Comparative efficacy of different inducing agents on breeding performance of a near threatened cyprinid Osteobrama belangeri in captivity

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\textbf{A B S T R A C T}

An experiment was conducted to breed a near threatened Cyprinid Osteobrama belangeri in captivity through hormonal induction. Carp pituitary extract (CPE) and three different GnRH based synthetic hormones viz., Ovaprim, Ovadite and Wova-FH were used as inducing agents. Experiment was conducted following a Completely Randomized Design (CRD). The brooders were injected with different doses of hormones and kept in the breeding hapas at 2:1 male to female ratio. All the inducing agents induced the fishes to breed, whereas no breeding was observed in the control set. Spawning success rate varied depending on the rate of inducement and the type of inducing agent used. CPE at a dose of 9 mg kg\(^{-1}\) of female and 3 mg kg\(^{-1}\) of male as well as the synthetic hormones at 0.5 ml kg\(^{-1}\) of female and 0.2 ml kg\(^{-1}\) of male broodings were found to be effective in inducing the fishes to breed in captivity. Efficacy of the synthetic hormones was significantly higher than that of CPE (\(P<0.05\)). Dose of hormone apparently affected the percentage of fertilization, egg output, hatching rate and spawn production. Administering an over-dose of the inducing agents caused early milting resulting in poor fertilization and under-dosing might cause late inducement in males. The present breeding protocol is simple and can be taken up by small breeders. It will be helpful in aquaculture and conservation of O. belangeri.

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1. Introduction

Osteobrama belangeri (Valenciennes, 1844) commonly known as “Pengha” is a medium sized carp of high food value especially in Manipur, India. The species reportedly represented up to 40\% of the total fishery of Loktak Lake (a Ramsar site) of Manipur a few decades ago (Behera et al., 2009). The species was later classified as “Extinct in the Wild” in 1997 (CAMP Report, 1998) and as “Near threatened” (IUCN, 2010). The abundance of the species declined due to habitat degradation, construction of dam, introduction of invasive species and overexploitation. Fish breeders of the state started induced breeding of this species through hormonal inducement because of its high consumer preference, price and demand. It fetches better price than the other carp species in domestic markets especially in Manipur, India and adjacent Myanmar.

O. belangeri breeds in riverine conditions during the Southwest monsoon season and it does not breed in confinement without hormone induction (Behera et al., 2010). Thus, seed production of the species in captivity is a major constraint. Therefore, improvement of induced breeding techniques for this species is needed for increasing its aquaculture production and conservation through ranching. However, limited attempts have been made to induce O. belangeri to breed and scientific information on this aspect is fragmented. Earlier breeding trials of this species were reported by Reddy (2000), Samarjit and Basudha (2007), Behera et al. (2008), Devi et al. (2009) and Behera et al. (2010, 2015). No report is available on the comparative efficacy of different inducing agents on breeding performance of the species in captivity. Selecting appropriate inducing agents will enhance the overall reproductive success. There is a need to assess the comparative efficacy of available inducing agents for induced breeding of the species so that

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the fish breeders can choose the inducing agents as per availability, convenience and economics. Against this background, we are reporting the comparative efficacy of four inducing agents (viz., carp pituitary extract, Ovaprim, Ovatide and Wova-FH) on the breeding performance of O. belangeri in captivity.

2. Materials and methods

2.1. Broodstock collection and maintenance

The captive breeding experiment was conducted at the Demonstration fish farm of the ICAR Research Complex for NEH Region, Manipur Centre, Imphal, Manipur, India. O. belangeri brooders (n = 80) were collected from Loktak Lake, Bishnupur district, Manipur fished using encircling net and transported alive to the farm in oxygenated polyethylene bags. The fishes were given KMnO₄ dip treatment before releasing them into earthen ponds. They were reared in earthen ponds (0.12 ha each, average depth 1.5 ± 0.12) at 1000 nos./ha located at the institute for a period of 8 months. During this period, they were fed ad libitum with soft aquatic weed (Azolla, Lemna, etc.) and locally available terrestrial fodders. They were also given supplementary feed (rice polish-mustard oil cake mixture at 1:1 ratio) @ 5% of body weight twice daily (in the morning and evening). Growth of brooders and water quality of the ponds were monitored at monthly intervals.

2.2. Hormonal preparation

Carp Pituitary Extract (CPE) was prepared from carp pituitary glands collected from freshly caught matured Indian major carp, Catla catla (available at the farm) following methods described by Thomas et al. (2003). Synthetic hormones viz., Ovaprim, Ovatide and Wova-FH were obtained from a local supplier (Agocons Agro Products Pvt. Ltd., Imphal, Manipur, India).

2.3. Experimental design

The present experiment followed a Completely Randomized Design (CRD) using twelve treatments in addition to a control performed in triplicates. Different hormonal preparations (prepared and commercial) used were Carp Pituitary Extract (CPE) and three GnRH based synthetic hormones viz., Ovaprim (OP), Ovatide (OT) and Wova-FH (WFH) at different doses to induce breed the fish. For CPE administration, fishes were applied with three doses (both preparatory & decisive dose for female and only decisive dose for male after 4 h of first injection) of freshly prepared heteroplastic CPE. Different treatments used in the experiment along with their individual doses for male and female brooders have been summarised in Table 1.

2.4. Captive breeding

Captive breeding of the experimental fishes was carried out during the peak of Southwest monsoon month of July. Healthy brooders maintained in the brood ponds were randomly selected based on their overall external appearance. These were segregated sex wise based on external morphology and transferred to nylon net hapas (3.0 × 2.5 × 1.5 m) for acclimatization. Males were identified by their slender body, rough dorsal surface of pectoral fins. In males, milt oozed out through the genital aperture on applying gentle pressure on the posterior abdomen. In females, the pectoral fins had smooth surface and had reddish, soft swollen and bulging belly. On applying gentle pressure on the posterior belly, eggs oozed out in some cases. The average body weight of the brooders used for the trial was 256 ± 4 g for females and 174 ± 3 g for males. Free oozing males and ripe females were paired in 2:1 ratio for spawning.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Treatments based on type of hormone and dose applied.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>Hormonal dosage to female</td>
</tr>
<tr>
<td>Control</td>
<td>No induction</td>
</tr>
<tr>
<td>T1</td>
<td>7 mg kg⁻¹ (2 + 5 mg kg⁻¹) of CPE</td>
</tr>
<tr>
<td>T2</td>
<td>9 mg kg⁻¹ (3 + 6 mg kg⁻¹) of CPE</td>
</tr>
<tr>
<td>T3</td>
<td>12 mg kg⁻¹ (4 + 8 mg kg⁻¹) of CPE</td>
</tr>
<tr>
<td>T4</td>
<td>0.3 ml kg⁻¹ of OP</td>
</tr>
<tr>
<td>T5</td>
<td>0.5 ml kg⁻¹ of OP</td>
</tr>
<tr>
<td>T6</td>
<td>0.7 ml kg⁻¹ of OP</td>
</tr>
<tr>
<td>T7</td>
<td>0.3 ml kg⁻¹ of OT</td>
</tr>
<tr>
<td>T8</td>
<td>0.5 ml kg⁻¹ of OT</td>
</tr>
<tr>
<td>T9</td>
<td>0.3 ml kg⁻¹ of OT</td>
</tr>
<tr>
<td>T10</td>
<td>0.2 ml kg⁻¹ of WFH</td>
</tr>
<tr>
<td>T11</td>
<td>0.5 ml kg⁻¹ of WFH</td>
</tr>
<tr>
<td>T12</td>
<td>0.7 ml kg⁻¹ of WFH</td>
</tr>
</tbody>
</table>

Note: CPE = Carp Pituitary Extract; OP = Ovaprim (OP); OT = Ovatide; WFH = Wova-FH.

Brooders in treatment groups were administered with three different doses of the prepared (combined dose for preparatory and decisive doses in case of CPE) and synthetic hormones (single dose) intramuscularly whereas the control group was left without any hormone administration. The injected brooders were immediately released into the breeding hapas.

2.5. Breeding performance

Effective fecundity of each female after spawning was determined through random sampling of released eggs in a 10 ml graduated measuring cylinder from the eggs released by the female. The total number of eggs in one ml were counted and multiplied with total volume of eggs released (Behera et al., 2009a, 2010). The fertilization rate of eggs was determined by randomly taking a sample of approximately 100 eggs from the total eggs in a glass petri dish (Behera et al., 2009a); fertilized eggs having intact nucleus were only considered for calculating percentage of fertilization. Ova diameter was measured by keeping approximately 20 eggs in a row along a measuring glass scale under a dissecting microscope (Behera et al., 2009a); total lengths of eggs were divided by the number of eggs to obtain mean diameter of each egg (Behera et al., 2009a). One day old hatchlings were maintained in circular Fibre Reinforced Plastic (FRP) tanks. Aeration (with air pumps) and partial water exchange was practiced daily to maintain water quality in the tanks.

Important water quality parameters of brood stock pond and breeding pond (the pond where breeding hapas were kept) were analyzed following APHA (1998). Care was taken to maintain the water quality within the favourable range in the brood stock (e.g., water temperature: 28.5 ± 2.5 °C, pH: 7.8 ± 0.32, dissolved oxygen: 6.5 ± 1.1 mg/L and free CO₂: 2.1 ± 0.7 mg/L) and breeding ponds (e.g., water temperature: 28.5 ± 2.5 °C, pH: 7.5 ± 0.35, dissolved oxygen: 6.8 ± 1.1 mg/L and free CO₂: 1.8 ± 0.6 mg/L).

2.6. Statistical analysis

The experimental data were statistically analyzed using SPSS software (version 16.0 for windows). One-way analysis of variance (One-way ANOVA) was used to compare significant differences, if any, between treatments. Significant differences between two means were tested using Duncan’s multiple range tests at P < 0.05. The results are presented as mean ± standard error (SE).
Table 2
Results of the captive breeding experiments of *O. belangeri* with CPE and GnRH based synthetic hormones.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Size of female (g)</th>
<th>Hormonal dosage to female (ml kg⁻¹ body weight)¹</th>
<th>Size of male (g)</th>
<th>Hormonal dosage to male (ml kg⁻¹ body weight)¹</th>
<th>Latency period (h)</th>
<th>Number of eggs by female</th>
<th>Fertilization (%)</th>
<th>Hatching (%)</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0 (Control)</td>
<td>254 ± 3</td>
<td>0</td>
<td>175 ± 4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>No spawning</td>
</tr>
<tr>
<td>T1 (CPE)</td>
<td>256 ± 3</td>
<td>7</td>
<td>174 ± 3</td>
<td>2</td>
<td>12</td>
<td>16400 ± 289¹</td>
<td>34.0 ± 1.2 h</td>
<td>43.8 ± 1.7 h</td>
<td>Partial spawning</td>
</tr>
<tr>
<td>T2 (CPE)</td>
<td>254 ± 3</td>
<td>9</td>
<td>178 ± 2</td>
<td>3</td>
<td>8</td>
<td>37367 ± 960¹</td>
<td>68.6 ± 2.4 d</td>
<td>62.7 ± 1.2 d</td>
<td>Complete spawning</td>
</tr>
<tr>
<td>T3 (CPE)</td>
<td>257 ± 3</td>
<td>12</td>
<td>174 ± 3</td>
<td>4</td>
<td>10</td>
<td>24533 ± 837²</td>
<td>53.5 ± 1.5 f</td>
<td>50.5 ± 1.1 f</td>
<td>Complete spawning</td>
</tr>
<tr>
<td>T4 (Ovaprim)</td>
<td>258 ± 3</td>
<td>0.3</td>
<td>177 ± 3</td>
<td>0.1</td>
<td>10</td>
<td>29600 ± 989²</td>
<td>54.7 ± 1.6 e</td>
<td>52.1 ± 1.2 f</td>
<td>Complete spawning</td>
</tr>
<tr>
<td>T5 (Ovaprim)</td>
<td>256 ± 2</td>
<td>0.5</td>
<td>174 ± 3</td>
<td>0.2</td>
<td>7</td>
<td>67333 ± 467³</td>
<td>95.5 ± 2.1 a</td>
<td>87.6 ± 1.4 a</td>
<td>Complete spawning</td>
</tr>
<tr>
<td>T6 (Ovaprim)</td>
<td>256 ± 2</td>
<td>0.7</td>
<td>176 ± 3</td>
<td>0.3</td>
<td>8</td>
<td>44700 ± 993⁴</td>
<td>78.3 ± 1.8 c</td>
<td>76.6 ± 1.6 b</td>
<td>Complete spawning</td>
</tr>
<tr>
<td>T7 (Ovatide)</td>
<td>255 ± 3</td>
<td>0.3</td>
<td>177 ± 2</td>
<td>0.1</td>
<td>10</td>
<td>30850 ± 264⁴</td>
<td>55.2 ± 1.9 e</td>
<td>58.9 ± 1.4 e</td>
<td>Partial spawning</td>
</tr>
<tr>
<td>T8 (Ovatide)</td>
<td>256 ± 3</td>
<td>0.4</td>
<td>174 ± 4</td>
<td>0.2</td>
<td>7</td>
<td>63400 ± 866⁵</td>
<td>93.4 ± 2.0 h</td>
<td>85.3 ± 2.0 a</td>
<td>Complete spawning</td>
</tr>
<tr>
<td>T9 (Ovatide)</td>
<td>255 ± 3</td>
<td>0.5</td>
<td>175 ± 3</td>
<td>0.3</td>
<td>8</td>
<td>46838 ± 962⁶</td>
<td>77.2 ± 2.1 c</td>
<td>74.9 ± 1.8 e</td>
<td>Complete spawning</td>
</tr>
<tr>
<td>T10 (Wova-FH)</td>
<td>255 ± 3</td>
<td>0.3</td>
<td>179 ± 3</td>
<td>0.1</td>
<td>10</td>
<td>21733 ± 167⁷</td>
<td>52.0 ± 1.2 f</td>
<td>52.6 ± 1.4 f</td>
<td>Partial spawning</td>
</tr>
<tr>
<td>T11 (Wova-FH)</td>
<td>257 ± 2</td>
<td>0.5</td>
<td>178 ± 3</td>
<td>0.2</td>
<td>7</td>
<td>64867 ± 721⁸</td>
<td>92.2 ± 2.3 b</td>
<td>86.3 ± 1.7 a</td>
<td>Complete spawning</td>
</tr>
<tr>
<td>T12 (Wova-FH)</td>
<td>255 ± 3</td>
<td>0.7</td>
<td>176 ± 3</td>
<td>0.3</td>
<td>8</td>
<td>43667 ± 611⁹</td>
<td>76.1 ± 1.3 c</td>
<td>72.5 ± 1.2 c</td>
<td>Complete spawning</td>
</tr>
</tbody>
</table>

P-value 0.778 – 0.595 – – 0.001 0.001 0.001 –

Note: Values in the same column with different superscript differ significantly (P < 0.05); Data expressed as Mean ± SE, n = 3; Egg production = Number of eggs released/female; Fertilization rate = 100 (no. eggs with faint streak/no. eggs in sample); Hatching rate = 100 (no. hatched eggs/no. tail bud embryos).

¹ mg/kg body weight in case of CPE.
3. Results

O. belangeri attained maturity at the age of 2+ years and were observed to be fully mature during the month of July. A varied degree of response of administered inducing agents was observed in different doses during the present experiment. Fertilization rates, latency period, egg output and hatching rate in response to different treatments have been summarised in (Table 2). The response to hormone inducement was very high; out of 39 female selected for induced breeding, 36 responded positively and produced viable eggs. Predictably, no spawning took place in control sets.

3.1. Breeding behaviour

Brooders showed mating behaviour after hormonal injection in all the treatments (except in the control), which varied between the treatments. Each female was paired with two males. Mating was preceded by elaborated courtship behaviour. We observed that the male rubbed its body against the female and released its milt over the eggs released by the female. Brooders showed mating behaviour after 3–4 h of injection in T5, T6, T8, T9, T11 and T12. It was delayed (seen after 7–8 h of injection) in T2, T3, T4, T7 and T10. In case of T1, breeding behaviour was seen only after 10 h of injection.

3.2. Latency period

Latency period varied significantly between the synthetic hormones injected group and the CPE injected group. Spawning took place as early as after 7 h of injection in T5, T8 and T11; 8 h in T2, T6 and T9; 10 h in T3, T4, T7 and T10, whereas CPE at lowest dose (T1) took the longest time (12 h). The result showed delayed latency period with administration of low dose of CPE than other groups.

3.3. Fertilization rate

Fertilization rate was significantly higher (P<0.05) at T5 (95.2 ± 2.1%), T8 (93.4 ± 2.0%) and T11 (92.2 ± 2.2%) using Ovaprim, Ovatide and Wova-FH injection respectively than that in the other treatments. However, there were no significant differences between T5, T8 and T11 (P<0.05). Fertilization rate in T2 (68.6 ± 2.4%) was significantly higher within the CPE injected group (P<0.05); it was also significantly lower than that in T5, T8 and T11 comprising synthetic hormone administration group (P<0.05). The results showed that fertilization rate was significantly higher with injection of synthetic hormones than with CPE.

3.4. Egg output

The number of eggs released by the female was significantly higher (P<0.05) at T5 (67,333 ± 467), T8 (63,400 ± 966) and T11 (64,867 ± 721) using Ovaprim, Ovatide and Wova-FH injection respectively than that by the other treatments. Egg output per female in T2 (37,367 ± 960) was significantly higher within the CPE injection group. But it was significantly lower in T2 than that in the T5, T8 and T11 of the synthetic hormone administration group. The fertilized eggs were spherical, translucent and demersal measuring 2.40 ± 0.03 mm in diameter and were non-adhesive. Unfertilized eggs were pale and opaque.

3.5. Hatching rate

Twitching movement of the embryos was observed within 8–9 h of spawning and the young ones hatched out within 14–16 h at 28.5 ± 2.5 °C. Estimated hatching rate was significantly higher (P<0.05) at T5 (87.7 ± 1.2%), T8 (85.3 ± 2.0%) and T11 (86.29 ± 1.65%) using Ovaprim, Ovatide and Wova-FH injections respectively than that in the other treatments. The hatching rate in T2 (71.9 ± 1.2%) was significantly higher within the CPE injection group even though the rate of hatching in T2 was significantly lower than that in T5, T8 and T11 of synthetic hormone administration group, suggesting synthetic hormone gave significantly higher hatching rate than that by CPE (P<0.05).

3.6. Hatching

Freshly hatched larvae measured 4.3 ± 0.2 mm in length and 1.6 ± 0.3 mg in weight. One-day-old hatchlings were maintained in nylon hapa and plastic toughs simultaneously. Movement of hatchlings was reasonably fast and their air bladders were prominently visible with regular fanning of pectoral fins. Mouth started developing after 2 days of hatching. The yolk sac was fully absorbed on the 4th day and hatchlings grew up to 6.4 ± 0.3 mm in length and 2.7 ± 0.2 mg in weight. After 4 days of hatching, the spawns were released into pre- prepared nursery tanks for further growth.

4. Discussion

The present investigation showed that O. belangeri attained sexual maturity in 2+ years and spawning can be induced in captivity by administration of CPE or synthetic hormones like Ovaprim, Ovatide and Wova-FH. Spawning response was comparable to that with major carps, in which 95–100% breeding response was easily achieved (Basavaraja et al., 1999). In our study, CPE at a dose of 9 mg kg−1 of female and 3 mg kg−1 of male and synthetic hormones at 0.5 ml kg−1 of female and 0.2 ml kg−1 of male brooders were found to be effective in inducing the species to spawn in captivity. However, the efficacy of the three synthetic hormones was significantly higher than that of CPE. Rath et al. (2007) did a comparative evaluation of CPE and 3 different GnRH based synthetic hormones on the induced breeding performance of Indian Major Carps (IMC), which support our present findings.

Difference in latency period was observed among different treatments in the present study. Higher latency period in CPE (7 mg kg−1 of female) and synthetic hormones at low dose of 0.1 ml kg−1 of body weight of female indicates difference in the mode of action of the hormone. Similar observation was reported by Pandey et al. (2002a) in L. rohita. Behera et al. (2007) also reported the longer latency period in low dose of synthetic hormone Ovaprim and Ovatide on induced breeding of Labeo bata. According to Billiard et al. (1984) and Peter et al. (1986), differences in dose requirement may be attributed to varied level of dopamine activity in fishes.

In our experiment, the administered hormonal dose apparently affected the rate of fertilization. Over-dosing of the inducing agents caused early milting; resulting in poor fertilization and under-dosing caused late inducement in males. Similar finding was reported by Routray et al. (2007) in their review paper. Pandey et al. (2002b) successfully conducted breeding of IMC using Ovatide at a dose of 0.4 ml kg−1 body weight for female and 0.1 ml kg−1 body weight for male. Behera et al. (2007) reported that the egg output per female, fertilization and hatching rate was the highest with Ovaprim at a dose of 0.5 ml kg−1 of female and 0.2 ml kg−1 of male body weight; in case of Ovatide it was of 0.4 ml kg−1 of female and 0.2 ml kg−1 of male body weight in L. bata.

The number of eggs released by the female O. belangeri (Number of eggs by female) with administration of CPE, Ovaprim, Ovatide and Wova-FH was quite high, which points to its high fecundity as compared to many carps available in India. In our study, water temperature in the breeding pool during the experiments was 28.5 ± 2.5 °C, which was favourable for breeding. The high egg out-
put per female in our experiment may also be attributed to the quality of brood stocks used for induced spawning.

The dose of hormone obviously affected hatching in O. belangeri. Optimal range of water quality parameters recorded in the breeding pond also might have contributed to increased hatching rates. Behera et al. (2007) reported that the hatching rate of L. bata was the highest when induced with Ovaprim and Ovatide along with optimum range of water quality in the experimental pond. Rath et al. (2007) also reported higher hatching rate in IMC through while administering Wova-FH at 0.4-0.5 ml kg⁻¹ of female body weight.

5. Conclusion

The commercial seed production of O. belangeri can be achieved in captivity through induced breeding using CPE or GnRH based synthetic hormones viz., Ovaprim, Ovatide and Wova-FH. However, the success rate of induced spawning will depend on the rate of induction and also the type of inducing agent used. The breeding protocol is simple and can be adopted by small scale carp breeders. Availability of a host of inducing agents for induced breeding O. belangeri is likely to be helpful in increased availability of commercial seed of the species for their use in expanding its aquaculture and conservation.

Conflicts of interest

None of the authors has conflicts of interest.

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