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Breeding Performances of *Anabas Testudineus* (Bloch) in Specially Designed Cemented Tanks

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ABSTRACT

The present study was carried out from January, 2009 to May, 2009. The objective of this study is to develop an economic and easily acceptable breeding technique of Anabas testudineus in Assam condition. The breeding process was performed in two specially designed cemented tanks having direct connection with the rearing pond by aqua pump to make available necessary live food and freshwater whenever necessary. The induced breeding was done by intramuscular injection of Ovaprim (2 ml/kg fish) near the caudal peduncle of brood fish. After 7-8 hours the brood fish released their eggs and sperms and after 25-27 hours, the eggs hatched. The fertilized eggs of the climbing perch were transparent and clear in appearance, round in shape and floating freely in the water surface. The newly hatched larvae were slender, semitransparent and measured about 2.95 mm in length. Two days after hatching, they started to feed on small food like Paramoecium and other supplied dry food.

Keywords: *Anabas testudineus,* induced breeding, larvae.

INTRODUCTION

This fish is highly esteemed for its highly nourishing quality and prolonged freshness out of water and a valuable diet for sick and convalescent [1]. It is also believed to have medicinal properties such as disease prevention; increasing male libido and slowing down the ageing process for females and it also has been proven to be an effective biological agent for controlling breeding of *Aedes* mosquitoes in stagnant drains [1]. This fish is suitable for cultivation in ponds, reservoirs and rice field. In recent times, because of higher value and market demand, this species has recently come under considerable pressure and is nearly extinct in some areas. This might also be due to environmental changes and over-fishing [2-3].

The major constraint in the culture of this species in large scale level is the non-availability of quality seeds from the natural habitat and also scarcity of matured brood fish. Hence it is expedient to introduce an enduring system of producing seeds of climbing perch through induced breeding as an alternative to the present unreliable dependence on wild seed collections, for supply to fish farmers engaged in aquaculture. To utilize and manage this species judiciously in culture system, understanding of reproductive biology, seed production and larval rearing techniques are very essential. Numerous studies on the biology of this candidate species have been available, which includes features as feeding [4-6], embryonic development, larval development and growth [7] induced breeding [8]. Healthy and sexually matured brood fishes were selected during the breeding season (2nd week of March to 2nd week of July) for breeding experiments. Free oozing males and ripe females were taken in the ratio of 2:1 respectively, for breeding. Breeding experiments were performed with Ovaprim to assess the reproductive performance of *A. testudineus* in captivity. The performance of breeding in natural condition was not studied in this experiment and there is no such report also.

Although *A. testudineus* are believed to be "difficult to breed" in laboratory conditions, reports of success with the induced breeding of this species are also available, though scanty [9-11]. However, these studies are restricted to analyze the overall breeding performances. Although some information regarding this species is available, adequate

knowledge on its breeding is yet to be unfolded. In view of above, the present study is aimed to investigate the breeding performance of *A. testudineus* under controlled condition by induced breeding with Ovaprim in specially designed cemented tanks.

This experiment can be applied for other small sized fishes like *Colisa* species, *Puntius* species, *Nandus nandus* etc. which are already bred by different workers in aquarium condition or in small breeding tanks as the facility of this experiment is better than aquarium condition or small breeding tanks.

MATERIALS AND METHODS

The present study was conducted from January, 2009 to May, 2009 at West Jalukbari in Guwahati, Assam, India under natural condition. This area lies between the latitude and longitude of $26^0$6/N and $91^0$48/E respectively. The brood fish for this work were brought from the nearest wetland and also collected from fish market at early morning just after the arrival of fresh fish. Utmost care had been taken to select and collect the healthy brood fish during the time of collection. The weight of the males ranged from 27.65 g to 33.87 g and the females from 35.34 g to 49.45 g. The collected brood fishes were stocked in the rearing pond which was connected to the specially designed cemented tanks through an electric aqua pump (Model: Tullu-60, Power: 250 W, Max. Dis.: 900 litres per hour) to collect water from the rearing pond to the tanks. The sizes of the cemented tanks were 150 cm X 60 cm X 75 cm. One double nozzle air pump of 5watt was also connected to the tanks to meet the dissolved oxygen demand especially during night and when the electric aqua pump was not in function. The air pump also helps to create a fluvial current within the tanks which is necessary for proper fusion of sperm and ova just after release by the brooders. The inlet pipes of the tanks were covered by 0.5 mm mesh size net to prevent entry of any predators of newly hatched larva like *Cyclops*. The tanks were also covered by metallic or plastic nets to prevent the escape of the brooders which become agitated after injection of hormones. One fourth of the surface area of each tank was covered by *Azolla* as the climbing perch prefers some area to use as shelter and also to give natural condition and to prevent penetration of direct sunlight in some areas of the tanks. The bottom of the tanks was also covered with fine sand upto five centimeters height collected from the river bed. One tray of the area slight lesser than the bottom of the tanks made up of metallic net (mesh size smaller than the body height of the brooders) were fitted at the bottom of the tanks which help to collect the brooders just after spawning by lifting the same without disturbing the eggs. As the eggs of the *A. testudineus* are non-adhesive and free floating in nature, so there is no chance of the damage of the fertilized eggs by the metallic net. Four outlet pipes were fitted at different heights i.e, 15 cm, 25 cm, 35 cm and 45 cm of each tank to maintain different depth of the water during different stages of development of fish larva. The outlet pipe at 15 cm point is required for the newly hatched larvae as the newly hatched larvae cannot cover water depth more than 15 cm. Other outlet pipes at different level are required if the larval development upto juvenile size has to be done at the same tanks. The inner mouths of all the outlet pipes were covered by nets to prevent escape of the larvae. Small meshed sized net was required for the outlet pipe of the lower portion to prevent the smaller sized larvae. The outer mouths of the outlet pipes were also fitted above the water surface of the rearing pond which indirectly helps to increase the dissolved oxygen rate. The water depth was also maintained by siphoning process with a rubber pipe covered with required mesh sized net in between the outlet pipes when needed. Before starting the experiment the water of the tanks were filtered by synthetic cloth (with 50 meshes to 1 cm) collected from the pond to avoid entry of predators of hatchlings like *Cyclops*. If the tanks are newly constructed, breeding experiment should be started minimum 15 days after filling with water to facilitate the growth of algae at the wall of the tanks which provide a natural environment to the brooders.

Four females and eight matured males were selected from the stock for induced breeding. The breeders were given intramuscular injections of Ovaprim (synthetic hormone available at market) with a rate of 2ml per kg body weight [12] and near the caudal peduncle. Slight high dose of the injection in case of female gave better result. All the breeders were released into two cemented tanks with the ratio of 1:2 in two equal halves (six numbers in each tank) until fertilization occurred. Water outlets of the tanks were covered with proper mesh size net. The mesh size was 0.5 mm at the beginning of tank nursing. After 10-12 hours, the breeders released their eggs and sperms and after 19-23 hours, the fertilised eggs were hatched. The temperature during the incubation period was 28-31^0C.

After hatching the breeders were immediately transferred into the stocking pond. The larvae were fed with the small sized natural food available in the tanks like *Paramoecium* etc. Artificial powder food, dried *Daphnia* powder available in the market along with yolk sac of the boiled egg of hen or duck were also supplied to the tank to avoid any scarcity of food item. Regular filtered water was supplied to the tanks from rearing ponds to avoid any contamination evolved from the supply of artificial food.

RESULTS AND DISCUSSION

After 10 to 15 minutes of injection of Ovaprim both male and female became active. The male fish chased the female. After sometime, the fishes became calm and went to the bottom of the cemented tank and remained side by side in the tank. They again agitated after 8-13 hours of injection and started rubbing their body to each other. Then both male and female fish nudged their snouts into the genitalia of each other. Their dorsal spines were in erected position. After about 10-12 hours of injection spawning started, which continued for six hours. Low temperature of about 28^0C and darkness are important factors for spawning. The aerator was then kept on and the unfertilized eggs gradually started to fertilize. When the brooders became sluggish, they were removed from the tank and kept in the rearing tank. The percentage of fertilization and percentage of ovulation was almost same. The latency period was 10 to 12 hours and the time taken to hatch was 9 to 11 hours. The mean percentage of survival of hatchling was 80-82%. Unlike the carp pituitary stimulation, both males and females of *Anabas* were injected with Ovaprim only once in the present experiment.

In *Anabas* the eggs are spherical and yellowish coloured measuring 0.7 mm in diameter with a single large oil globule. The egg is highly laden with yolk, which remains at the vegetal pole. Fertilization took place within 10-12 hours after spawning and the fertilized eggs were usually floating freely on the water surface. The rate of fertilization varied from 90-95%. The unfertilized eggs were non adhesive and were found scattered in the tank. Thirty five to forty minutes after the laying of eggs the first cleavage commences. Within nineteen to twenty third hours, the larva bursts out of the egg case. The larva measures 2.95 mm in length. Different stages with their morphological characters are shown below in Table-1.

Table-I: Different stages of development with their morphological characters-

Stage	Observations
Resting Phase (Male gonads)	Gonads are pinkish coloured, elongated and slender structure. Seminiferous lobules are small and packed with spermatogonia. This stage extends from August to January.
Resting Phase (Female gonads)	Presence of small filamentous pinkish white coloured ovaries. This stage extends from August to January.
Maturing Phase (Male gonads)	Gonads become slightly swollen with pinkish colour. This stage extends from last part of January to first part of March.
Maturing Phase (Female gonads)	In this period ovaries are increased in volume and become opaque and slight yellowish coloured. This stage extends from last part of January to first part of March.
Male gonads (March)	Seminiferous tubules increased in size filled up with sperms. This stage extends from March to May.
Female gonads (March)	Well vascularized ovaries increased to fill the whole of body cavity in the months of March to May
Matured Male	The matured males are reddish coloured and possess some bands at their lateral sides which become distinct during breeding season and generally longer and brighter than the female.
Matured Female	The fully ripe females have bulging yellowish abdomen with a prominent bulging at the vent resembling genital papilla
Egg (Two celled stage)	The cell dividing into two blastomers of nearly equal size after 30 minutes of fertilization
Just before hatching	After 9^{th} hour of fertilization the differentiation of the embryo begins and the organs like tail, abdomen and head are formed
Newly hatched larvae	After 28^{th} to 30^{th} hour of fertilization the tail was the first organ to come out from the egg followed by other organs. The average total length of newly hatched larvae was 2.95mm (from 20 individuals).

Mookerjee and Mazumdar [13] have described sexual dimorphism during breeding season on the basis of colouration. But this type of colour difference was not noticed in any of the specimen examined. Adult fish showed sexual maturity. Sexes are apart by girth, as that of female is larger particularly when in spawning condition. Males are somewhat darker in colour and have more of a knife-edged anal fin than female. Among other feature of sexual dimorphism the pectoral fin of male becomes rough during breeding season. The genital papilla rather pointed and narrow with free

oozing milt while on applying pressure laterally on the abdomen [14].

The size at sexual maturity of female *A. testudineus* was 12.25 ± 1.20 cm (mean ± SD) in total length and 40.20 ± 5.32 g. in body weight. The females were almost immobile in the tank throughout the inducing process but, shortly before ovulation, started erratic swimming repeatedly. After breeding all brooders were survived. Brooders showed chasing behavior after 8-13 hours of injection of Ovaprim at the dose of 2 ml per kg body weight. Each female was found to be paired with a single male. At all times the more active and aggressive male paired with the female and other male was found passive and idle in the corner of the tank. Mating was preceded by elaborate courtship. It was observed that the male rubbed its body with female and released its milt and the eggs were fertilized externally. The spawners bred 10 to 12 hours after the injections but always in dark period. Fertilization was never less than 90% and practically all the fertilized eggs had hatched 19 to 23 hours after spawning. Parental care was not noticed in this species. The fertilized eggs of *A. testudineus* were floating, rounded, white cream bright and clear in appearance. The fertilized eggs had a diameter ranged from 0.56 to 0.80mm. The fertilization rate varied from 90 to 95%. In the present observation numbers of eggs released by the female were ranged from 12,084 to 48,477 numbers indicating high fecundity. The fecundity seems high as compared with other reports available in India. Khan and Mukhopadhyay [15] observed fecundity ranging from 10002 to 36477 in size range of 99-169 mm. However, Banerjee and Prasad [9] reported the fecundity of 4588 to 34993 in Bihar region in the fish size range 73-182 per 8.4-100.2g. The total lengths of newly hatched larvae were 2.95 mm. Fertilized eggs were hatched out after 9-13 hour of spawning. The period of hatching out was 21 hour 30 minutes at the water temperature of 28.0-31^{0}C. This is similar to other fishes such as green catfish, red-tail cat fish and siamese gourami, which have hatching times out of 18 hour, 23 hour 40 minutes and 22 hour 10 minutes, respectively, at the water temperature 27.0-30.5^{0}C [16-17]. No nest building activity was observed in the spawning tank.

A paradox is that the *Cyclops*, otherwise a preferred item of food for the fish larvae, can on the other hand prey on it sucking through its soft body wall. The harmful role of *Cyclops* to fish spawn has been highlighted by Lakshmanan [18] who noticed that *Cyclops* in density of 0.17 ml/l of water could destroy 255 of the major carp spawn within 24 hour of its introduction in the medium. Spawn of *Anabas,* less than half of the dimension of spawn of the major carp, is naturally more susceptible to the attack. Pestilent nature of *Cyclops* is not unusual as Marshall and Orr [19] have observed that in addition to the filtering process of feeding; a copepod can seize individual organisms and suck out their contents. Dehadrai and Banerji [20] have referred to the risk from *Cyclops* to the survival of spawn of air-breathing fishes in general. Through the series of laboratory trials the density at which the *Cyclops* can become substantially harmful to *Anabas* spawn was ascertained as 1,500 numbers per litre and the stage at which the spawn can ward off the attack was found to be the length over 7 mm.

Notwithstanding the risk from *Cyclpos*, micro-crustacea and rotifers are the preferred items of food for the larvae of *Anabas* right from the start of the feeding behavior which it manifests within 36 hours of the hatching.

The yolk sacs were completely absorbed within 92 to 96 hours after hatching. All larval mouths were open at 28 to 30 hours after hatching and at 32 to 36 hour after hatching the fish started feeding.

The newly hatched larvae higher than 2 mm in size, sluggish and buoyant are disposed to destruction by the larger broods of *Anabas* itself besides other harmful organisms. Ultimate number of survival in a brood of *Anabas* is found limited by belligerent tendency between the individuals in the same brood marked by the fact that nearly 3% of them out-grow the rest as 'shoot' fry.

The yolk absorption period for newly hatched larval climbing perch (almost 4 days after hatching) was found to be similar to other fishes. Amornsakun *et al.* [21] reported the yolk absorption of larval green catfish, *Mystus nemurus,* was complete at 3 days after hatching at water temperature of 25-30^{0}C. Amornsakun [16] reported the yolk absorption of larval red-tail catfish was complete at 4.3 days after hatching at water temperatures of 28.0-30.5^{0}C.

The larvae of rabbit fish, *Syganus guttatus* have rapid development of eye, mouth and alimentary canal during the yolk-sac stage which makes it possible for the larvae to feed before the yolk is completely absorbed [22]. In this study through microscopic observation, it was found that climbing perch larvae started to feed at 36 hours after hatching with their remaining yolk-sacs at around 50% of the initial volume.

The hatchlings were stocked at the rate of two larvae per litre of water (1 cubic metre = 1000 litres) in the tanks, which was just double to the stock density applied by Banerji and Prasad, [9]. Supply of minute plankton dominated by rotifers as food was sustainingly maintained in the tanks.

CONCLUSION

This study reveals an economic and easily acceptable breeding technique of *A. testudineus*. The poor fish farmers with limited facility can use this technique for production of the seeds of one of the most economically important fish in this region. Based on the present experiments, synthetic hormone (Ovaprim) at 2.0 ml per kg body weight dose is recommended (slightly higher in female) in order to stimulate spawning. Therefore, it is recommended that the seed of *A. testudineus* could be produced in economic way through proper induced breeding practice.

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