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Original Article

Comparative efficacy of different doses of inducing agent on breeding performance of a near threatened catfish pabda (*Ompok bimaculatus* Bloch, 1794) in West Bengal

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Abstract
In India, diversification in shrimp culture area is required due to climate changes, fluctuation in resources, and its marginal profit. Near Threatened pabda (*Ompok bimaculatus*) may be an alternative aquaculture species which can fulfil all the demands for the shrimp farmers. In this study, healthy, disease-free, 12-month reared brood fishes were collected and stocked in eight tanks (8×4×2 feet) sex wise (130 pairs) with shower facilities. Synthetic hormone was used to induce both male and female fishes at 0.3 and 0.6 ml (T1); 0.5 and 0.7 ml (T2) and 0.7 and 0.8 ml (T3) kg^{-1} body weight (bw) respectively, having 3 replicates for each treatment. Ovulation period (7 to 11 hours), relative fecundity (105.20±39.93 eggs g^{-1} bw of female), fertilization rates (67.37±20.73 to 75.88±24.84%), hatching rates (62.57±18.46 to 79.67±26.68%) and survival rates (55.01±14.93 to 65.62±19.91%) were observed. Among treatments, T2 exhibited better breeding performance but no significant differences with other treatments were observed. Important water quality parameters like temperature (28.27±1.68°C), pH (7.10±0.44), dissolved oxygen (4.25±0.37 mg L^{-1}), hardness (210.96±9.31 mg L^{-1}) and alkalinity (202.78±8.00 mg L^{-1}) were also recorded during this experiment. After 48 hours of hatching, hatchlings were reared in aerated tanks for another four days with close observation and fries were harvested 24 days of rearing. From the present experiment, T2 exhibited best effective dose for successful induced breeding of pabda.

Keywords: catfish; induced breeding; larval rearing; pabda; shrimp culture

1 | INTRODUCTION

Diversification in aquaculture is very much needed in today's aspect in the world, to harmonize with climate changes, fluctuation in resources and food security issues for the nations. Aquaculture is the world's fastest growing food producing sector (Bartley 2005), through diversification and more species integration in culture system, can enhance its production in sustainable way. India is having 15 to 20 commercial potential fish species but Indian major carps, shrimp, and prawn are only emphasized (Singh *et al.* 2019). More than 87% of fish production comes from carp culture, though there is enormous scope for species diversification, to reach that goal (GBIF Secretariat 2023). In India, primarily West Bengal had tried some trials on minor carps and minnows, included in polyculture systems (Ayyappan and Jena 2003; Roos *et al.* 2007) for better production but neglected many endemic fish species (NFDB 2016) that have much potential impact on both culture and conservation aspects. Changing from low value carps to high value fish species culture (Ahmed 2009; Saha 2003) and focusing on native fish species integration make profit maximisation (FAO 1999; Kohinoor *et al.* 1997). Pabda *O. bimaculatus* (Bloch 1794) is recently gaining importance as a promising candidate diversified catfish (Talwar and Jhingran 1991; Ponniah and Sarkar 2000; Ayyappan *et al.* 2001; Lakra *et al.* 2010).

Pabda is a non-airbreathing, potamodromous threatened catfish in siluroid of Indian sub-continent (IUCN 2011) having delicious taste, high quality flesh with soft bone, excellent nutritional profile and high in micronutrients (Roos *et al.* 2007; Halwart 2008; Beveridge *et al.* 2013; Alam *et al.* 2016; Biswas *et al.* 2018; Paul *et al.* 2018; Rawat *et al.* 2018; Dhar *et al.* 2019; Arambam *et al.* 2020; Biswas *et al.* 2022). They have elongated body and are laterally compressed with dorso-ventrally flattened head, snout rounded and have two pair of barbells; have superior mouth with lower jaw, caudal fin is forked with rounded lobes and pectoral fin with smooth spines (Talwar and Jhingran 1991). They are carnivorous in nature and found in freshwater with slow to moderate water flow, lotic areas like ponds, canals, lakes, shallow river and stream (Sridhar *et al.* 1998; Banik *et al.* 2011; Debnath *et al.* 2013) with plenty of floating and submerged aquatic weed which supports in both hideout and shelter (Chakrabarti *et al.* 2012). Pabda fish are found in several countries like Bangladesh, Pakistan, Sri Lanka, Nepal, Indonesia, Malaysia, Myanmar, Thailand, Vietnam and North-eastern states of India like West Bengal, Bihar and Odisha (Day 1981; Talwar and Jhingran 1991; Jayaram 1999; Chakrabarty *et al.* 2007; Jayaram 2009; IUCN 2010; Ng *et al.* 2010; Banik *et al.* 2011; Biswas *et al.* 2018; Dhar *et al.* 2019). Among in the genus *Ompok*, *O. bimaculatus* grow larger in size (NBFGR 2011) and therefore, called boalipabda. The pabda species is suitable for both monoculture and polyculture-based farming where 30% crude protein-based feed has to be applied (Debnath *et al.* 2015; Debnath *et al.* 2018).

This species is under high risk of extinction due to habitat loss, water pollution, ecological variations and is registered as endangered species in India (Ng *et al.* 2010; Sarma *et al.* 2012; IUCN 2019). To increase fish population in nature, captive breeding, (Molur and Walker 1998) an essential requirement for mass seed production, culture, management and conservation of fish population is required (Vijayakumar 2010). Successful induced breeding required sexual identification, that can be done by observing secondary sexual dimorphic characteristics (Rao *et al.* 1986; Banik *et al.* 2011; Sarkar *et al.* 2017; Biswas *et al.* 2018) on breeding season mainly June to August. By using synthetic hormone, induced breeding can be done successfully (Sridhar *et al.* 1998; Chakrabarty *et al.* 2007; Chakrabarti *et al.* 2012; Debnath *et al.* 2013; Raizada *et al.* 2013; Biswas *et al.* 2018; NFDB 2018). Individuals of 80 g body weight (bw) for males and 120 g for females are considered suitable for induced breeding (Singh *et al.* 2019). Captive breeding of *Ompok* spp. received wide attentions (e.g. Bhowmick *et al.* 2000; Mukherjee *et al.* 2002; Chakrabarty *et al.* 2005; Sarkar *et al.* 2005; Chakrabarti *et al.* 2009; Purkayastha *et al.* 2012). However, a few attempts have been made on the captive breeding of *O. bimaculatus* (Sridhar *et al.* 1998; Banik 2010; Banik *et al.* 2011; Raizada *et al.* 2013; Hussan *et al.* 2020). Such breeding programme can improve the stock and ensure survival of a threatened fish species, as observed for pengba, *Osteobrama belangeri* (Das *et al.* 2016).

Successful breeding and culture of pabda fish require good water quality parameters including temperature (20 – 26°C), pH (6 – 8), DO (4 – 5 mgL^{-1}), hardness (4 – 28 degree of general hardness, dGH) and alkalinity (120 – 150 ppm) and nutritionally balanced food having ≥32% protein (Pawar *et al.* 2023). Culture pond should be prepared very well before introducing the fish. For that, 4000 kg ha^{-1} cow dung and 40 kg ha^{-1} single super phosphate (SSP) were applied and after stocking, intermittent fertilisation should be carried out in every 15 days interval with cow dung at 500 kg ha^{-1} and SSP 20 kg ha^{-1} to maintain the pond fertility (Hussan *et al.* 2020). 10% aquatic weed is to be maintained covering the surface area as it provides a good hide-out for fishes and controls water temperature in summer. Good level of oxygen in pond should be maintained by installing aerator in pond and run at least three hours daily. In early stage of pabda, there might be a huge mortality due to predation. Thus, supply of specific feed in larval stages should be ensured otherwise, seed supply would not be up to the mark in commercial level. Construction of flow-through tub system or cemented tank hatchery might be used for hatching purpose (Sridhar *et al.* 1998; Debnath *et al.* 2013). The focused area of the present experiment was to check and compare the breeding performance of pabda (*O. bimaculatus*) in captive conditions for better and quality seed production towards fulfilment of farmer's demand.

2 | METHODOLOGY

2.1 Brooder management

This breeding programme was carried out at Puran Hatchery (21.9924°N 87.8893°E), Nandigram, Purba Medinipur during May to October 2021. Brood fishes were reared for 12 months prior to breeding by providing commercial feed (32% CP) with plenty of natural food using organic juice and important water quality parameters like pH (7.5), dissolved oxygen (4 – 5 mg L^{-1}), water hardness (200 mg L^{-1}) and alkalinity (200 mg L^{-1}) was maintained properly.

2.2 Collection of brood fish

We collected disease free, healthy, and mature male brooder (*n* = 130) with an average length and weight (21 cm and 65 g respectively) and female brooder (*n* = 130) with an average length and weight of 23 cm and 90 g respectively from the culture fish pond in May 2021. The collected fishes were disinfected with suitable sanitizer (using methylene blue at 1 ppm) for 5 – 10 minutes, then washed with freshwater and were released separately in different tanks based on sexes. They were acclimatised, separately for male and females, in tank (size 8×4×2 feet) with continuous aeration and eight individuals at a time and heavy shower. The following water quality parameters (Table 1) were maintained in breeding tank (APHA 1995). For better breeding management, sexing of brooder is required in the beginning. Pabda are bisexual and can be distinguished easily during the breeding season (Figure 1; Table 2).

TABLE 1 Water quality parameters to be observed for this breeding programme (*n* = 8).

Parameters	Mean ± SD value
Temperature (°C)	28.27 ± 1.68
pH	7.10 ± 0.44
Dissolved oxygen (DO, mg L^{-1})	4.25 ± 0.37
Hardness (mg L^{-1})	210.96 ± 9.31
Alkalinity (mg L^{-1})	202.78 ± 8.00

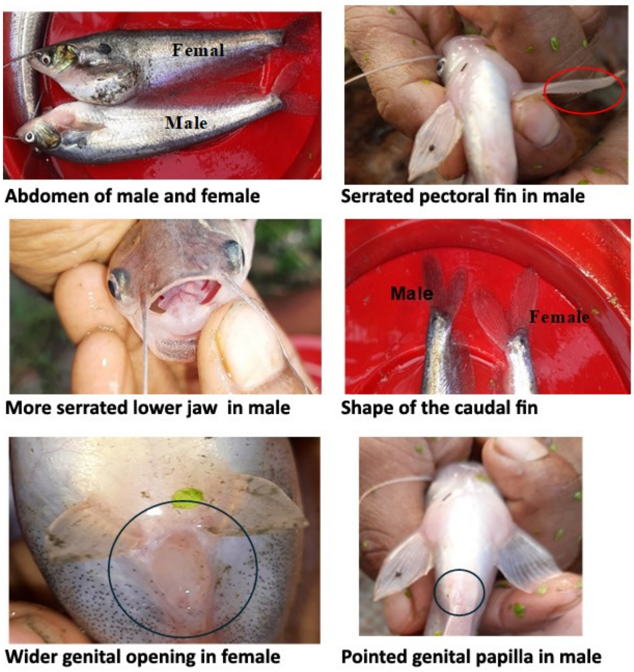

FIGURE 1 Sexing of pabda *Ompok bimaculatus*.

2.3 Estimation of fecundity

The gravimetric method (Haniffa and Sridhar 2002) was employed to estimate the relative fecundity of females. During estimation of fecundity, three 1 g egg samples from the ovary were collected and eggs were counted. The mean egg count number was then multiplied by the total weight ovary to determine the fecundity.

TABLE 2 Identification of mature male and female individuals of *Ompok bimaculatus*.

Male	Female
Pectoral fins are serration	Large and smooth pectoral fin
For individuals of same age, male is smaller than female	Bigger in size
Slender in shape, short body	Soft, round, and bulged abdomen, fleshy
Pointed, conical shaped elongated genital papilla	Genital papilla with reddish vent

2.4 Induced breeding

After acclimating them under a shower, selected healthy and mature brooders were injected with 'Spawn-pro,' a synthetic hormone. Both male and female fish were dosed at rates of 0.3 ml and 0.6 ml (T1), 0.5 ml and 0.7 ml (T2), and 0.7 ml and 0.8 ml (T3) per kg of body weight (bw), respectively. Each treatment had 3 replicates. The injected fish were stocked in breeding tanks with heavy showers for 2 – 4 hours. Spawning was observed 7 – 11 hours after the injection.

2.5 Determination of ovulation rate

The ovulation rate of *O. bimaculatus* was calculated using the following formula: Ovulation rate (%) = (No. of fish ovulated / Total No. of fish injected) × 100

2.6 Determination of fertilization rate

The fertilization rate of *O. bimaculatus* was calculated by the following formula: Fertilization rate (%) = (No. of fertilized eggs / Total No. of eggs laid) × 100

2.7 Determination of hatching rate

The hatching rate was calculated by visual method through observation of the hatchlings. The following formula was employed: Hatching rate (%) = (No. of hatchlings / Total No. of eggs fertilized) × 100

2.8 Determination of survival rate

The survival rate was determined by the following formula: Survival rate (%) = (No. of hatchlings survived / Total No. of hatchlings) × 100

2.9 Nursery pond preparation

Nursery pond for *O. bimaculatus* fish rearing was prepared the same way as nursery pond prepared for carp fry and fingerling rearing. For better management practices, 1 m water height and 0.02 to 0.04 ha of pond area should be maintained (Jhingran and Natarajan 1988). In case of presence of water, we applied mahua oil cake first, after six days, mustard oil cake (MOC) at 150 – 225 kg ha^{-1} and lime at 150 – 200 kg ha^{-1} were applied. After that, raw cow dung (at 4000 – 5000 kg ha^{-1}) was spread over the nursery pond. Inorganic fertiliser, urea and single super phosphate (SSP; 1 : 2) at the rate of 37.5 kg ha^{-1} was applied after five to six days of liming. Within 15 – 16 days, the pond was ready to be used for stocking *O. bimaculatus* spawn at the rate of 0.5 – 0.75 million ha^{-1}.

2.10 Data collection

We recorded water temperature (°C), pH, dissolved oxygen (DO, mg L^{-1}), hardness (mg L^{-1}) and alkalinity (mg L^{-1}) using the API FW Master Test Kit (United States). Relative fecundity (No. of eggs kg^{-1} body weight), fertilisation rate (%), hatching rate (%), latency period (hour) and fish survival rate (%) were also recorder.

2.11 Data analysis

Student's t-test and Fisher/Snedecor's F test were used to identify differences between treatments. Pearson's correlation was employed to determine the relationship between variables. Statistical tests were performed with MS-Excel software. The mean values were compared according to DMRT test (Gomez and Gomez 1984).

3 | RESULTS AND DISCUSSION

3.1 Relation between relative fecundity and hormonal doses

The relative fecundity of *O. bimaculatus* varied across treatment groups. In T2 treatment it was observed that, fecundity with doses of 0.7 ml kg^{-1} of bw was highest (105.20 ± 39.93 eggs g^{-1} bw of female) and in T1, the lowest was recorded at the dose of 0.6 ml kg^{-1} of bw (54.77 ± 15.64 eggs g^{-1} bw of female) (Table 3). A positive linear correlation (r^2 = 0.9158; p < 0.01, n = 130) was observed between fecundity with doses and it was significant at 1% level (Figure 2).

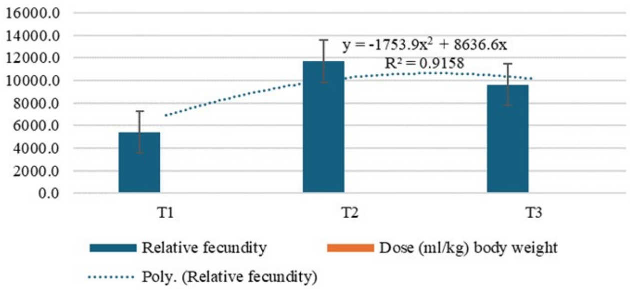

FIGURE 2 Relation between relative fecundity (eggs g^{-1} body weight of female) and hormonal doses (ml kg^{-1} body weight) in female.

TABLE 3 Results of induced breeding programme (mean ± SD) of pabda, *Ompok bimaculatus*.

Treatment	Relative fecundity (eggs g^{-1} bw)	Fertilisation rate (%)	Hatching rate (%)	Survival rate (%)
T1	54.77 ±15.64	67.37 ± 20.73	62.57 ± 18.46	55.01 ± 14.93
T2	105.20 ±39.93	75.88 ± 24.84	79.67 ± 26.68	65.62 ± 19.91
T3	84.96 ±29.73	74.63 ± 24.33	73.79 ± 23.81	63.28 ± 18.92

In this trial, one-year mature male (length 21 cm and weight 65 g) and female (length 23 cm and weight 90 g) were used for breeding programme. Same sized and matured one-year male and female fishes were used by Qayyum and Qasim (1965), Rao *et al.* (1986) and Mishra *et al.* (2013). Induced breeding of pabda was successfully done by Ovaprim at the same doses (0.5 ml kg^{-1} bw in both male and female) (Sridhar *et al.* 1998; Debnath *et al.* 2013). Other researcher observed that the best induced breeding result were found at 0.5 – 1.0 ml kg^{-1} for male and 1.0 – 1.5 ml kg^{-1} for female by inducing the same Ovaprim synthetic hormone. In 2011, Banik *et al.* (2011), also used Ovaprim hormone as an inducing agent and best results were obtained at 1 ml kg^{-1} for females and 0.5 ml kg^{-1} for males. But in these trials, a different hormone (i.e. Spawn pro) was used as an inducing agent. Hussan *et al.* (2020) and Parameswaran *et al.* (1970) observed the relative fecundity in pabda as 131–186 and 221 eggs g^{-1} bw respectively in females. Therefore, our findings were lower when compared to the earlier studies.

3.2 Relation between fertilisation rate and hormone doses

It was found that fertilisation rate varied with hormonal doses and fertilisation rate of T1, T2 and T3 were found as 67.37 ± 20.73, 75.88 ± 24.84 and 74.63 ± 24.33% respectively (Table 3) respectively. The best fertilisation was observed in T2 treatment (91.2%) and the lowest was 79.6% in T1 treatment.

Formerly, some researcher observed the fertilisation rate (%) in *O. bimaculatus* and it was found between 70 and 90% when Ovaprim hormone was used (Banik *et al.* 2011; Chaturvedi *et al.* 2013; Raizada *et al.* 2013). This was similar to the present findings as the highest fertilisation rate in this study was 91.2% (in T2) and the lowest was 79.6% (in T1).

3.3 Hatching rate (%) in different treatments

Hatching took place in 18 – 22 hours after fertilisation. Water temperature of hatching tank was maintained at 28.27 ± 1.68°C, which is ideal for hatching in these trials. Soft water with less alkalinity (202.78 ± 8.00 mg L^{-1}) is conducive for a better embryonic development and better hatching rate. The newly hatched larvae were cylindrical in shape, transparent, devoid of mouth and have pectoral fins and body pigments. Yolk sac was pale greenish in colour and gets absorbed in 2 – 3 days depending on the temperature. The water level in the hatching tank was also very crucial for successful hatching of *O. bimaculatus*. It was maintained from 1 – 7 days with 3 – 4 cm to 12 – 15 cm with mild water exchange.

The hatching rates were 62.57 ± 18.46, 79.67 ± 26.68 and 73.79 ± 23.81% in T1, T2 and T3 respectively (Table 3). It was significantly correlated (r^2 = 0.8968, p < 0.05; n = 130) with water temperature. The hatching rate was the highest in T2 followed by T3 and T1 (Figure 3). The hatching rate varied across literature, usually between 65 and 90% (Chaturvedi *et al.* 2013; Raizada *et al.* 2013; Hussan *et al.* 2020). Raizada *et al.* (2013) found slightly higher hatching rate (85 – 95%) in *O. bimaculatus* which was comparable to the present finding. Hatching time was 21 ± 1 hours and always depend upon water temperature.

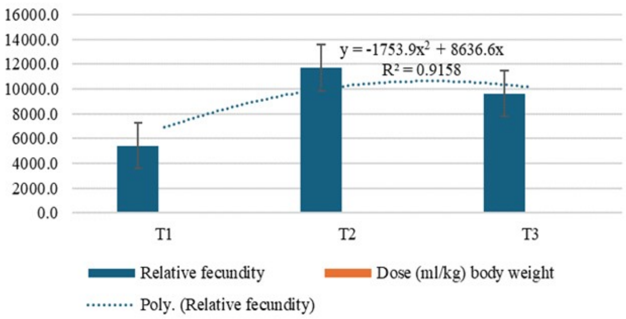

FIGURE 3 Relation between hatching rate (%) in study treatments (T1 – T3).

3.4 Survival rate

The highest survival rate of *O. bimaculatus* was 65.62 ± 19.91% in T2 and the lowest was 55.01 ± 14.93% in T1 (Table 3). A positive correlation (0.742) was observed and significant. These results indicate that survival rate (%) did not depend on the hatching rate or fertilisation rate; rather, it depends on water quality management in rearing tanks. DO, pH, alkalinity and hardness of the water were important in this regard. Water level in rearing tanks had an important role and proper hiding space encysts shooter segregation for every two days interval. First feeding was started after yolk sac absorption; it should start within 48 hours after hatching. Screened zooplankton was applied for the first four days with low water flow. Then spawn was released in well prepared nursery pond. Stocking density was also important for getting a better survival. Debnath *et al.* (2014) observed that 6 larvae L^{-1} water ensured best survival rate. Hussan *et al.* (2020) reported the survival rate of 43.8 – 58.5% in *O. bimaculatus*. In this study, 30 – 40 larvae L^{-1} water (T2) was found best.

4 | CONCLUSIONS

The findings of this present study recommend that Spawn pro hormone dose T2 (0.7 ml and 0.5 ml kg^{-1} body weight) resulted in the highest spawning rate, egg production, fertilisation, hatching rate and survival rate in *O. bimaculatus*. This breeding protocol does not require any high cost of investment, so it can be adopted by any entrepreneur. Those who are interested in seed production of catfishes can also use this study findings; it would be also helpful in species restoration and conservation programmes.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

AUTHORS' CONTRIBUTION

SP research design, primary data collection and validation; BG statistical analysis and draft manuscript (MS) writing; TKG research design, and critical review and finalisation of the MS; PB validation of data, review and statistical analysis.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on a reasonable request from the author.

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