



Captive breeding and Embryonic development of Endangered, *Osteobrama belangeri* (Val., 1844) under Mid-hill condition in North east India

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Received: 8 September 2016; Accepted: 16 June 2017

ABSTRACT

Osteobrama belangeri (Valenciennes) -an endangered indigenous medium carp of Northeast India was first introduced in the hill state of Meghalaya in 2011 to evaluate its performance under mid hill condition (900m above msl) in an effort to conserve this highly esteemed species through captive breeding and aquaculture. The species is endemic to the State of Manipur in the Northeast India bordering with Myanmar and is locally known as Pengba. In July 2014, *Osteobrama belangeri* –attained full maturity at the age of 30 months under pond condition and could be induced bred in captivity by means of an inducing agent, Gonopro-FH (Salmon GnRH). In this study, a maiden attempt has been made to describe different embryonic development stages of *O. belangeri* in controlled conditions. The paper also highlights a low-cost simple protocol for successful seed production of *O. belangeri* in an effort to conserve and promote the highly esteemed fish as a candidate species for mid hill aquaculture.

Key words: Captive breeding, Embryonic development, Mid hill aquaculture, *Osteobrama belangeri*

North Eastern Region of India is located between 21.57°N–29.30°N latitude and 89.46°E – 97.30°E longitude. The region, comprising eight states, namely Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura represent a diverse freshwater ecosystem and is considered as one of the hot spots of fresh water fish biodiversity in the world (Kottelat and Whitten 1996). There has been a wide variation in the number of fishes reported from this region ranging from 172 (Ghose and Lipton 1982) to 267 (Sen 2000). Although there are several potential fish species for aquaculture in the region, by and large, fish seeds of about 11–12 species are commonly produced by seed producers for aquaculture in the region (Das 2014). Use of aquaculture for conservation and recovery of endangered fish populations is highlighted by many scientists. There are several constraints in development of the fisheries and aquaculture sector in the hill states of Northeast region. In addition to difficult terrain, non-availability of quality fish seeds of suitable species in time, cold climatic conditions and lack of appropriate infrastructure facilities are identified as some of the limiting factors in expanding aquaculture.

Captive breeding for conservation and recovery of endangered fish population is gradually finding importance (Das 2000). In the state of Meghalaya, under mid altitude (900 m above mean seal level) a period of about 6-7 months in a year has been found suitable for growth of carp species (Das 2013) as temperature plays an important role in fish growth.

India harbours several indigenous major, medium and minor carp species in natural water bodies. Some of these carps species have been cultured on large scale in order to meet demand for human consumption as well as for conservation by initiating seed production through induced breeding technique using carp pituitary extract (Chaudhury and Alikunhi 1957). Potentiality of induced breeding of indigenous and exotic carps in India has been accelerated after introduction of Ovaprim as an ovulating agent (Nandeeshha *et al.* 1990) and in later years many other ready to use fish breeding hormones (Reddy and Thakur 1998, Das 2004) made significant impact on fish seeds industry of the country.

Osteobrama belangeri is an indigenous medium carp endemic to the state of Manipur in Northeast India. The species which is locally known as Pengba attracts good consumer demand and market price (USD 10–15/kg) for its taste. *O. belangeri* is reported to migrate from Chindwin river of Myanmar to the upstream of Imphal river of Manipur for breeding in flood plains in early monsoon. There are reports of occurrence of this fish in the Loktak lake and other adjoining lakes and tributaries of Manipur. However, in past few decades the population of Pengba in the natural waters of Manipur declined significantly and has now been listed as one of the endangered fish species of India (CAMP Report 1998). *O. belangeri* is a herbivore fish species feeding mainly on algae, aquatic plants, zoo plankton, diatoms etc. The average marketable size of the species range from 100 to 500 g.

Realizing the importance of *O. belangeri* in the region, a maiden effort was undertaken in 2011 to introduce the

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species in the state of Meghalaya as a potential candidate species for mid hill (900–1200 m above msl) aquaculture. The effort is not only to conserve this highly esteemed fish species through captive breeding and aquaculture but also to enhance fish production of the region. In July 2014, for first time the species was successfully induced bred in captivity for seed production under the mid hill condition using Gonopro-FH in a simple, low-cost and farmer friendly hatching system at the fish farm of ICAR RC for NEH region, Umiam, Meghalaya, India (Das and Ngachan 2014). Gonopro-FH- a synthetic peptide analogue (Salmon GnRH) is another potential inducing agent for fish breeding. Motila *et al.* (2014) successfully bred giant zebra fish, *Devario acquirpinatus* under captive condition through oral application of Gonopro-FH along with feed.

A number of attempts had been initiated in the past by several researchers to increase its population by induced breeding techniques in captivity using different ovulating agents like carp pituitary extract (Reddy 2000), carp pituitary extract and Ovaprim (Singh and Basudha 2007), Ovatide (Devi *et al.* 2009), Wova-FH (Behera *et al.* 2010) but no attempt was made under mid hill condition. Despite availability of adequate quantity of quality seeds of *O. belangeri* is still a far cry. Except Manipur no other major Indian states currently produce seeds of Pengba for aquaculture.

MATERIALS AND METHODS

In 2011, about 500 nos. of fry size (2–3 cm in length) *Osteobrama belangeri* were transported to ICAR Fish farm at Umiam, Meghalaya located at an altitude of about 900m above msl from Manipur by air and road. They were reared under monoculture system in an experimental earthen pond (20m length × 20m width × 1.5 m depth) until they attained maturity. The fishes were daily fed with azolla and supplementary feed (rice polish: mustard oil cake: fish meal at 1:1:1 w/w) to apparent satiation.

After a 30-month-rearing period, fully matured brooders of *O. belangeri* were harvested from the pond with a drag net for induced breeding trials. The Gonadosomatic Index, GSI was calculated following the standard formula: $[\text{gonad weight}/\text{total body weight}] \times 100$.

Altogether ten trials were conducted during July–August to confirm the best time for seed production under mid hill condition. Each breeding set comprised of one female and two males.

The weights of female and male brooders ranged from 110 to 220 g and 80 to 110 g respectively. The average weight of female brooder was 162.5 g while the male brooder was 97.1 g. The inducing agent, Gonopro-FH was administered in single dose to the brooders at different doses in the evening hours between 16.0 to 16.30hr. The injected breeding pairs (one female with two males) were released in a breeding hapa (1.5 × 2.5 × 3.0 m) fitted in an earthen pond. The brooders were removed from the hapas early in the morning and the eggs were collected manually to transfer the fertilized eggs to locally made hatching devices for

incubation. A simple hatching device was designed by modifying a 17 litres capacity plastic bucket (45cm in diameter). The fertilized eggs were kept in motion by fitting a water inlet at the bottom of the bucket and an outlet pipe to drain out the overflow water. In addition, the water was continuously oxygenated through a simple air pump commonly used in aquarium. The rate of water flow in the hatching bucket varied between 267 to 625 ml/minute during the incubation period. Water temperature, which is an important factor for successful breeding and hatching of fish eggs was recorded with a thermometer for every trial.

Fertilization rate was estimated randomly by taking 3 to 4 samples from the total eggs produced by the female in every trial following the formula given by Suquet *et al.* (2005):

$$\text{Fertilization rate (\%)} = \frac{\text{(no. of eggs at gastrula stage} \times 100)}{\text{total no. of eggs}}$$

Hatching rate was measured at the time of harvesting of spawn after 72 h of hatching i.e on fourth day of hatching. The number of spawns (4-day-old larvae) present in 1ml was recorded randomly 3 times. Hatching rate was measured according to the formula of Suquet *et al.* (2005):

$$\text{Hatching rate (\%)} = \frac{\text{(no. of spawn} \times 100)}{\text{total no. of eggs transferred for incubation.}}$$

The embryological developmental stages of *O. belangeri* were recorded (Table 1) soon after fertilization till hatching with the help of a binocular microscope (Magnus MSZ – TR, Olympus) and digital camera (Olympus).

The induced breeding data of *O. belangeri* were analysed statistically using prism 4 version.

RESULTS AND DISCUSSION

Under pond condition, Pengba was observed to attain full maturity in a growing period of 30 months. Male matured earlier than female. However, the peak breeding season was found to be very short (first week of July to second week of August) under mid hill condition. The month of July is the best time for induced breeding of matured Pengba. Similar to other carp species, Pengba also demonstrates secondary sexual characteristics such as roughness or softness of pectoral fins, slender or bulged abdomen etc. during the breeding season. The fully matured male freely oozes milt and the female releases a few ova when pressed gently along the abdomen. The Gonadosomatic Index, GSI varied between 19.8 to 23.15%.

In the breeding experiment, 10 gravid females and 20 fully matured males were selected and based on past experience, a single dose of hormone analogue Gonopro – FH was administered to both female and male fish. The female weighing between 110 g - 220 g total body weight received a dose of 0.7 to 0.8ml/kg body weight whereas the males received a dose of 0.4ml- 0.5ml/kg body weight.

Out of the 10 females, only 7 females responded with complete spawning. Latency period was 9.1 ± 0.09 h at a

Table 1. Developmental stages of embryo and fry of *Osteobrama belangeri*

Developmental stage	Time(h)	Plate	Remark
Fertilized egg	00.00	1.a	Spherical, non adhesive, translucent and demersal.
Two cell stage	00.20	1.b	First cleavage.
Four cell stage	00.30	1.c	Second cleavage.
Eight cell stage	00.47	1.d	Third cleavage.
Sixteen cell stage	01.12	1.e	Fourth cleavage.
Multicellular stage	01.31	1.f	Fast division of cells geometrically
Morula stage	02.34	1.g	A group of blastomeres in a cap shape in animal pole due to repeated cleavage.
Early gastrula stage	03.33	1.h	Blastoderm started invading yolk by spreading over the yolk in the form of a thin layer.
Middle gastrula stage	04.50	1.i	Formation of germinal ring around the yolk and half of yolk was occupied by blastoderm.
Late gastrula stage	05.51	1.j	Almost yolk was covered by blasoderm.
Yolk plug stage	06.46	1.k	Gradual spreading of germinal layer over yolk was completed. Rudiments of head and tail were differentiated.
organogenesis	07.20	1.l & m	Head and tail bud were differentiated; embryo became elongated and encircled the yolk.
Hatching stage	14.00	1.n	Head was prominent with rudiment eye vesicles; formation of somites and yolk sac was seen clearly.
Just before hatching	18.00	1.o	Eye vesicles were quite prominent, yolk sac was differentiated as primary and secondary lobes; tail was free from yolk sac; continuous and vigorous rotational movement of the embryo with beating caudal portion was noticed.
Just hatched out fry	19.00	1.p	Just hatched out sac fry was not pigmented and its size was 3.1 ± 0.04 mm.
3 day old fry		1.q	The size of 3 day old fry was 5.04 ± 0.02 mm. Eyes were darkly pigmented and on dorsal portion of the body become sparsely pigmented with dark spots. The fry became active with fanning of its pectoral fins, the air sac was prominently seen and opening of its mouth started. Absorption of yolk sac was nearly to complete.

mean water temperature of 25.3 ± 0.18 °C and at a pH range of 6.3 to 6.5. One of the female broods weighing 180 g released the highest number of eggs (Approximately 65,136 numbers) indicating high fecundity of the fish. The highest hatching rate of 98.92% was recorded at a water temperature of 25°C. The fertilized eggs are non adhesive, spherical, translucent and demersal measuring about 2.3 ± 0.06 mm in diameter. The average incubation period of eggs was observed to be 19.6 ± 0.4 hours while the average hatching rate was 61.9% at water temperature that ranged from 19.0 to 26 °C during incubation. In two of the trails, the low hatching percentage could be attributed to low water temperature (19.0° C to 20.5°C). Statistical analysis of correlation between female body weight and number of eggs spawned by a female individual was found insignificant ($r = 0.5304$; $P=0.2198$).

Different stages of embryological development are presented in the Fig 1; 2- cell stage and 4- cell stage were observed after 20 and 30 min of fertilization respectively. This was followed by 8- cell stage at 47 min and 16- cell stage at 72 min. Morula stage was recorded after 2 h 34 min of fertilization. Organogenesis was observed at 12 h of incubation with appearance of head and tail rudiments. Formation of somites, rudiment eyes and optic vesicle were prominent at around 14 h of incubation. After 15 h of incubation slight movement of embryo was recorded just behind the primary yolk sac and gradually moderate twisting movement of the embryo was clearly observed. After 18 h

the whole embryo could rotate inside the egg shell. In about 19 h hatching started and lasted for about 1–2 h. The size of just hatch out sac fry was 3.1 ± 0.04 mm and 3-day-old larvae measured 5.04 ± 0.02 mm.

In the present study it was found that Gonopro-FH was an effective inducing agent for induced breeding of *Osteobrama belangeri*. In our experiment a breeding hapa and a locally designed portable plastic bucket were used for egg laying, incubation and hatching. The use of portable rural model hatcheries for carp breeding and hatching has been reported by many researchers earlier (Venugopal 1990, Das *et al.* 1994, Das *et al.* 1998, Das 2002, Das 2003). In Orissa, induced breeding of *O. belangeri* with carp pituitary extract gave 75 – 80% of fertilization rate and incubation period lasted for 13 – 14 h (Reddy 2000).

In Manipur, an ovulating agent “Ovatide” was found to be effective in induced breeding of *O. belangeri* and recorded more than 90% fertilization rate and nearly 90% average hatching rate. The study also recorded a latency period of 8 h and an incubation period of 26 h at 28 – 29 °C (Devi *et al.* 2009). In another study, when Wova-FH - a potent ovulating agent was used @ 0.5ml/kg body weight of female, a fertilization rate of above 90%, a hatching rate of 85% and a latency period of 7 h with incubation period of 14 – 16 h at 28 °C were recorded (Behera *et al.* 2010). In the present experiment, the highest hatching rate of 98.92% was recorded at a water temperature of 25°C while the least hatching percentage of 5.94% was recorded in one of the

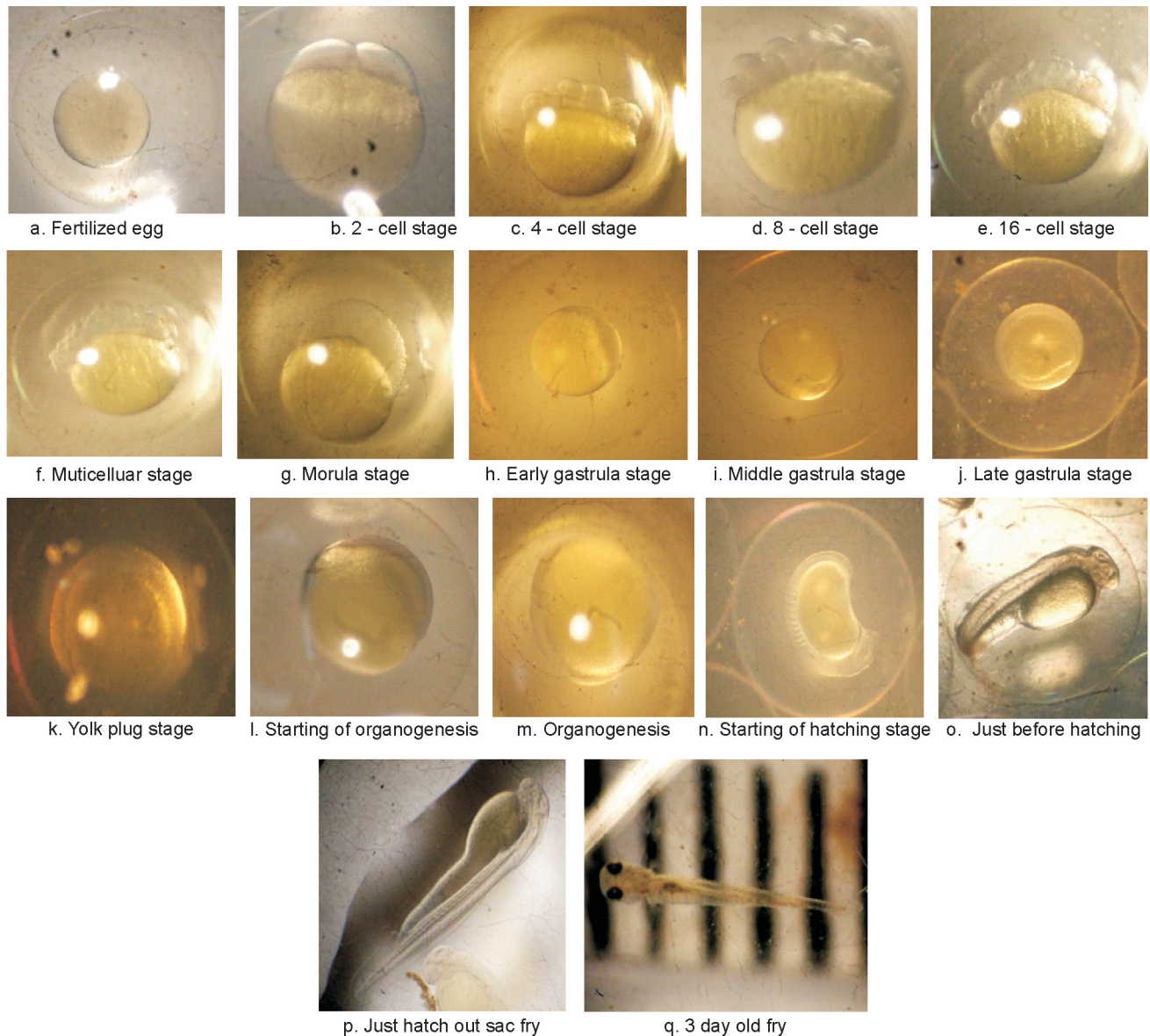


Fig. 1. Embryonic developmental stages and fry of *Osteobrama belangeri*, Pengba; a. Fertilized egg b. 2 - cell stage c. 4 - cell stage; d. 8 - cell stage e. 16 - cell stage f. Muticellular stage; g. Morula stage h. Early gastrula stage i. Middle gastrula stage; j. Late gastrula stage k. Yolk plug stage l. Starting of organogenesis; m. Organogenesis n. Starting of hatching stage o. Just before hatching; p. Just hatch out sac fry q. 3 day old fry.

breeding experiments which may be attributed to low water temperature (19–20°C). The average hatching rate for the eggs incubated in locally designed portable hatcheries was 61.9%. In a similar study, Das *et al* (1994) reported an average hatching percentage of 53% when eggs of *Puntius javanicus* were incubated in a low-cost, laboratory designed plastic tubs. In a similar study, low hatching rate was observed in scale carps when eggs were incubated at low water temperatures (Kaur and Toor 1980). Eggs of *Cyprinus carpio* also could not hatch when they were incubated in water temperature at 20°C (Hakim and Gamal 2009).

In the present study, it was observed that July with water temperature of around 25°C is the most suitable or the peak breeding season for the pengba fish (*O. belangeri*) under the mid hill climatic condition. The study has also

highlighted the different embryological developmental stages of *O. belangeri* under the mid hill climatic condition for the first time. The simple protocol presented in this study for production *O. belangeri* can easily be adopted by the farmers with minimum resources in order to conserve this species through aquaculture.

ACKNOWLEDGEMENT

The study was a part of the Institute project undertaken by the first author. The support and encouragement received from the Director, ICAR Research complex for NEH Region, Umiam, Meghalaya is gratefully acknowledged.

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