Asian Fisheries Science **22**(2009):1107-1115
ISSN: 0116-6514
E-ISSN: 2073-3720
https://doi.org/10.33997/j.afs.2009.22.4.005

Asian Fisheries Society, Selangor, Malaysia

Induced Spawning and Hatching of *Osteobrama belangeri* (Valenciennes) Using Ovatide, an Ovulating Agent

G.A. DEVI[1,2], G.S. DEVI[2], O.B. SINGH[3], S. MUNILKUMAR[2*] and A.K. REDDY[2]

[1]College of Fisheries, Central Agricultural University
Lembucherra, Tripura west 799 210 India
[2]Central Institute of Fisheries Education (Deemed University)
Indian Council of Agricultural Research
Seven Bungalows, Versova, Mumbai – 400 061 India
[3]Directorate of Fisheries, Government of Manipur
Imphal, India

Abstract

Osteobrama belangeri (Valenciennes), locally known as *Pengba*, is a highly esteemed and endangered indigenous minor carp of Manipur state in northeast India. A trial on induced spawning and hatching of this species was conducted using Ovatide, an ovulating agent. Complete spawning was observed within 8 hours and larvae hatched in 26 hours at 28-29°C. Results showed 95.0 ± 1.05% fertilization and 88.8 ± 1.04% hatching rate. The paper discusses the efficacy of the ovulating agent, Ovatide, and the potential for large scale seed production of *O. belangeri* to promote commercial culture as well as for conservation of this species.

Introduction

Most of the important species for aquaculture such as Asiatic carps, mullets, milk fish, etc are open water breeders and breed under the influence of environmental stimuli. They become refractory when subjected to

* Corresponding author. Tel.: +91 22 2636 1446, Fax: +91 22 2636 1573
E-mail address: munilkumar@gmail.com

confinement in ponds. They either do not mature completely or become incapable to reproduce in the confined environment. The stocking materials (seed) of these, therefore, have to be collected from their natural breeding grounds. There are constraints regarding poor quality seeds due to mixing of many other undesired species, insufficient quantities, etc and as a result, wide scale expansion of aquaculture is not possible. The major breakthrough achieved by Chaudhury and Alikunhi (1957) in induced breeding of Indian major carps using pituitary extracts has greatly contributed for the rapid development of carp culture in India. With over 90% of the population being fish consumers, there is a huge gap between demand and supply in Manipur, situated in the north eastern region of India. Of late, there seems to be an urgency to take up aquaculture activity on a larger scale, however, large scale quality seed production is difficult in the state. However, most of the seeds of Indian major carps and Chinese carps are produced by farmers using pituitary extracts in traditional hapas.

Osteobrama belangeri (local name *Pengba*) is indigenous to the state of Manipur. The state is located between 23°83'N to 25°68'N latitude and 93°03'E to 94°78'E longitude sharing a long border with Myanmar in the east. Imphal valley where most of the aquaculture activity is undertaken is located at an altitude of 790 m above mean sea level with a temperature range of 0 to 38°C. *Osteobraama belangeri* is almost extinct in the wild and it fetches a very high market price in Manipur. Even though *O. belangeri* is a highly esteemed fish in Manipur, wide scale culture of the fish is yet to be seen. The major constraints are the scarcity of seeds and suitable feed for its culture. Though there are unpublished reports on the breeding of *O. belangeri*, there is no standardized breeding technique using new synthetic ovulating agents. Earlier breeding trials of this species were reported by Reddy (2000), Munilkumar and Nandeesha (2007) and Samarjit and Basudha (2007). The state fisheries department has reported to have bred this fish with fish pituitary gland extracts. However, this experiment is the first attempt towards the commercialization of induced breeding of *Pengba* using synthetic hormone "Ovatide" at the field level.

In India, trials with Ovaprim which contain LH – RH analogues prepared by M/s Syndel Laboratories were very successful (Nandeesha et al. 1990). However the major prohibitive factors for Indian farmers were its high cost and its thick density that cause difficulty in injection. This prompted a Mumbai based pharmaceutical company, M/s Hemmo pharma to develop an indigenous hormonal formulation called "Ovatide" which was made more affordable (Reddy and Thakur 1998). The main composition of Ovatide is a synthetic peptide protein which is analogues i.e. struc-

turally related to the naturally occurring gonadotropin releasing hormone (GnRH) and a dopamine antagonist. It contains 20 µg of sGnRH and 10 mg of Domperidone per ml and appeared as an attractive inducing agent for such induced breeding programmes, and was found to be cost effective and successful as Ovaprim (Thakur and Reddy 1998). The study was under taken with the assistance of the enthusiastic and helpful state fisheries department personnel to standardize an induced breeding technique of *Pengba* for large scale seed production at the farmers' level.

Materials and Methods

Field trials with the new hormonal product-Ovatide for fish breeding were carried out at two different farms, one in a government fish farm at Lamphel, Imphal district and another at Tomba & Sons Fish Farm (Pvt.) at Ningombam, Imphal. *Pengba* broodstock for induced breeding trials were obtained from the farm ponds of the respective fish farms. The samples of Ovatide were made available to the Central Institute of Fisheries Education, Mumbai from M/s Hemmo Pharma in 10 ml vials. Ovatide contains 20 µg of sGnRH and 10 mg of Domperidone per ml.

Breeding trials were conducted in traditional cloth *hapas* of 2.0 x 1.0 x 1.0 m dimension and 16 no. mesh size. Hapas were fixed in ponds having stagnant water. Most of the breeding trials were taken by using the already available infrastructural facilities at the respective fish farms. Matured females and males were selected from the brood stock ponds following the method suggested by Jhingran and Pullin (1988) and kept in breeding hapas fixed in ponds 2-3 hours prior to the administration of hormone without aeration. The breeding set consisted males to female in a 2:1 ratio. The average weight of the female brooders was 0.17 kg ranging from 0.13 – 0.20 kg while the average weight of males was 0.12 kg ranging from 0.10 – 0.15 kg. Injections were administered intramuscularly in the region of the caudal peduncle above the lateral line with 2 ml hypodermic syringe and no. 22 needle. The doses of hormone were 0.6 $ml \bullet kg^{-1}$ for females and 0.3 $ml \bullet kg^{-1}$ for males. The injected fishes were released in the breeding hapas. Generally, fishes were injected between 14.00 and 18.00 hours. After the breeding, the water hardened eggs were transferred to double walled hapas for hatching. The double walled hapas were fixed in the same pond where breeding took place.

The eggs were collected from breeding hapas between 5.00 and 6.00 hours. The eggs were measured with 15 to 20 litre plastic buckets and a one litre mug was used to transfer the eggs into the double walled egg incubation hapas that were fixed in the ponds. The dimension of the outer hapa was 2.25 x 1.25 x 1.25 m with 20 mm mesh size while that of the inner hapa was 2.0 x 1.0 x 1.0 m with 1.5 mm mesh size. In each inner hapa, eggs from each set were stocked which ranged from 0.07 to 0.12 million. Fecundity is the number of eggs laid in a single spawn by an oviparous female fish (McFadden et al. 1965). The fertilization rate was estimated randomly taking 3 to 4 samples from each inner hapa by using the following formula as given by Suquet et al. (2005):

Fertilization rate (%) = (No. of fertilized eggs at gastrula stage X 100)/Total no. of eggs

The hatching percentage was calculated after harvesting the spawn on the 3rd day. The spawn was measured using measuring cups of a known volume. For estimation of hatching percentage, two samples of 1 ml each were counted individually from each hatching hapa. The formula used was as given by Suquet et al. (2005):

Hatching rate (%) = (No. of spawn X 100)/Total no. of eggs transferred for incubation

Data were analyzed using SPSS (16.0) version software to test the significance of variance and to obtain standard error of the means. One way analysis of variance was employed to test the performances of Tomba & Sons Fish Farm and the Manipur Govt. Fish Seed Farm on egg production and hatching performance at 0.05% level of significance.

Results

A summary of the results of trials on induced breeding of *pengba* using Ovatide are presented in tables 1 and 2. Complete spawning was observed within 8 hours ranging from 7.20 – 8.15 hours and larvae hatched out in 26 hours within a range of 25.15 – 26.40 hours at 28-29^{o}C. Breeding of 5 females with 10 males resulted in 95.0 ±1.04% fertilization and 88.8 ±1.04% hatching rate on the average. The average working fecundity was 527.6±18.69 thousand eggs•kg^{-1} of female broodstock. The average number of spawn produced per kg fish was 443.2 ±16.19 thousands.

Table 1. Summary of results on induced breeding of *O. belangeri* using Ovatide at Manipur Government Fish Farm and Tomba and Sons Fish Farm (private farm)

Parameters	Values are in Mean (± S.E.)			
	Manipur Govt. Fish Farm	Tomba and Sons Fish Farm (private farm)	Average of both fish farms	P-Value
Mean Female body weight (kg)	0.17 ± 0.02	0.17 ± 0.04	0.17 ± 0.02	0.88
Mean Male body weight (kg)	0.26 ± 0.02	0.20 ± 0.00	0.23 ± 0.04	0.16
Female dose (ml•kg^{-1})	0.60 ± 0.00	0.60 ± 0.00	0.6 ± 0.00	-
Male dose (ml•kg^{-1})	0.30 ± 0.00	0.30 ± 0.00	0.3 ± 0.00	-
Latency period (hrs)	7.62 ± 0.19	7.68 ± 0.48	7.64 ± 0.18	0.90
Working Fecundity ('000 eggs•kg^{-1})	546.0 ± 27.22	500.0 ± 0.00	527.6 ± 18.69	0.28
Fertilized eggs ('000 eggs•kg^{-1})	89.00 ± 13.58	77.50 ± 14.50	84.40 ± 9.18	0.61
Fertilization (%)	95.33 ± 1.2	94.50 ± 2.50	95.0 ± 1.05	0.75
Hatchlings production ('000 hatchlings•kg^{-1})	456.7 ± 21.7	423.0 ± 23.0	443.2 ± 16.19	0.38
Hatching rate (%)	88.33 ± 1.08	89.51 ± 2.55	88.8 ± 1.04	0.65

Discussion

The results clearly showed that even though breeding and hatching were done in traditional hapas, egg production, fertilization and hatching rates were quite high and these compared well with the average performance of carp breeding using a portable circular hatchery system as reported by Venugopal (1990). In terms of egg, the fish used at Manipur Government Fish Seed (MGFS) Farm gave a better result i.e. 546.0±27.22 thousands as compared with the fish bred at Tomba & Sons Fish (TSF) Farm, i.e. 500.0±0.0 thousand per kg, even though the results were not significant statistically (P>0.05). The fertilization rate was almost similar and high at the farms i.e. 94.5±2.50 % at TSF Farm and 95.3±1.20% at MGFS Farm. The average hatchling production per kilogram body weight at TSF Farm and MGFS Farm were 423.0±23.00 thousands and 456.7±21.70 thousands respectively. The hatching percentages were 89.5±2.55 % and 88.3±1.08 % at TSF Farm and MGFS Farm respectively. The latency period at both farms ranged from 7.15 to 8.15 hours.

Das et al (1994) reported induced breeding of *Puntius javanicus* which is a similar species with similar food and feeding habits as well as size. The study revealed the possibility of simplifying spawning and hatch-

Table 2. Trials on induced breeding of *O. belangeri* using Ovatide (OT) at Tomba & Sons Fish Farm (private farm) and Manipur Government Fish Seed Farm

Set no	Weight of fish (kg)		Average dose given (ml•kg^{-1})		Latency period (hrs.)	Total no. of eggs ('000)	Average no. of eggs ('000•kg^{-1})	Fertilization rate (%)	Total no. of good eggs ('000)	Average no. of good eggs ('000•kg^{-1})	Total spawn recovered ('000)	Average no. of spawns recovered ('000•kg^{-1})	Hatching rate (%)
	♀	♂	♀	♂									
Tomba & Sons Fish Farm													
Set 1	0.20	0.10 0.10	0.6	0.3	8.15	100.0	500.0	92.0	92.0	400.0	80.0	400.0	86.96
Set 2	0.13	0.10 0.10	0.6	0.3	7.20	65.0	500.0	97.0	63.0	485.0	58.0	446.0	92.06
Manipur Government Fish Farm													
Set 3	0.16	0.12 0.10	0.6	0.3	7.40	84.0	525.0	93.0	78.0	488.0	70.0	437.0	89.74
Set 4	0.15	0.15 0.10	0.6	0.3	8.00	77.0	513.0	96.0	73.0	492.0	65.0	433.0	89.04
Set 5	0.20	0.15 0.15	0.6	0.3	7.45	120.0	600.0	97.0	116.0	582.0	100.0	500.0	86.21
Mean values ±Std Error	0.17 ± 0.01	0.23 ± 0.02	-	-	7.6 ± 0.18	89.2 ± 9.56	527.6 ± 18.67	95.0 ± 1.05	84.4 ± 9.18	501.4 ± 20.91	74.6 ± 7.29	443.2 ± 16.19	88.8 ± 1.04

ing techniques of this species with a single dose of an ovulating agent "Ovaprim" [(S-GnRH+Domperidone; (Syndel Laboratory, Canada)]. The doses given were 0.35 ml•kg^{-1} for females (340 g) and 0.15 ml•kg^{-1} for males (105 g). The fish spawned within 4-5 hours with percentage of hatching ranging between 38.8 and 64.8% with an average of 53%. The fecundity of the fish weighing 240 g female was 169.41 thousands per kg with a fertilization rate of 73.24%. Hatching took place within 13-14 hours. In the present experiment, the doses of the ovulating agent "Ovatide" were 0.6 ml.kg^{-1} for female (0.17 ± 0.02 kg) and 0.3 ml•kg^{-1}for males (0.23 ± 0.04 kg). Breeding of *O. belangeri* is reported to have bred with 0.2ml•kg^{-1} initial dose and 0.4 ml•kg^{-1} resolving dose for females and a single dose of 0.4 ml•kg^{-1} for males at private farms (Singh, personal communication). Spawning is observed within 6 hours with a fecundity of 250,000•kg^{-1}. The fertilization rate is reported to be above 90% with 80% hatching rate within an incubation period of one and a half days at 24°C. Reddy (2000) reported the captive breeding of *O. belangeri* in Orissa with carp pituitary extracts with similar results of 75-80% fertilization rate and hatching within 13-14 hours. In the present experiment done in Manipur, complete hatching was observed within 26 hours at 28-29^{o}C. Variations may have been due to different environmental parameters at different places.

The major breakthrough achieved by Chaudhury and Alikunhi (1957) in induced breeding of the Indian major carps using pituitary extracts has greatly contributed to the rapid development of carp culture in India without having to depend heavily on the riverine collections of larvae /postlarvae, fry and fingerlings. According to Tripathi and Khan (1990), one of the promising hormones to induce breed carp was Luteinising Hormone-Releasing Hormone (LH-RH) and its analogues. Subsequent studies on the possibility of using LH-RH analogue combined with a dopamine antagonist for stimulation of gonadotropin release from carp pituitary in China led to the development of "Linpe method" (Peter et al. 1988). Based on these studies, Syndel Laboratories in Canada developed the product named "Ovaprim", which contains sGnRH and the dopamine antagonist domperidone. The successful application of Ovaprim in induced breeding of fishes in India is a well accepted fact (Nandeesha et al. 1990). In the meantime, Ovatide that has a similar chemical composition and lesser cost has also been equally effective in induced breeding of several species of fishes in India (Reddy and Thakur 1998). The successful induced breeding of *O. belangeri* with the help of Ovatide in traditional hapas indicates the possibility of adopting the technique by farmers with relative ease. The good results of *Pengba* breeding shows that commercial breeding pro-

grammes can be taken up for large scale commercial farming of this species as well as for stocking the natural waters as conservation strategy.

Acknowledgement

The assistance rendered by the farm owners and the officials of the State Fisheries Directorate, especially Mr N. Biramani Singh, former state Director of Fisheries, Manipur, Mr. Sagar Chandra Mandal, Assistant Professor, College of Fisheries, Agartala and Mr. S. Surchandra Singh, a progressive farmer is gratefully acknowledged.

References

Chaudhury, H. and K.H. Alikunhi. 1957. Observations on the spawning of Indian carps by hormone injections. Current Science 26:381-382.

Das, S.K., B.K. Bhattacharjya and K. Sarma. 1994. Induced spawning and hatching of Tawes, *Puntius javanicus* (Bleeker). Asian Fisheries Science 7:191-194.

Jhingran, V.G and R.S.V. Pullin. 1988. A hatchery manual for the common, Chinese and Indian major Carps. Asian Development Bank, Manila. 191 p.

McFadden, J.T., E.L. Cooper and J.K. Anderson. 1965. Some effect of environment on egg production in brown trout (Samo trutta). Limnology and Oceanography 10:88-95

Munilkumar, S. and M.C. Nandeesha. 2007. Aquaculture practices in Northeast India: Current status and future directions. Fish Physiology and Biochemistry 33:399-412.

Nandeesha, M.C., S.K. Das, E. Nanthaniel and T.J. Varghese. 1990. Project report on breeding of carps with Ovaprim in India. Special Publication No.4.AFSIB, Mangalore, 41 p.

Peter, R.E., H.R. Lin and G. Van der Kraak. 1988. Induced ovulation and spawning of cultured freshwater fish in China: advances in application of GnRH analogues and dopamine antagonists. Aquaculture 74:1-10.

Reddy, P.V.G.K. 2000. Captive breeding of *Osteobrama belangiri* (Val.)- A threatened food species . In: Fish Biodiversity of North East India. (ed. A.G. Ponniah and U.K. Sarkar), pp 122-123. NATP Publ.2. National Bureau of Fish Genetics Resources, Lucknow, India.

Reddy, A.K and N.K. Thakur. 1998. Ovatide- a new hormonal preparation for induced fish breeding. Fishing Chimes 18(4):28-30.

Samarjit, N.S and C. Basudha. 2007. Induced spawning of *Osteobrama belangeri* (Val.), a critically endangered fish in India using carp pituitary and Ovaprim. Aquacult (2):231-236.

Suquet, M., Y. Normant, J.L. Gaignon, L. Quemener and C. Fauvel. 2005. Effect of water temperature on individual reproductive activity of Pollack (*Pollachius pollacius*). Aquaculture 243:113-120

Thakur, N.K. and A.K. Reddy. 1998. Repeated field trials with new hormonal formulation - Ovatide for fish breeding. Central Institute of Fisheries Education, Mumbai. 48 p.

Tripathi, S.D. and H.A. Khan. 1990. Carp seed production technology – a review. In: Carp seed production technology. (ed. P. Keshavanath and K.V.Radhakrishnan), pp. 1-11. AFIB Special Publication No.2. Asian Fisheries Society, Indian Branch, Mangalore, India.

Venugopal, G. 1990. Field observations on the efficacy of "portable circular hatchery- a rural model". In: Carp seed production technology (ed. P. Keshavanath and K.V. Radhakrishnan), pp. 57-60. AFIB Special Publication 2. Asian Fisheries Society, Indian Branch, Mangalore.

Received: 19 March 2007; Accepted: 14 May 2009