



Captive breeding of a near threatened fish, pengba *Osteobrama belangeri* (Valenciennes, 1844) using three different inducing agents

J. RAYMOND JANI ANGEL¹, V. K. TIWARI², P. P. SURESH BABU³, KIRAN DUBE RAWAT²
BOBY IGNATIUS⁴, R. B. PRAMOD KIRAN⁵, S. DAM ROY¹, R. CHARAN¹, DEEPTI R. NAIR²
P. SRINIVASA RAO³ AND K. B. SREERAMAMURTY³

¹ICAR-Central Island Agricultural Research Institute, Post Box No. 181, Port Blair - 744 101
Andaman and Nicobar Islands, India

²ICAR-Central Institute of Fisheries Education, Versova, Mumbai - 400 061, Maharashtra, India

³Kakinada Research Centre of ICAR-Central Institute of Fisheries Education, Kakinada - 533 001
Andhra Pradesh, India

⁴ICAR-Central Marine Fisheries Research Institute, Post Box No. 1603, Ernakulam North P. O., Kochi - 682 018
Kerala, India

⁵Department of Aquatic Biology and Fisheries, University of Kerala, Thiruvananthapuram, Kerala, India
e-mail: raymondicar007@gmail.com

ABSTRACT

Farm reared pengba, *Osteobrama belangeri* were induced to spawn in captivity during August, 2012 by injecting three different synthetic hormones, Ovaprim, Ovatide and Gonopro-FH. Single dose (1 ml kg⁻¹ body weight) of each hormone was administered and results were recorded. Spawning was observed within 8 h after injection. Hatching of eggs were observed after 22±2 h of incubation at 27±1°C. The mean fertilization rate was 84.05±0.36% for Ovaprim, 79.17±3.95% for Ovatide and 84.85±0.89% for Gonopro-FH treated fish. The mean hatching rate was 84.69±1.73% with Ovaprim, 75.01±1.92% with Ovatide and 86.52±0.88% with Gonopro-FH. Gonopro-FH and Ovaprim gave 5.67 and 4.88% higher fertilization rate as well as 11.5 and 9.69% more hatching rate of eggs respectively as compared to Ovatide. Ovaprim and Gonopro-FH were found to be more effective in induced breeding of *O. belangeri*.

Keywords: Gonopro-FH, *Osteobrama belangeri*, Ovaprim, Ovatide, Pengba

Introduction

The major technological breakthrough achieved in the induced breeding of Indian major carps (IMC) by Chaudhury and Alikunhi (1957) led to the rapid development of carp farming in India. Development of synthetic hormones for induced breeding of fishes helped farmers to overcome the need to sacrifice brooders and laborious hypophysation process. The synthetic hormone, Ovaprim (M/s Syndel Laboratories, Canada) has been successfully used (Nandeesh et al., 1990) since 1990 for mass production of fish seed. In 1997, Ovatide, an indigenous synthetic hormone which is more affordable than Ovaprim, was formulated by M/s Hemmo Pharma, Mumbai. The active ingredient in both Ovaprim and Ovatide is same and the major prohibitive factor for Indian farmers to use Ovaprim was its high cost and its thick consistency that caused difficulty in injection. Recently, Yurembam et al. (2014) reported the use of another synthetic hormone, Gonopro-FH (salmon-GnRH-a) marketed by Amrit Pharmaceuticals, Aurangabad, India

as oral administration for induced breeding of giant zebra fish *Devario acquirinnatus*.

Osteobrama belangeri (Valenciennes, 1844) (Family: Cyprinidae; Order: Cypriniformes) a medium sized carp locally known as 'pengba', is the state fish of Manipur, India and a local delicacy. The species is distributed in the rivers and lakes of the state. It is also found in Yunnan, China and Myanmar (Basudha and Vishwanath, 1999). The Indian stocks of pengba are usually referred to as moderate to highly vulnerable and have been variably categorised as "Extinct in wild" in the Conservation Assessment and Management Plan (CAMP, 1999), as "Threatened" (Reddy, 2000), "Near Threatened" (Vishwanath, 2010) and as "Endangered" (Suresh, 2000; Menon, 2004). Even though *O. belangeri* has become extinct from the wild in Manipur, it still exists in Myanmar. In the past, it formed a big fishery in Loktak Lake. Over the last 45 years its wild population has undergone a drastic decline and is presently found missing in the Loktak Lake and other water bodies of central plains of Manipur. It is perhaps due

to the loss of habitat after the construction of Ithai Barrage on the Imphal River for supply of water to the Loktak Hydro-electric Project. The barrage possibly prevented the breeding migration of the fish from the Southern parts of Manipur River, which ultimately flows into the Chindwin in Myanmar (Dinesh and Mema, 2012).

It is widely accepted that the long term sustainability of fisheries and aquaculture of a nearly threatened species can be achieved only through the development of captive seed production technologies. Standardisation of breeding protocol for large scale seed production of endangered fish species is critical in this development. In this context, with a view to restore the population of *O. belangeri* in the wild and to develop broodfishes for stock enhancement and management programme, induced breeding and larval rearing of *O. belangeri* was attempted. Efforts were also made to compare the efficiency of three different commercially available inducing agents at a uniform dose for successful spawning.

Materials and methods

Broodstock rearing

Experiments were conducted in the Freshwater Fish Farm at the Kakinada Centre of ICAR-Central Institute of Fisheries Education, Balabhadrapuram, Andhra Pradesh. Mature female and male brooders of 2+ year class were reared separately in two different earthen ponds and fed with supplementary feed consisting of groundnut oil cake and rice bran (1:1) at 5% of body weight per day.

Selection of brooders

Females of *O. belangeri* are comparatively larger than their male counterpart. Unlike in many other carps, sexual dimorphism in this species is difficult to be identified by feeling the roughness on inner side of pectoral fin. Mature females were observed with bulged belly region, slightly swollen genital aperture and oozing ova upon gentle pressure on the abdomen (Fig. 1a) while mature males were observed with freely oozing milt (Fig. 1b).



(a)



(b)

Fig. 1. Sexual dimorphism in *Osteobrama belangeri*. (a) mature female, (b) mature male

Males and females were selected in the ratio 3:2. A total of 27 males (173.67 ± 5.25 g; 26.61 ± 0.45 cm) and 18 females (419.53 ± 11.48 g; 30.13 ± 0.36 cm) were used for the experimental trials

Induced breeding

In the present study, three different inducing agents *viz.*, Ovaprim, Ovatide and Gonopro-FH were used for evaluating the comparative spawning and reproductive performance of pengba. Two males and three females were used in each breeding sets. Uniform dose (1 ml kg^{-1} body weight, BW) of each inducing agent was administered to the experimental fishes in respective sets by a single intramuscular injection between the base of dorsal fin and lateral line, in the evening hours (17.30 hrs). The injected fishes were transferred to breeding hapas ($3 \times 2 \times 2$ m) affixed in the nearby canal, to ensure continuous flow of water throughout the latency period. The breeding experiments were conducted in the month of August, 2012 with conducive weather conditions for breeding.

The occurrence of spawning was monitored periodically by checking for the presence of eggs in the breeding hapa. Egg collection was done in the following morning at around 8.00 hrs (Fig. 2). Fecundity, fertilization and hatching rates were calculated by random sampling and counting.

Fertilization and hatching

The total number of eggs and fertilization percentage were determined by analysing 3 samples of 1ml aliquots. The eggs were then transferred to an incubation pool in the hatchery (Fig. 3).

The hatchlings were reared in the incubation pool for three days till complete yolk absorption and then the spawn were transferred to cement ponds for nursery rearing. The spawn were counted using standard 50 cm^3 aluminium cups, taking an average of 1000 nos. of spawn per cubic cm (50,000 per cup). The physico-chemical parameters of canal and incubation pool water were analysed following standard methods (APHA, 2012).



Fig. 2. Collection of eggs

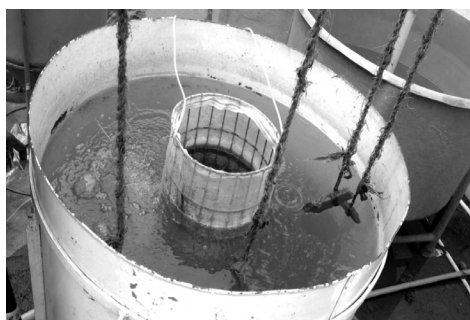


Fig. 3. Hatching of eggs in the incubation pool

Data analysis

The fertilization rate and hatching rates of different treatments were compared for the three different inducing agents using one way ANOVA and Tukey’s test. Data was analysed using Statistical package for the Social Sciences (SPSS) version 16.0.

Results and discussion

The present experiment was aimed to determine the effectiveness of three commercially available preparations in induced breeding of *O. belangeri* and the results are summarised in terms of average no. of eggs per female, fertilization rate and hatching rate (Table 1). The physico-chemical parameters of water were well within the optimum level for breeding and larval rearing of the species. The water temperature, dissolved oxygen (DO), total alkalinity and pH recorded were 26 - 30°C, 6 -7 ppm, 90 - 110 ppm and 8 - 8.5, for the canal water and 26 - 28°C, 7 - 7.5 ppm, 110 - 130 ppm and 7.5 - 7.9 in the incubation pool, respectively.

Complete spawning was observed within 8 h after the administration of hormones. Latency period of

7.15 to 8.15 h was observed for the same species by Devi *et al.* (2009) using Ovatide as inducing agent. The fertilized eggs of *O. belangeri* were observed to be spherical, non-adhesive and demersal. The mean fecundity and mean fertilization rate obtained after induced breeding with Ovaprim was 2.46 lakh eggs per female and 84.054±0.36%, whereas it was 2.27 lakh eggs per female and 79.17±3.95% with Ovatide and 2.68 lakh eggs per female and 84.85±0.89% with Gonopro-FH respectively.

The mean fertilization rate of eggs was found to be high in treatments induced with Gonopro-FH and Ovaprim compared to that of Ovatide (Fig. 4), however, the difference was not statistically significant ($p < 0.05$). Hatching took place at 22±2 h post-fertilization. The mean hatching rate obtained after induced breeding was 84.69±1.73% with Ovaprim; 75.01±1.91% with Ovatide and 86.52±0.88%.with Gonopro-FH. Absorption of yolk sac was completed within the next 72 h.

The mean hatching rate of eggs was found to be significantly high ($p < 0.05$) in treatments induced with Gonopro-FH and Ovaprim compared to that of Ovatide (Fig. 4). The positive response of *O. belangeri* to Gonopro-FH and Ovaprim indicated the effectiveness of these preparations in induced breeding of the species.

Induced breeding technique is arguably the most important research achievement in the field of finfish aquaculture to ensure regular supply of stocking material. Several preparations have been tried for induced spawning

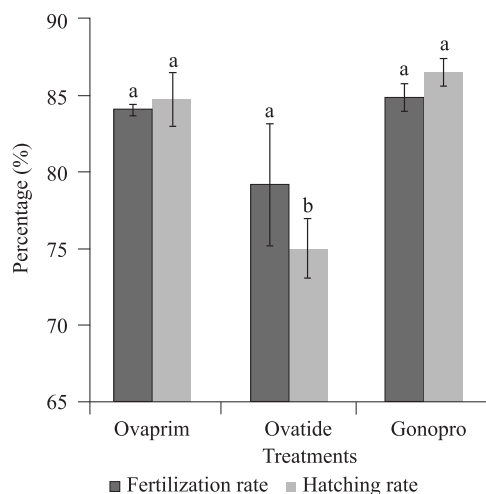


Fig. 4. Fertilization and hatching rates in *O. belangeri* with different inducing agents

Table.1. Fecundity, fertilization rate and hatching rate recorded in *O. belangeri* with Ovaprim, Ovatide and Gonopro-FH

Inducing agent	Average no. of eggs per female	Percentage fertilization	Percentage hatching
Ovaprim	246204.86±5950.96 ^{ab}	84.05±0.36 ^a	84.70±1.73 ^a
Ovatide	227236.27±9146.74 ^b	79.17±3.95 ^a	75.01±1.91 ^b
Gonopro-FH	268138.35±4949.87 ^a	84.84±0.89 ^a	86.51±0.88 ^a

in fishes with various levels of success (Harvey and Hoar, 1979). Induced breeding of many Indian fishes were attempted by several workers (Ramaswamy and Sundaraj, 1969; Zairin *et al.*, 1992; Alok *et al.*, 1998), however, the level of success varied based on the combination of the fish species and inducing agent. A study on the comparison of spawning success with different inducing agents in relation to different species of IMCs revealed that the spawning success (fecundity and fertilization rate) of *Catla catla* is more with Ovaprim, whereas, the results were better with Ovatide in *Labeo rohita* and *Cirrhinus mrigala* (Asha and Kamaldeep, 2004). Reddy and Mathur (2000) also reported better results with ovatide in *L. rohita* and *C. mrigala* as compared to *C. catla*. Chauhan *et al.* (1999) reported breeding success of *L. rohita* at par when induced to breed with Ovaprim and Ovatide. Gurpreet and Sudhanshu (2012) proved that Ovaprim gave 5.83 and 12.95% better performances over Ovatide and carp pituitary extract in terms of hatching rate of *L. rohita* eggs. The difference in effective dosage among different species is attributed to the varied levels of dopamine activity (Billard *et al.*, 1983; Peter *et al.*, 1986). Devi *et al.* (2009) attempted induced spawning and hatching of *O. belangeri* in its natural habitat using Ovatide (0.6 ml kg⁻¹ for females and 0.3 ml kg⁻¹ for males) and reported 95.0±1.05% fertilization and 88.8±1.04% hatching rates. Since the current experiment was carried out at a site far away from the natural habitat of the species, a higher dose of the hormonal preparations were administered; however, the fertilization and hatching rates observed were found less when compared to the reports of Devi *et al.* (2009). A dosage of 1ml kg⁻¹ body weight of Ovaprim was recommended for artificial spawning of *Clarias gariepinus* in a similar experiment conducted by Achionye and Israel (2012). Ovaprim at 1ml kg⁻¹ gave better results in terms of fertilization rate and hatching rate in koi carp (Ghosh *et al.*, 2012). Sharma *et al.* (2010) recommended 1ml of Ovatide per kg body weight of female broodfish to be optimum among the three experimental doses, 0.6, 0.8 and 1ml kg⁻¹, for best breeding performance and egg quality in *Clarias batrachus*.

The results of the present study clearly demonstrate the possibility of using synthetic preparations like Ovaprim and Gonopro-FH for effective induced spawning and seed production of *Osteobrama belangeri*, which may be used for stock enhancement as well as for ranching of the species in natural water bodies. Being a species of commercial importance, the protocol for captive breeding of *O. belangeri* is likely to pave way towards commercialisation of the technology, which might introduce an exciting entrepreneurial area in aquaculture.

References

- Achionye-Nzeh, C. G. and Israel, O. 2012. Ovaprim doses effects on eggs of African mudfish, *Clarias gariepinus*. *Int. J. Life Sci. Pharma Res.*, 2(2): 6-9.
- Alok, D., Krishnan, T., Talwar, G. D. and Garg, L. C. 1998. Multiple induced spawning of the Indian catfish *Heteropneustes fossilis* (Bloch) using D-Lys salmon gonadotropin releasing hormone analog. *Aquaculture*, 29: 252-254.
- APHA 2012. *Standard methods for the examination of water and waste water*: 22nd edn. American Public Health Association, Washington DC, 1360 pp.
- Asha Dhawan and Kamaldeep Kaur 2004. Comparative efficacy of Ovaprim and ovatide in carp breeding. *Indian J. Fish.*, 51(2): 227-228.
- Basudha, C. H. and Vishwanath, W. 1999. Food and feeding habits of an endemic carp, *Osteobrama belangeri* (Val.) in Manipur. *Indian J. Fish.*, 46(1): 71-77.
- Billard, R., Alagarawami, K., Peter, R. E. and Breton, B. 1983. Potentialisation per le pimozone des effets du LH-RH-A sur la secretion gonadotrope hypophysaire l'ovulation et la spermiation chez la carpe commune (*Cyprinus carpio*). *C. R. Acad. Sci. Paris*, 296:181-184.
- CAMP 1999. Freshwater fishes of India: Report summary, 1998, *Zoo's Print J.*, 14:1-27.
- Chaudhury, H. and Alikunhi, K. H. 1957. Observations on the spawning of Indian carps by hormone injections. *Current Sci.*, 26: 381-382.
- Chauhan, R. S., Singh, V. K. and Singh, U. P. 1999. Performance of Ovatide – a new spawning formulation in induced breeding of *Labeo rohita* in Tarai agro-climatic region. *National seminar on Sustainable Aquaculture*, 21-22, Jan, 1999, Punjab Agricultural University, Ludhiana, p. 50.
- Devi, G. A., Devi, G. S., Singh, O. B., Munilkumar, S. and Reddy, A. K. 2009. Induced spawning and hatching of *Osteobrama belangeri* (Valenciennes) using Ovatide, an ovulating Agent. *Asian Fish. Sci.*, 22(4): 1107-1115.
- Dinesh Singh, K. and Mema Devi, W. 2012. Ultrastructure study of egg membrane in *Osteobrama belangeri*, Val. *IOSR J. Agr. Vet. Sci. (IOSR-JAVS)*, 1(4): 49-51.
- Ghosh, A. K., Biswas, S., Sarder, L., Sabbir, W. and Rahaman, S. M. B. 2012. Induced breeding, embryonic and larval development of koi carp (*Cyprinus carpio*) in Khulna, Bangladesh. *Mesopot. J. Mar. Sci.*, 27(1): 1-14.
- Gurpreet, S. T. and Sudhanshu, R. 2012. An economically viable approach for Induced breeding of *Labeo rohita* by Ovatide, Ovaprim and carp pituitary extract. *IOSR J. Agr. Vet. Sci. (IOSR-JAVS)*, 1(1): 30-32.
- Harvey, B. J. and Hoar, W. S. 1979. *The theory and practice of induced breeding in fish*, 48 pp.
- Menon, A. G. K. 2004. Threatened fishes of India and their conservation. *Zool. Soc. India*, 170 pp.

- Nandeesh, M. C., Das, S. K., Nanthaniel, E. and Varghese, T. J. 1990. Project report on breeding of carps with Ovaprim in India. *Special Publication No. 4. Asian Fisheries Society Indian Branch, Mangalore*, 41 pp.
- Peter, R. E., Chang, J. P., Nahorniak, C. S., Omeljaniuk, R. J., Sokolowska, M., Shih, S. R. and Billard, R. 1986. Interaction of catecholamines and sGnRH in regulation of gonadotropin secretion in teleost fish. *Recent Prog. Horm. Res.*, 42: 513-548.
- Ramaswamy, L. S. and Sundararaj, B. I. 1969 Induced spawning in the Indian catfish *Heteropneustes fossilis* with pituitary injection. *Acta Anat.*, 31: 551-562.
- Reddy, P. V. G. K. 2000. Captive breeding of *Osteobrama belangeri* (Val.): a threatened food fish. In: Ponniah, A. G and Sarkar, U. K. (Eds.), *Proceedings of fish biodiversity in North-east India*, National Bureau of Fish Genetic Resources, India, 228 pp.
- Reddy, A. K. and Mathur, K. B. 2000. Ovatide - a highly potent inducing agent for breeding of carps. *First Indian Science Congress*, 21-23 September, 2000, Punjab University, Chandigarh.
- Sharma, K., Yadava, N. K. and Jindal, M. 2010. Effect of different doses of ovatide on the breeding performance of *Clarias batrachus* (Linn.). *Livestock research for rural development*, vol. 22, Article 69.
- Suresh, V. R. 2000. Food habits and composite culture potential of an endangered medium carp *Osteobrama belangeri* (Val.) in Manipur. In: Ponniah, A. G. and Sarkar, U. K. (Eds.), *Proceedings of fish biodiversity in North-east India*, National Bureau of Fish Genetic Resources, India, 134 pp.
- Vishwanath, W. 2010. *Osteobrama belangeri*. In: *IUCN, 2012, IUCN Red List of Threatened Species, version 2012.1*.
- Yurembam Motilan, Yumnam Bedajit and Waikam Vishwanath, 2014. Induced breeding of giant zebra fish, *Devario acquipinnatus* (Mc Clelland, 1839) by oral delivery of synthetic gonadotropin releasing hormone analogue (Salmon-GnRH-a) Gonopro-FH. *Int. J. Scientific Res.*, 3(1): 502-503.
- Zairin, M., Furukawa, K. and Aida, K. 1992. Induction of ovulation by HCG in the tropical walking catfish *Clarias batrachus* under 23- 25°C. *Bull. Japan Soc. Sci. Fish.*, 59: 1681-1695.

Date of Receipt : 08.03.2013

Date of Acceptance : 05.09.2015