (T3) proved most effective. The optimal HATCHME® dosage in T3 (0.3 ml kg^{-1} for males and 0.6 ml kg^{-1} for females) yielded the best reproductive outcomes, with an average egg output of 50,931±747, fertilisation rate of 82.7±0.88% and hatching rate of 80.3±0.82%. A reduced latency period of 7 h and a shorter incubation period of 20.2 h further validated the efficacy of this dosage, suggesting that specific hormone levels can significantly enhance reproductive performance of *O. belangeri*. This result corroborates the observations of Das *et al.* (2016), who reported improved breeding performance in *O. belangeri* with similar hormone treatments. Devi *et al.* (2009) used Ovatide at doses of 0.6 ml kg^{-1} for females and 0.3 ml kg^{-1} for males, achieving a fertilisation rate of 95%, a hatching rate of 85% and a latency period of 8 h, with hatching occurring at 26 h under 28-29°C. Compared to our treatments, the doses and latency period are almost similar, but the temperature, incubation period, fertilisation and hatching rates are slightly higher than those in our treatments. Another study reported that Gonopro-FH at 0.7 ml kg^{-1} in females and 0.4 ml kg^{-1} in males, with a latency period of 9 h and an incubation period of 19 h at 25°C, achieved more than 90% fertilisation rate and 98% hatching rate and was found to be highly efficient in the induced breeding of Pengba (Das and Singh, 2017). The doses, fertilisation and hatching rates are significantly higher than those in our treatments, although the latency period is similar to T2, while the temperature is slightly lower than in our treatments. Angel *et al.* (2015) maintained male and female brooders of Pengba separately and achieved fertilisation and hatching rates by using Ovaprim 84 and 84%, Ovatide 79 and 75% and Gonopro-FH 84 and 86% respectively with an 8 h latency period and a 22 h incubation period at 26-28°C, respectively. In comparison to our treatments, the brooders were maintained together in a cement tank; fertilisation and hatching rates by Ovatide were similar to T2, but were higher with the other two inducing agents (Ovaprim and Gonopro-FH) than in our treatment. In our study, the fertilisation and hatching rates were found to be higher at doses of 0.3 ml kg^{-1} for males and 0.6 ml kg^{-1} for females (T3), followed by 0.2 ml kg^{-1} for males and 0.4 ml kg^{-1} for females (T1) and 0.25 ml kg^{-1} for males and 0.5 ml kg^{-1} for females (T2). These findings are similar to those of Das *et al.* (2016), who conducted artificial breeding using three different inducing agents (Ovatide, Ovaprim, Wova-FH) in the induced breeding of Pengba. Behera *et al.* (2010) reported that Wova-FH was highly effective in the induced breeding of Pengba at doses of 0.5 ml kg^{-1} for females and 0.3 ml kg^{-1} for males, achieving a fertilisation rate above 90% and a hatching rate of 85% with a latency period of 7 h and an incubation period of 14-16 h. In comparison to our treatments, the doses are similar to T2 and the latency period is similar to T3, but the fertilisation and hatching rates are higher than in all our treatments. Biswas *et al.* (2019) maintained Pengba broodfish in a tank for about one week, feeding them a pelleted diet with 25-30% protein and an average brood weight of 150-200 g and used Ovaprim as an inducing agent at a dose of 0.25 ml kg^{-1} body weight for males and 0.5 ml kg^{-1} body weight for females, with hatching occurring 18-20 h at 26-30°C post-fertilisation. Compared to our study, the duration of broodfish maintenance was one week longer in their study and the protein percentage in feed was also higher (35%) in their study. However, the doses, temperature and incubation period were slightly similar to our study. Behera *et al.* (2015) collected Pengba brooders from Loktak Lake and maintained them in earthen ponds. Subsequently, mature brooders were collected from the earthen ponds by repeated netting and induced by Wova-FH at doses of 0.6 ml kg^{-1} for females and 0.3 ml kg^{-1} for males, with chasing behaviour commencing 5-6 h post-injection and hatching occurring 18 h after fertilisation at 24-26°C. Compared to our study, Pengba was raised in earthen ponds along with other carps until maturity; after that, mature brooders were collected by netting and maintained in a cement tank for about two weeks for gonadal development and timing of maturation. However, the doses were similar to T3, but the chasing behaviour started 1-2 h later than in our study, while hatching occurred 2-3 h earlier at a lower temperature than in our study. Angel *et al.* (2015) recorded optimal physico-chemical water parameters such as, water temperature of 26-28°C, DO 7-7.5 mg l^{-1}, alkalinity 110-130 mg l^{-1} and pH 7.5-7.9 during the captive breeding of Pengba. Biswas *et al.* (2019) reported that the optimum water quality parameters during larval rearing were a water temperature of 24-30°C, DO >4 mg l^{-1}, alkalinity 80-120 mg l^{-1} and pH 7.5-8.0, which is very similar to our study.

Water quality parameters also played a crucial role in reproductive success. Although there were no significant differences in water temperature across treatments, variations in pH, dissolved oxygen (DO), salinity, alkalinity and hardness were notable ($p<0.05$). T3 exhibited slightly elevated levels of pH, DO, alkalinity and hardness, which were associated with superior reproductive outcomes. These findings are consistent with previous research emphasising the critical importance of maintaining optimal water quality for successful induced breeding (Gupta *et al.,* 2008). Correlation analyses further highlighted the strong relationships between water quality parameters and reproductive success metrics. DO showed a very strong positive correlation ($r = 0.97$) with egg output and a very strong negative correlation ($r = -0.98$) with latency and incubation periods. Similarly, pH, alkalinity and hardness demonstrated strong positive correlations with reproductive success, underscoring the importance of maintaining optimal levels of these parameters to enhance reproductive efficiency. Despite the lack of significant differences in temperature across treatments, T3 showed a positive correlation between temperature and reproductive parameters, suggesting that temperature may play a nuanced role within the broader context of water quality and improve the reproductive performance. This observation aligns with the work of Rajts *et al.* (2023), which highlighted the benefits of hormonal induction in improving reproductive outcomes for small indigenous fish species. The findings also support Sarma and Mohan (2024), who emphasised the necessity of optimising environmental conditions for the sustainable aquaculture of coldwater fishes, including *O. belangeri*. Further reinforcement comes from Gurjar *et al.* (2023), who discussed sustainable management practices for coldwater fish diversity in India. Behavioural observations from our study indicate that mating behaviour, which commenced approximately 2-3 h after hormone injection, is essential for successful spawning. This finding is corroborated by Biswas *et al.* (2019), who reported similar spawning behaviours in response to hormonal treatments. Iqbal *et al.* (2021) reported that, in induced breeding of carps, temperature is the most important water quality parameter which influences the reproductive parameters such as ovulation rate, spawning response, fertilisation and hatching. Gupta *et al.* (2008) suggested that the optimum water quality needs for IMC seed production through induced breeding are 24-29°C temperature, 6.5-8.5 pH, 5.0-6.0 mg l^{-1} dissolved oxygen and less than 150 mg l^{-1} total alkalinity, . These conditions align with the recommendations of Jayasankar *et al.* (2018), who emphasised the importance of maintaining optimal water conditions for successful freshwater aquaculture. Mohapatra *et al.* (2018) reported that temperature ranges of 27-34°C reduced the spawning response time and significantly improved fertilisation and hatching rates in induced breeding of rohu (92.7%) and mrigal (97.5%) while other water quality parameters *viz.,* DO (3.0-5.5 mg l^{-1}), pH (7.2-8.25), total alkalinity (54.0-80.0 mg l^{-1}) and total hardness (46.0-94.0 mg l^{-1}) were found to be not optimal. However, the results clearly indicate that temperature and pH are the most critical water quality parameters influencing the induced breeding of carps. Chakrabarty *et al.* (2016) reported that elevated temperatures reduced the spawning response time (latency period) and enhanced fertilisation as well as hatching rates in carps. Their findings showed that higher temperature ranges (28.3-32.6°C) and a pH of 7.1-7.2 rapidly improved reproductive performance in *L. rohita,* even when other water quality parameters were below optimum levels. Similarly, in our study, increased temperature and pH were found to significantly influence key reproductive parameters in *O. belangeri.* Effective brooder management, including well-aerated tanks and optimal water conditions, significantly enhanced reproductive performance, in line with recommendations by Sahoo *et al.* (2023). The study by Laskar *et al.* (2024) provided a valuable framework for understanding the taxonomic and distributional issues affecting *Osteobrama* species in India, emphasising the importance of accurate species identification in breeding programs. Additionally, the integrative approach of Debnath and Yengkokpam (2024) highlighted the importance of conservation practices for floodplain wetlands, which are crucial habitats for *O. belangeri*. Statistical analysis revealed strong positive correlations between brooder weight and reproductive outcomes, such as egg output and hatching rates and negative correlations with latency and incubation periods. These correlations reinforce the findings of Das *et al.* (2022) and Rakkannan *et al.* (2023), highlighting the role of optimal broodstock weight and water quality in improving reproductive efficiency.

Our findings demonstrate a significant improvement in the breeding efficiency of *O. belangeri* through the precise application of HATCHME® and optimised water quality parameters, setting a new benchmark in reproductive enhancement. This study offers valuable insights into the combined effects of synthetic hormone dosages and water quality on breeding success of *O. belangeri*. The results contribute significantly to the understanding of induced breeding techniques and offer practical guidance for the conservation and sustainable aquaculture of this endangered species. Future research should focus on fine tuning the balance between hormonal treatments and environmental conditions to further improve breeding outcomes and support effective population management of *O. belangeri*.

The study revealed that induced breeding of the endangered species, Pengba, can be successfully achieved using the locally available synthetic hormone, HATCHME®. This approach offers a cost-effective and accessible option to other existing hormone treatments. Critical factors influencing reproductive success include the precise hormone dosage (0.3 ml kg^{-1} for males, 0.6 ml kg^{-1} for females) and proper regulation of water quality. Higher water temperatures >27°C, were found to enhance breeding success. Under hormone treatment, a single mature female of pengba can produce between 40,000-55,000 eggs during the peak breeding season. Key factors for improving reproductive outcomes include, selecting mature broodfish, administering accurate hormone dosages and maintaining strict control over water quality parameters. The use of induced breeding technology with HATCHME® enables fish farmers to adopt sustainable practices that support species conservation while also harnessing the commercial and nutritional benefits of pengba. Although the results are promising, further research is needed to conduct in-depth comparisons with other synthetic hormones and to assess their broader ecological impacts.

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