E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2017; 5(1): 428-432

www.fisheriesjournal.com
Received: 18-11-2016
Accepted: 19-12-2016

CH Basudha
ICAR Research Complex for NEH Region, Manipur Centre, Lamphelpat, Imphal, Manipur, India

NG Singh
Department of Agricultural Statistics, College of Agriculture, Central Agriculture University, Iroisemba, Imphal, Manipur, India

N Sobita Devi
ICAR Research Complex for NEH Region, Manipur Centre, Lamphelpat, Imphal, Manipur, India

CH Sinthoileima
ICAR Research Complex for NEH Region, Manipur Centre, Lamphelpat, Imphal, Manipur, India

Correspondence
CH Basudha
ICAR Research Complex for NEH Region, Manipur Centre, Lamphelpat, Imphal, Manipur, India

Induced breeding and embryonic development of an indigenous fish *Bangana dero* (Hamilton) in captivity using wova FH

CH Basudha, NG Singh, N Sobita Devi and CH Sinthoileima

Abstract
Bangana dero (Hamilton) is one of the most popular indigenous minor carps in the north eastern states of India. Induced breeding of *B. dero* was conducted by using Wova-FH in different doses @ 3.0ml, 5.0ml and 7.0ml per kg of female brooders in sets A, B and C respectively. All the males in each set were given similar dose @ 0.2ml/kg body weight. The fishes were spawns in different latency period i.e., 7 to 10 hours at 26°C. Fertilized eggs were hatched out after 12-18 hours of fertilization at 25.2-26.8°C. Stages of embryonic development were discussed in detail. Statistical analysis was worked out to determine the relation between the hormone dosage with egg output, fertilization rate and hatching rate. The highest number of egg output (17,790 eggs), fertilization (87.33%) and hatching rate (87.73%) were found in fish with hormone @ 0.5ml/kg female and significantly higher ($P<0.05$) than the other doses.

Keywords: *Bangana dero*, captive breeding, Wova FH, embryonic development

1. Introduction
Bangana dero (Hamilton, 1822) common name is *Kalabans* known as *Ngaton* in fry and fingerling stages and *khabak* in yearling and advanced stages in Manipuri, *Khital* (Tangkhul), *Ngatai* (Myanamar). Hamilton [7] described *Cyprinus, B. dero* from Brahmaputra River, India. It has treated as *Labeo* for sometime [22]. Subgenus *Bangana* has been upgraded to the genus status, and [12] recognized the species as *Bangana dero*. It is an endemic to Asia inland waters. Maximum standard length is 40cm. It is one of the most popular indigenous minor carps in the north eastern states of India. It is distributed throughout the Himalayan foothills, in India, Nepal, China [25] and Shri Lanka [23]. It is also found in Iran [5], Afghanistan [17] and Bangladesh [19]. There are reports on that the species also found in some of the national parks of Nepal, e.g., Koshi Tappu Wildlife Reserve, Chitwan National Park and Karnali National Park [21]. There is a need to improved habitat protection at sites where this species is known to occur. Further survey work is needed to determine whether or not this species is experiencing a decline, or is undergoing natural population fluctuations.
B. dero is an important food and game fish and has a ready demand in the local market fetch the triple prices of that of IMC. The fish occurs in the waters of the Litan, Iril, Thoubal, Sekmai rivers and its adjoining lakes in Manipur. It breeds during the south west monsoon season. Shoals of advanced fries and fingerlings of this fish species occur in the months of October and November every year. During last 1-2 decade the occurrence of fries and fingerlings of this high priced fish become reduced drastically. According to CITES (2013) [4] this fish species is categorized as Least Concern (LC).
B. dero is bottom feeder feeds on insect's larvae, molluscs, algae, zooplanktons, detritus bisexual and sexes can be distinguished during the breeding season. The pectoral fin of male has rough dorsal and the female dorsal side of the pectoral is smooth. The genital aperture of the female is reddish and swollen and has soft belly. In the case of male, the genital opening is not prominent however, when applying gentle pressure on the belly it oozes milt. The fish matured at the 2^+ years and males mature earlier than the females. It is riverine and seasonal spawners and spawns during south west monsoon (June-August). They spawned at the adjacent shallow inundated terrains during flood times. Adults inhabit torrential hill-streams in shallow waters. They migrate to warmer regions of lakes and streams during winter [2].

The present study aimed to investigate the effective dose of a synthetic hormone, Wova-FH for induced breeding of *B. dero* to find out the efficiency of spawning, fertilization and hatchability of the fish in captivity. The study also attempted to include the embryonic development and larval stages of this fish.

2. Materials and Methods

2.1. Brood fish

The experiment was conducted at ICAR Manipur Centre. Yearlings of *B. dero* 40-60g body weight (n=57) were collected from the Sekmai river at Sekmaijin during October-November, 2009 and transported in oxygenated poly bags. The fishes were stocked in earthern ponds having 10m x 12m with a water depth of 1.5m. Fishes were fed with pelleted diet having 30% crude protein at 3% body weight per day. One month prior the breeding experiment the male and female brooders were segregated and reared in separate ponds. *B. dero* is bisexual and sexes can be distinguished during the breeding season. The pectoral fin of fully matured male showed rough dorsal surface and the female dorsal side of the pectoral was smooth. The genital aperture of the female was reddish and swollen and has soft belly. On applying gentle pressure on the belly, eggs oozes out. In the case of male, the genital opening was not prominent however, when applying gentle pressure on the belly it oozes milt. The fish matured at the 2^{+} years and males mature earlier than the females.

2.2. Experimental design

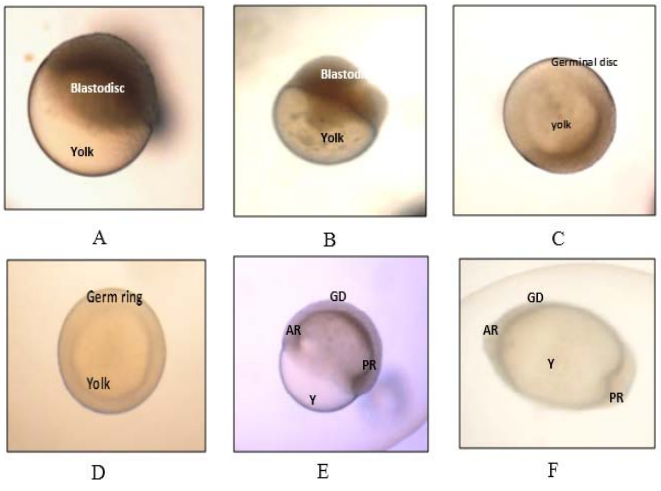
The breeding experiments were carried out in the month of July 2010. The brooders were collected from the earthern ponds by netting and transferred into fibreglass tanks for acclimatization for 5-6 hours. The three different doses of hormone, Wova-FH (Biostadt Agrisciences, Wockhardt Life Sciences, Mumbai India) were 0.3ml/kg, 0.5mlkg and 0.7ml/kg for A, B and C sets respectively for the females. All the males were given a similar dose of hormone @ 0.2ml/kg in all the treatments (Table 1).

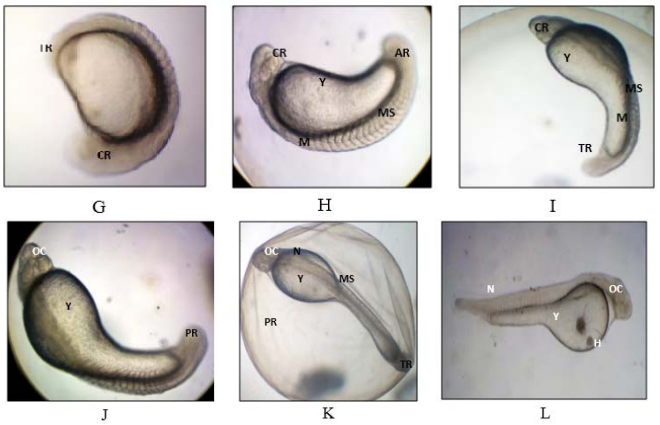
Table 1: Effect of hormone on egg output, fertilization and hatching rate of *Bangana dero* breeding

Sets	Hormone levels (ml/kg)	Latency period (hr)	Egg output (,000)	Fertilization (%)	Hatching (%)
A	0.3	10	13.79	53.93	70.00
B	0.5	7	17.49	87.33	87.73
C	0.7	8	13.30	62.73	76.40
SEm (±)			1.04	1.57	1.91
C.D $_{0.05}$			2.89	4.36	5.30
C.D. $_{0.01}$			NS	7.23	8.79

Table 2: Embryonic developmental stages of *Bangana dero* at water temperature of 25.2-26.8°C

Time (hour)	Development stage observed
0.30	Two celled stage
0.45	4- celled stage
1.10	16 celled stage
1.30	32 celled stage
1.40	Morula
2.0	Germ ring form
2.4	Gastrulation
7.0	Observation of embryo profile
8.16	The formation of somite head and tail bud stage
9.40	Somite stage completed
11.18	Prime stage (twitching movement)
12.10	Hatching 10%
12.40	Hatching 50%
18.00	Hatching 100%





Embryonic Development of *Bangana dero*

Plate: A. Early blastula stage, B. Mid blastula stage, C & D Gastrula stage germ ring formation, E. hpf embryo (late gastrula stage), F somite segmentation begin, G. 12 somites embryo with head and tail buds stage, H. somite stage (Embryo with caudal fin stage and elongated yolk), I Embryo with heart pulsation stage with complete tail, J 11.0 hours embryo with stretched tail, H. Prime stage (Heart pulsation stage embryo about to hatch out), L. 18.0 hours of hatch out embryo with yolk.

2.3. Selection of Brooders

36 brooder fishes were arranged in three sets at the ratio of 1:2 (Female:Male) in duplicate for each dose of hormone. Free oozing males and fully matured females having 66.0±2.3g were selected for this experiment. A female was considered ripe with well distended abdomen and eggs oozed out freely when gently press the abdomen while the genital papilla of ripe male was elongated, reddish in colour with oozing milt.

2.4. Hormone injection

Selected male and female in each set were injected require

doses using a 1ml graduated syringe intramuscularly at an angle of 45º at the dorsal side of caudal peduncle. Soon after hormone administration the fishes were release in breeding hapas.

After spawning, effective fecundity of each female was determined by random sampling of eggs in a 10ml graduated measuring cylinder from the total eggs released by the female. The number of total eggs in 1ml were counted and multiplied with total volume of egg released. The fertilization rate of eggs was determined by randomly tanking sample of approximately 100 eggs in a glass petridish. Fertilized were having intact nucleus inside the clear egg cells. The diameter of ova was measured by keeping the fertilized eggs in a row along the measuring scale under microscope (Olympus CX31). The diameter of each egg was calculated as total length of 20 eggs divided by numbers of eggs. After spawning the spent fishes were removed from each respective breeding *hapas*. The fertilized eggs were then transferred in hatching trays for hatching.

The water quality parameters of breeding ponds were analysed as per APHA [1] and maintained at water temperature 26±2.0°C, pH (7.5±0.1), dissolved oxygen (6.2±2.0ppm), total alkalinity (110±2.5ppm), and free carbon dioxide (2.2±0.2ppm).

2.5. Embryonic and Larval stages observation

Samples of eggs before fertilization and post fertilization at every 30- min interval were taken for further studies. In the present study, the developmental stages were divided into embryonic, larval and post larval development. The embryonic stage occurs inside the chorion and ends in hatching. The larval stage was characterized by nutritive contribution of the yolk sac and the stage ends when the larva becomes capable of exogenous feeding. The post larval stage begins immediately upon absorption of the yolk sac and was characterized by autonomous feeding. After, the yolk sac absorption, the larvae were fed with zooplankton.

Developmental time from post fertilization was rounded to the nearest minute until the morula stage and then to the nearest hour. The age of the larvae was denoted as hour after activation. Descriptions of the developing stages were made by examining live specimens under light microscope (Olympus CX31) and microphotographs of the developmental stages of eggs and larvae were taken. The specimens were measured by placing them over a slide having 1.0 mm graph paper at the bottom. Five to ten specimens were used to describe each stage. Five to ten egg specimens were used to describe each stage.

2.6. Calculation

Fertilization rate (%) = [(Number of fertilized eggs)/(total number of eggs counted)] x 100

Hatching rate (%) = [(number of eggs hatched)/(total number of eggs in the batch)] x 100

2.7. Statistical Analysis

Statistical analysis was worked out by using SPSS version 16.0 for windows. One-way ANOVA was used to analyse the variance to determine the relation between the hormone dosage with different parameters like fertilization rate, egg output, and hatching rate [6].

3. Results

The result of induced breeding of *B. dero* using different three doses of Wova-FH is presented in Table 1. The highest egg output, fertilization rate and hatching rate were found in set B and significantly higher than the egg output of set A and set C. However, there were no significant difference in egg output, fertilization and hatching rates in set A and C at ($P<0.05$).

Spawning commenced 7-10 hrs after injection and was completed within 4-5 hours. The fertilized eggs were bluish white in colour, demersal and translucent. Unfertilized eggs were paler and opaque. Fertilized eggs were hatched out after 12-18 hours of fertilization at temperatures of 25.2-26.8°C. The hatchlings were transparent and measured 3.20- 3.80 mm of total length with a large oval head, a well defined yolk sac and a short tail.

Embryonic development of *B. dero* was characteristics by the spherical pale cream-colored globular eggs of 0.9 to 1.0 mm. After fertilization the eggs absorbed water and become slightly bluish white in colour, demersal translucent and spherical in shape and measuring 1.8±0.2 mm in diameter. After few minutes of fertilization, cell division started. The blastodisc was contracted and a white dome was formed on the upper surface of the yolk. At about 30 min of fertilization, the first cleavage occurred, forming two large and approximately equal blastomeres. Twenty minutes later the second cleavage appeared. At about 1hour and 30minutes cleavage becomes irregular and in four hours' time the blastoderm was composed of a mass of relatively small but distinct cells. After one thirty minutes of development the cells have became relatively smaller owing to rapid cell division, and the margin of the blastoderm has begun to extend slightly farther over the yolk at 1hr 4 min. marula stage observed. At about 2 hours germ ring formation were observed. Gastrulating stage was observed at 2 hrs 40 min with 75% epiboly. Somite stage, segmentation begins at 3 hrs 20 min and completed of segmentation was observed at 9 hrs 4 min. The brain was clearly visible and the optic evaginations were somewhat oval extending posteriorly from the primary cerebral lobe. At 10 hrs 30 mins, development and appearance of heart observed. 11hrs18 mins to 12 hours, the embryo head formed a complete circle around the circumference of the yolk. Its tail was lying either to the right or left of the head and was often bent at an angle across the front of the latter. The pectoral fins appeared as fleshy folds lying over the yolk sac at the level of the auditory vesicle. Rhythmic movements occurred freely within the egg capsule. At 12 hrs 40 mins newly hatched larva of 1.3-1.7 mm was observed. The larvae took a sessile form and did not show free movement. Development of the larva and post larva eventually rupture the egg capsule. Total length was 1.3-1.7 mm. No visible pigmentation was found. The yolk sac beard large scattered. Newly-hatched larva freed itself by violent smashing actions of the tail. Swimming movements were somewhat restricted owing to the mass of yolk material. After 11- 13 hrs of hatching the fin folds were seen continuously around the tail. The vent and gill rudiments were formed. Gut was straight to slightly curve in anterior portion. Air bladder was shallow, behind pectoral region, which develop into two chambers in the post larval stage. The larvae attained a very little free movement with the help of fins. 2-4 day's old larva: The total length of the larvae was 2.5- 2.9 mm. The embryo showed a distinct reduction in the size of the yolk sac which had now become almost tubular due to its greater absorption anteriorly. 4-5 days old larva, total length of the larvae was 4.4- 4.8 mm.

4. Discussion

Induced breeding is a technique whereby sexually matured breeder fish are stimulated by using reproductive hormone or their synthetic analogs in brood fish through injection or supply in diet. The stimulation promotes timely release of sperms and gametes[14]. Induced breeding in captivity of many indigenous fish species were conducted successfully using different hormone *A. testudineus* using GnRH based drugs [10, 22], ovaprim [13]; *Clarias batrachus* using ovatide [20]. In the present study, Wova FH was used and most effective dose for *B. dero* was found to be 0.5ml/kg. The highest egg output in *B. dero* was found in set B i.e, wova- FH dose of 0.5ml/kg body weight and significantly higher than the egg output of set A (0.3ml/kg) and set C (0.7ml/kg). However, there was no significant difference in egg output in set A and C at ($P<0.05$). However spawning took places at lower dose of 0.3ml/kg as well as higher dose of 0.7ml/kg and similar to those reported by other workers for *A. testudineus* [13]. Nandeesha *et al.* (1990) [15] and Haniffa *et al.* (1996) [8] also used different doses of ovaprim (0.3-0.6ml/kg body weight) for induced spawning in carps and murrels. The results showed that complete spawning of *B. dero* occurred at the doses of 0.3ml/kg to 0.7ml/kg and the dose of hormone affected significantly the percentage of fertilization, egg output, hatching rate and hatching production respectively.

From the present experiment, 0.5ml/kg body weight of female and 0.2ml/kg body weight for male exhibited encouraging results for induced spawning and hatching and may be used as a standard dose. It can be utilized for re-establishment, conservation of this fish species. It can be recommended for commercial seed production of *B. dero* in captivity also.

In the present study, the latency period of *B. dero* was 7-10 hours. Udit *et al.* (2014) [24] observed the latency period of *Puntius sarana* was also to be 8 to 9 hours. However, the latency period of *Ompok pabda* was found to be 6 to 8 hours when ovatide hormone used [18]. The latency period was also found to be 5 to 6 hours in *Botia dario* when injected with 0.025ml WOVA-FH. According to George and Chapman (2013) [9] developmental rates are extremely temperature dependent and small changes in temperature can have large effects on developmental time and temperature difference of > 3ºC resulted in a 15-17 hour difference in hatching time.

The fertilized eggs of *B. dero* were transparent and unfertilized ones were opaque and white. Similar type of captive breeding, embryonic development, fecundity, fertilization rate and hatching rate were reported [11]. The initial stages of embryonic development are almost identical for both lower and higher level of invertebrates. Fish embryonic development consists of seven stages leading to hatching. These stages are period of zygote, cleavage, blastula, gastrula, the segmentation, pharyngula period and finally hatching. The egg hatched out in 18±2 h after fertilization at 26±0.5ºC and yolk-sac was completely absorbed in 48 hours of post fertilization. The survival of larvae reduced after 5 days. The fertilization was recorded 70-87%. The blastodisc was clearly distinct from the yolk-sac. The development in egg started within half hour of fertilization. Many workers described the larval and embryonic development of varieties of fish species. *Ompok bimaculatus* development was described by Parmeswaran *et al.*, (1967) [16] and *O. pbda* by Chakrabarty *et al.*, (2007) [3]. Within 48-72 hours the external indicators of the fish developed gills, jaw and pectoral fin have been grown at an accelerated rate. Once development has completed the fish was ready to hatch.

The results of the present study will provide highly relevant information for the designing and improvement of environmental condition for breeding and hatching operation of *B. dero* which will lead to a better understanding for quality fish seed production of this fish species in the region.

5. Acknowledgements

The authors acknowledge the financial support from Department of Science and Technology, Government of India (Sanction order No. DST/TDD/2K12/167 dt. 25/05/2014). The authors are thankful to Dr. S.V. Ngachan, Director, ICAR Research Complex for providing necessary facilities for conducting the study.

6. References

1. APHA. Standard methods examination of Water and wastewater. American Public Health Association, New York, 20th editions. 1998, 141.
2. Basudha Ch, Geetakumari Kh, Sobita Devi N, Sureshchandra Singh N, Ngalaton A, Singh IM. *Bangana dero* (Hamilton 1822): Production Technology for diversified aquaculture in North East India. Technical Bulletin No.RCM(TB)-**10**, ICAR Research Complex for NEH Region, Manipur Centre, Lamphelpat Imphal. 2016; 795004: 35.
3. Chakrabarty NM, Mondal SC, Chakrabarty PP. Artificial breeding and rearing of butter fish *Ompok pabda*-a significant mile stone in technology advancement. Fishing chimes. 2007; 26(10):134-136.
4. CITES. Appendices I, II and III valid. UNEP. 2013, 46.
5. Coad BW. Freshwater fishes of Iran. Acta Sci. Nat. Acad. Sci. Brno. 1995; 29(1):1-64.
6. Duncan DV. Multiple ranges and multiple F-test, Bionomics. 1955; 11:1-42.
7. Hamilton F. An account of the fishes found in the river Ganges and its branches. Archibald Constable & Co., Hurst, Robinson & Co. 1822; 1(7):1-405.
8. Haniffa MA, Merlin Rose T, Shaik MJ. Induced of ovulation in *Channa striatus* by salmon gonadotropin releasing hormone analogue (sGNRH). Fish. Chimes. 1996; 16:23-24.
9. George AE, Chapman DC. Aspects of Embryonic and Larval Development in Bighead Carp *Hypophthalmichthys nobilis* and Silver Carp *Hypophthalmichthys molitrix*. PLoS One. 2013; 8(8).e73829. doi:10.1371/journal.pone.0073829
10. Loh JY, Ting AY. Comparative study of analogue hormones and embryonic, larvae and juvenile development on the induced breeding of climbing perch, (*Anabus testitudineus*, Bloch, 1792)). Intern. J. Fish. Aquat. Sci. 2015; 2:277-278.
11. Kimmel CB, Willium WB, Kimmel SR, Ullmann B, Schilling TF. Stages of embryonic development of the zebra fish. Developmental Dynamic. 1995; 203:250-310.
12. Kullander OS. Fang F, Delliny B, Ahlander E. The fishes of Kashmir Valley. In: River Jhelum, Kashmir valley, Impacts on aquatic environment. Lenart Nyman (ed.). 1999, 99-163.
13. Kumar K, Mohanty UK, Dasgupta S, Sahu AK. Induced spawning of *Anabus (A. testudineus*, Bloch) under captivity in pre-monsoon and monsoon months. J. Inland Fish. Soc. India. 2010; 42(2):8-13.
14. Murthy KS, Venkateshwaralu M, Kiran BR. Induced

breeding of Indian major, minor and cyprinid carps in India: an overview JIARM1. 2013; (6):165-193.
15. Nandeesha MC, Das SK, Nathaniel DE, Varghese TJ, Shetty HPC. Breeding of carp with ovaprim standardization of dosage and nationwide demonstration (abstract). World Aquaculture Society, Halifax, Canada. 1990, 81.
16. Parameswaran S, Selvaraj C, Radhakrishan S. A review of the freshwater fishes of the genus *Ompok*. J Zool Soc India. 1967; 19(1-2):89-98.
17. Petr T. Coldwater fish and fisheries in Afghanistan. In: T.Petr (ed.) Fish and fisheries at high altitudes: Asia. FAO Fish. Tech. Pap, No. 385. FAO Rome. 1999; 304:138-148.
18. Purukayastha S, Sarma S, Sarkar UK, Lakra W, Gupta S, Biswas SP. Captive breeding of endangered *Ompok pabda* with ovatide. J. Appl. Aquac. 2012; 24(1):42-48.
19. Rahman AKA. Freshwater fishes of Bangladesh. Zoological Society of Bangladesh. Department of Zoology, University of Dhaka. 1989.
20. Sharma K, Yadava NK, Jindal M. Effect of different doses of ovatide on the breeding performance of *Clarias batrachus* (Linn.). Livestock Res. Rural Dev. 2010; 22(4):69.
21. Shrestha J. Coldwater fish and fisheries in Nepal. FAO Fish. Tech Pap. 1999; 385:13-40.
22. Singh YB, Saha H, Mandal B, Tandel R. Breeding of climbing perch (*Anabus testudineus* Bloch, 1972) induced with ovatide. The Isareli Journal of Aquaculture-Bamidegeh. 2012; 64:1-4.
23. Talwar PK, Jhingaran AG. Inland fishes of India and adjacent countries. A.A. Balkema Rotterdam. 1991; 1:541.
24. Udit UK, Reddy AK, Kumar P, Rather MA, Das R, Singh DK. Induced breeding, embryonic and larval development of critically endangered fish *Puntius sarana* (Hamilton, 1822) under captive conditions. The journal of Animal & Plant Sciencs. 2014; 24(1):159-166.
25. Zhu SO. Synopsis of freshwater fishes of China. Jiangsu Science and Technology Publishing House. 1995; 1(4):1-549.