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RESEARCH ARTICLE

Captive Breeding and Embryonic Development of Butter Catfish (*Ompok bimaculatus*, Bloch 1794), a Threatened Fish of Indian Sub-continent in Northern India

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Abstract The captive breeding of butter catfish (*Ompok bimaculatus,* Bloch 1794), a threatened silurid of Indian sub-continent was successfully carried out at the National Bureau of Fish Genetic Resources, Lucknow. Ten induced breeding trials conducted on the fish revealed that the fish can be naturally spawned in controlled conditions using sGnRH analogue and dopamine antagonist. The most suitable and economical dose estimated was 0.7 ml kg^{-1} body weight for female and 0.5 ml kg^{-1} for male. The latency period for spawning was 7–8 h at temperature 27 ± 0.5 °C and fertilization and hatching rates were found in the range of 75–90 % and 80–90 % respectively in flow-through system. The egg hatched out in 21 ± 1 h post fertilization (hpf) at temperature 27 ± 0.5 °C and yolk-sac was completely absorbed in 48 hpf. The larval survival reduced considerably after 5 days and was recorded 10.4 % after 10 days rearing, reason being, poor food acceptability and cannibalism. The fish responded well when injected with hormonal doses within 36 h of procurement from the pond but thereafter did not respond probably due to stress factor. These trials may be useful in standardizing the ex-situ breeding protocols for *O. bimaculatus*.

Keywords *Ompok bimaculatus* · Threatened fish · Butterfish · Captive breeding · Embryonic development

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Introduction

The butter catfish, *Ompok bimaculatus* (Bloch, 1794) is a threatened silurid of Indian sub-continent [1]. It is commonly found in India, Pakistan, Bangladesh, Myanmar, Sri Lanka and Afghanistan [2–4]. The fish is widely distributed in the plains and submontane regions and commonly found in streams, rivers, canals, bheels, Jheels, reservoirs and tanks [5]. The fish breeds in large water bodies particularly flowing rivers and do not spawn under captive conditions. It is considered a delicacy in many parts of India, particularly north-eastern states where it fetches very high market price and has been prioritized as a fish for freshwater aquaculture [6]. However, the population of this species has declined tremendously in last 5 decades to more than 50 % [7, 8] and it has been listed among the 120 endangered fish species of India [9]. In order to meet the demand and also to reduce pressure on natural resources for survival of the species, the ex-situ breeding has been considered a useful tool in the conservation of the species [10, 11]. Hence, there is a need for the development of ex-situ seed production technology so that the species can be cultured as well as used for ranching programmes for its conservation. The Government of Tripura has declared this fish as the State fish to gear up its conservation in natural waters and exploring aquaculture potential. The life history and biology of *O. bimaculatus* has been described by various workers [12–15]. A good number of workers had taken up trials on captive breeding of *O. pabda* [11, 16–21], however, a few attempts have been made on *O. bimaculatus*, where captive breeding has been carried out either by stripping or using aquatic substratum like *Eichhornia crassipes* and *Hydrilla verticellata* [8, 22, 23]. However, these practices are not only cumbersome but also show the sacrifice of the male fish. Therefore in the present

study, the captive breeding trials were conducted to assess if the fish can be spawned naturally in controlled hatchery conditions with or without substratum. Such work is of practical importance for up-scaling into simple farmer friendly practices for captive propagation of *O. bimaculatus*.

Material and Methods

The juveniles of *O. bimaculatus* were collected from river Gomti in Lucknow during January 2012 and reared in an earthen pond of 0.02 ha along with the brooders of rohu (*Labeo rohita*). The fishes were fed commercial floating fish pellets (ABIS, Proteins 32 %, Fat 3 %, Fibre 4 %, Moisture 10 %) of 2 mm size daily specially made for carps. No other feed was given to *O. bimaculatus*. The juveniles of *O. bimaculatus* were assessed for development of maturity every month and were found completely mature in the 1st week of July 2012. Fully mature brooders were harvested from the pond and shifted in the hatchery shed in FRP tank of size $6' \times 4' \times 3'$ with flow-through arrangement water system from a deep tube well. Induced breeding trials were then conducted in three sets of trial using sGnRH analogue and domperidone (Ovaprim; manufacturer Mac Mohan Pharma Ltd., Hyderabad, India) as inducing hormone. In the first trial, 4 pairs (Pair nos. I–IV) of brooders (each pair comprising of one female and two males) were injected with varying doses of hormone (0.5 ml to 0.8 ml per kg body weight) in case of female fishes. All the four pairs were given hormone injection on the day of harvesting from the pond and provided shelter of submerged weed, *Hydrilla reticulata* to serve both as natural shelter and substratum for egg sticking. The second trial was taken up after 4 days of the first trial, in which three pairs of brooders (Pair nos. V–VII) harvested at the time of first trial and stored in FRP tank for 4 days were given hormonal injection @ 0.7 ml kg^{-1} bw in females. The dose of hormone was selected on the basis of successful spawning obtained in the first trial. No shelter of aquatic vegetation was provided in this trial. In the third trial, three pairs (Pair nos. VIII–X) of freshly pond harvested brooders were given hormonal injection at the same rate as in trial no. 2 in order to ascertain whether cause of failure of spawning in trial no. 2 was due to stress condition resulting on account of keeping the brooders in captive conditions in FRP tank for 4 days or otherwise (Table 1). Further in order to confirm whether there was any effect of providing shelter of aquatic vegetation on induced spawning, these pairs were kept without any shelter. All the brooders (both females and males) were given single injection of hormone at the same time. The males were administered hormone @ 0.5 ml kg^{-1} bw in all the pairs. Each brooder pair was kept in FRP tanks of 300 l capacity having provision for flow-through water system (exchange rate 50 l/h) from a deep tube well water source. The tanks were lined with a bolting silk hapa for filtration of water as well as for easy harvesting of larvae and covered from top either with green house netting of 75 % darkness (pair nos. I, III, V, VII and VIII) or translucent mosquito netting cloth (pair nos. II, IV, VI, IX and X) to observe the affect of light condition during spawning activity. Translucent netting at the top also provided facility to observe the spawning behaviour of fish.

Spawning behaviour was observed in those breeding sets that were covered with translucent mosquito netting cloth. The egg hatching and larval rearing was taken up in the same tanks that were used for spawning. The fertilization rate was counted by collecting random sample of eggs from the tank bottom. The developmental stages of eggs were recorded using compound microscope with digital camera (Labomed). 20 eggs were procured from the breeding tank and observed every hour under the microscope for embryonic development. The yolk-sac absorbed larvae were harvested from the hapa, counted and again released in the same FRP tank. Ocular micrometer pre-calibrated with stage micrometer was used to measure the size of eggs and the larvae. In total 10 eggs or larvae ($n = 10$) were measured to get the average size. The yolk-sac larvae were fed *ab-libitum* daily with plankton collected from a pond and boiled hen's egg yolk. The fry were harvested on 10th day and counted for survival before release in the cement tank for further rearing. The water quality of hatchery was measured for temperature, pH, electrical conductivity (EC), total hardness and dissolved oxygen using multi water parameter (Thermo).

Results and Discussion

The brooders attained maturity in the 1st week of July when females were found to have large bulged abdomen, prominently on left side of the body with round light pinkish genital papilla. The males on the other hand had long pointed genital papilla; stouter and rough pectoral fins in comparison to females. The other secondary sexual characteristics observed were the same [23]. There are a few reports to confirm that *Ompok* spp. matures in May and June in carp culture ponds in North-eastern part of India [16, 21, 22] but such literature does not exist for northern regions and will be useful in further studies.

Effect of Hormonal Doses

The most appropriate and economical dose of sGnRH analogue and dopamine antagonist was estimated to be 0.7 ml kg^{-1} bw for females and 0.5 ml kg^{-1} bw for males as all the fishes injected with 0.7 and 0.8 ml kg^{-1} bw

Table 1 Induced breeding details of *Ompok bimaculatus*

Pair no.	Female weight (g)	Hormone dose (ml kg^{-1})	Male weight (g)	Hormone dose (ml kg^{-1})	Spawning result	Latency period (h)	Fertilization (%)	Hatching duration (h)	Spawn procured d (no)	Remarks
I	55	0.5	50 45	0.5	No spawning	–	–	–	–	Fishes were induced bred same day after collection from pond. Tanks were provided with shelter of *H. verticellata*. Pair nos. I & III were covered with green netting and II & IV with translucent cloth.
II	60	0.6	60 45	0.5	No spawning	–	–	–	–	
III	60	0.7	55 50	0.5	Spawned completely	7.30	85	22	900	
IV	70	0.8	70 55	0.5	Spawned completely	8.00	90	22	1,200	
V	40	0.7	35 35	0.5	No spawning	–	–	–		The fishes were stored in FRP tank for 4 days prior to induced breeding. No shelter was provided. Pairs V & VII were covered with green netting and VI with translucent cloth.
VI	35	0.7	30 30	0.5	No spawning	–	–	–		
VII	35	0.7	85 35	0.5	No spawning	–	–	–		
VIII	35	0.7	50 30	0.5	Spawned completely	7.30	95	22	450	Fishes were induced bred same day after collection from pond. No shelter provided. Pair nos. VIII provided green netting and IX & X translucent cloth.
IX	30	0.7	35 30	0.5	Spawned completely	7.30	95	22	350	
X	35	0.7	30 35	0.5	Spawned completely	8.00	95	22	450	

spawned naturally irrespective of light or dark condition and with or without substrate conditions in the spawning tanks. The females that were given low hormonal dose of 0.5 and 0.6 ml kg^{-1} bw did not respond both in light and dark conditions and also with substrates condition in spite of showing vigorous spawning behaviour. Sridhar et al. [8] however, used dose rate of 0.5 ml kg^{-1} bw of similar hormonal formulation for breeding one pair of this species in cement tanks provided with aquatic vegetation of *H. reticulata* and *E. crassipes,* whereas, Banik et al. [23] used varying doses of Ovaprim, ranging from 0.5 to 1.5 ml kg^{-1} bw, without any significant difference in egg laying, fertilization and hatchling production using stripping for artificial mixing of gametes. The fish has been reported to breed first time in captivity using pituitary hormone [13] and thereafter with synthetic hormone, Ovaprim by Sridhar et al [8] and Banik et al. [23]. In comparison to *O. bimaculatus*, there are a good number of reports on captive spawning of *O. pabda*. Hormonal formulations, Ovaprim and Ovatide have been reported to precipitate spawning in *O. pabda* with dose rates of 1.0–1.5 ml kg^{-1} [17, 19], 0.5–2.0 ml kg^{-1} [24], 0.4–0.6 ml kg^{-1} [20], 0.4–0.7 ml kg^{-1} [21] and in the case of *O. malabaricus* 0.5 ml kg^{-1} [25] under captive conditions and thus a dose rate ranging from 0.4–2.0 mg kg^{-1} bw suggested by the earlier workers is confusing particularly when these workers adopted stripping method for the extraction of ova and milt by sacrificing the males. Since, there is no earlier report of natural spawning of this species in clear water by application of inducing hormones; the suggested hormonal dose rate and the present method of spawning in clear water will be highly suitable in the controlled conditions.

Effect of Light, Darkness and Stress Conditions on Spawning

Out of five sets kept in darkness, only two sets spawned and laid fertilized eggs. Similarly, under light condition three sets out of five spawned and laid fertilized eggs. Hence, no significance could be established with relation to effect of light and darkness on induced spawning. Probably, the reason of non-spawning could be linked to stressed condition or low hormone dose as breeding did not occur in those pairs that were either kept in FRP tanks for more than 4 days prior to hormonal treatment or subjected to low hormonal doses (Table 1).

Breeding Behavior

Initially, all the fishes were found resting on one corner of the tank. On the arousal of breeding stimulus after around 6 h of giving hormone injection, the fishes started moving all around the tank with short resting at the bottom corners of the tank and occasional chasing by dominant/bigger male to smaller ones. Within half an hour, the chasing activity of dominant/larger males became more aggressive when female fish started moving at the water surface. At this time, on one hand the female was chased by both the males and on the other hand, dominant/larger male used to chase the smaller male also to push him away from female thus showing competitiveness for spawning. This was followed by release of eggs by female at the water surface when the following male(s) released the milt at the water surface immediately with its jerky swimming and fanning movements. The spawning activity persisted for around 45 min till female released all the eggs. Thus, the spawning was completed in 7.30–8.00 h of giving hormonal injection. The fishes then came to resting stage and gradually settled at one corner of the tank. The spawning movements were observed similar in all the sets instead of the fact whether the female fish spawned eggs or not.

Egg Quality and Embryonic Development

The eggs were found pale yellow, demersal and sticked to substratum (in case with *Hydrilla*) as well settled at the tank bottom, however, all of them settled at the bottom more particularly on tank corners or hapa folds when no substratum was provided. They were approximately 1.3 ± 0.1 mm in diameter, roughly rounded and due to a coating of little adhesiveness, trapped within debris. The fertilization was recorded 85–95 %. The blastodisc was clearly distinct from the yolk-sac (Fig. 1a). The development in egg started within an hour of egg laying, however, due to sticky nature of egg and large size together with heavy weight of yolk-sac, the formation of initial cell development up to morula stage was not distinct in the embryo and hence could not be photographed under the microscope. The development in embryo became distinct from morula stage onwards that reached within 3 hpf (Fig. 1b). At this stage the crown of the blastoderm starts spreading over the yolk in the form of a thin layer and the anterior and posterior ends of the embryo became differentiated and the embryo reached blastula stage within 4 hpf (Fig. 1c). The blastoderm further spread over the yolk and formed a germinal ring and more than half portion of the yolk was invaded and the head and tail ends of embryo became clearly distinguishable within 5 hpf (Fig. 1d). The embryo thereafter reached gastrula stage with complete closing of blastopore within 6 hpf (Fig. 1e). The further elongation of blastoderm gave rise to a bean-shape to the developing embryo in 12 hpf (Fig. 1f). At this stage, the antero-posterior axis was distinguishable in broader cephalic region and narrow end as tail region. The anterior protuberance formed a head fold and the posterior part

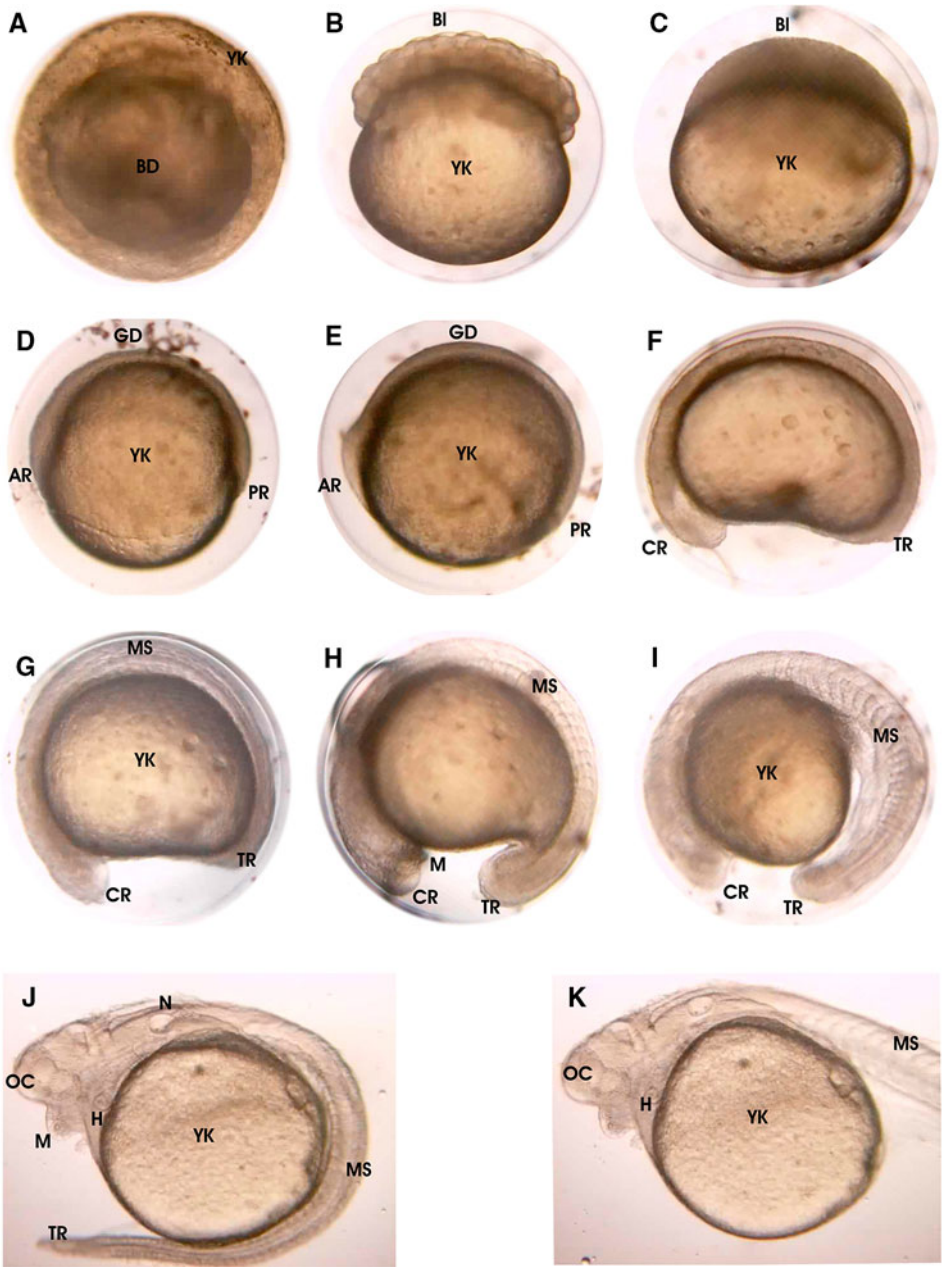

Fig. 1 Embryonic development of *O. bimaculatus*. *Plate a* Blastodisc, *Plate b* Morula stage, *Plate c* Blastula stage, *Plate d* Gastrula stage,*Plate e* 6 hpf embryo, *Plate f* 12 hpf bean-shape embryo, *Plate g* 14 hpf embryo, *Plate h* 16hpf embryo, *Plate i* 17 hpf embryo, *Plate j* 20 hpg embryo with complete tail, *Plate k* 20 hr embryo with stretched tail. *AR* anterior region, *Bl* blastoderm, *BD* blastodisc, *CR* cephalic region, *GD* germinal disc, *H* heart, *MS* mesodermal somites, *M* mouth position, *N* notochord, *OC* optic cups, *PR* posterior region, *TR* tail region, *Yk* yolk-sac

elongated to form the tail fold. At 14 hpf, the cephalic region became prominent with slight elongation from the yolk mass. The optic cups, brain, notochord and mesodermal somites were discernible; however, they were not very prominent (Fig. 1g). At 16 hpf, both head and tail ends further elongated and separated from the yolk-sac. At this stage, the optic cups, brain, heart, notochord and somites (15–16) were highly discernible. The heart beat also initiated at this stage but was very slow (Fig. 1h). The tail started detaching from the yolk-sac at 17 hpf from the middle portion. However, the end points of tail remain attached at this stage. Head further elongated in size showing all parts of brain, optic vesicle, heart, notochord and 24–25 somites. The beating of heart intensified and tail showing rhythmic movement on both sides one by one (Fig. 1i). The head region became more widened thereafter and showed clearly the mouth region, all parts of brain and heart (Fig. 1j). The lashing movement of tail also intensified leading to hatching of embryo from the vitelline membrane at 20 hpf at 27 ± 1 °C (Fig. 1k). The larval and embryonic development of *O. bimaculatus* was described first by Parmeswaran [5]. The overall embryonic

development in case of *O. bimaculatus* was found similar to *O. pabda* as reported by Chakrabarty et al. [26, 27]. Not much variations in hatching time (20 hpf at 27 ± 1 °C) was observed in the present study as the hatching was carried out in a flow-through system with facility of fresh tube well water from a deep bore well which was comparatively better from the findings of Chakrabarty et al. [26], who reported this to range between 20 and 30 hpf at temperature range of 27–30 °C in case of *O. pabda*. The hatchlings were 2.5 ± 0.1 mm in total length. They swarm very fast from the bottom reaching to water surface (depth 1.5–2.0 feet) and rest on their lateral side due to heavy yolk attached to their body. The yolk-sac was completely absorbed within 48 hpf and larvae started movements in search of food.

Larval Production

Out of five sets (total weight of female fishes 240 g) that spawned completely, a total of 3,350 yolk-sac absorbed larvae were produced (Table 1). The survival of the larvae was quite high till 5 days, however, disappearance of larvae along with little number of dead ones was observed from 5th day onwards. After rearing for 10 days, a total of 350 (10.44 %) fry were obtained which varied largely in length ranging from 10 to 25 mm having well developed body shape similar to the adult. Two pairs of barbels, all fins and mouth became prominent at this stage. The disappearance of larvae from 5 days onwards and severe size variation thereafter indicated that this species observe high rate of cannibalism from day 5 onwards. The larger size fry (shooters) were never found swimming in the water during day time and were procured only at the time of harvesting as they remain hiding inside the folds of hapa. Post larval mortality (40–50 %) in case of *O. pabda* in nylon hapa has also been observed between 7 and 10 days of rearing and the cause of mortality was assigned non-acceptance of feed [21].

Clear Water vs Shelter of Aquatic Vegetation

No difference in breeding response was observed between clear water and *Hydrilla* plotted tanks. The visibility of eggs and hatchlings was prominent in the clear water system, whereas, a lot of dirt sticked around the egg surfaces in *Hydrilla* plotted tanks making it difficult to observe the developmental stages. Therefore, the method of breeding the fish in clear water in flow-through system is better option where eggs could be easily observed for counting of number, fertilization rate and embryo development. However, the benefits of putting aquatic vegetation for larval rearing may be a better option for reducing cannibalism as pointed out by Sridhar et al [8] that needs to be checked out in future studies.

Water Quality

The water quality with temperature 27 ± 1 °C, pH 7.5 ± 0.1, EC 634 ± 4 μS cm^{-1}, total hardness 311 ± 5 mg l^{-1} and DO 5.4 ± 0.4 mg l^{-1} was in normal range for breeding of freshwater fishes.

Conclusion

The present single unit of flow-through system for captive breeding and larval rearing of *O. bimaculatus* was found simple, cost and space effective. It also provided easy opportunity for observing breeding and spawning behavior of fish and made egg and spawn collection simpler. In clear water system, the eggs were also found free from dirt that assembles all around the eggs due to its sticky nature hence observing them under the microscope for recording embryonic development became possible. Since, the early fry is cannibalistic in nature, the present facility could provide easy approach for size grading and removal of shooters. However, further work is required to improve survival rates from spawn to fry through culture of suitable live feeds and formulation of diets.

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