Aquaculture Research, 2005, **36,** 941–945 doi: 10.1111/j.1365-2109.2005.01281.x

Captive breeding of climbing perch *Anabas testudineus* (Bloch, 1792) with Wova-FH for conservation and aquaculture

Uttam Kumar Sarkar, Prashant Kumar Deepak, Dhurendra Kapoor, Raje Singh Negi, Samir Kumar Paul & Sreeprakash Singh

National Bureau of Fish Genetic Resources, Dilkusha, Lucknow, Uttar Pradesh, India

Correspondence: U Kumar Sarkar, National Bureau of Fish Genetic Resources, Canal Ring Road, Dilkusha, Lucknow 226002, Uttar Pradesh, India. E-mail: usarkar1@rediffmail.com

Abstract

Induced breeding of climbing perch, *Anabas testudineus* was conducted by synthetic hormone Wova-FH in the intensity level of 0.1, 0.2 and 0.3 mL kg^{-1} of body weight respectively. The brooders were injected one time and left to spawn in the spawning hapa in the sex ratio between male and female as 2:1. It was found that at all the intensity level hormone Wova-FH could enhance the fishes to breed and lay eggs whereas no breeding was observed in control set. The spawning time, quantity of the brooder spawn, fertilization rate, hatching rate and survival rate were quantified in each set of experiment. The egg output/female was significantly higher in 0.3 mL in comparison with 0.1 and 0.2 mL kg^{-1} of body weight. The statistical analysis showed significant ($P \leq 0.05$) effect between hormone dose on fertilization rate, egg output and hatching rate. The present experiment suggests that Wova-FH at the dose of 0.3 mL kg^{-1} body weight of fish is more effective which might be considered for raising captive population.

Keywords: Climbing perch, *Anabas testudineus*, artificial spawning and hatching, Wova-FH

Introduction

Climbing perch *Anabas testudineus* locally called as '*Koi*' is a small sized food fish, which inhabits both freshwater and brackish waters of Indian subcontinent and South east Asia. The fish exhibits obligatory air-gulping behaviour, omnivorous in habit and travels through ephemeral inflowing waterways, and thus often ends up on the land when the rain ceases. Climbing perch appear to be visual feeders, feeding primarily during the day. Over their native range, climbing perch occur mainly in low lying swamps, marshy lands, lakes, canals, pools, small pits and puddles (Talwar & Jhingran 1991).

The first reports concerning attempts at artificial hypophysation of climbing perch *A. testudineus* and the results of its controlled spawning were published in the 1970s (Khan 1972; Banerjee & Prasad 1974; Khan & Mukhopadhyay 1975), the carp pituitary having been used in induced stimulation. Recent review of literature shows that no attempts have been made after 1970s to breed *A. testudineus* except Banerjee and Thakur (1981). The effect of Wova-FH for inducing fish has been attempted to Indian Major Carps (unpublished). No experiments have so far been conducted on artificial breeding of A. *testudineus* with new synthetic hormone Wova-FH.

Among air breathing fishes *A. testudineus* is considered a delicacy in the eastern, northeastern and southern states of India and demand for this fish is very high for its fine flavour, restorage value, prolonged freshness out of water and a valuable diet for sick and convalescent. The present market value ranged from $2 to 3 per kg. The fish has a wide range of distribution in the freshwaters and is prevalent in the derelict and swampy waters. Because of reduced abundance in the wild fish is presently categorized as vulnerable (Vu) as per International Union for Conservation of Nature and Natural Resources (IUCN) criterion. It is omnivorous in nature and attains sexual maturity in the first year. Although culture, breeding and larval rearing technology of the major carps has been developed for the decades,

other species having commercial importance have been ignored. Recently, *A. testudineus* is considered as one of the potential new candidate species for aquaculture and captive breeding (Ponniah & Sarkar 2000; Ayyappan, Raizada & Reddy 2001). The aim of the present study was to determine the efficiency of synthetic hormone Wova-FH on reproduction and breeding behaviour of *A. testudineus* and to standardize the doses for conservation and aquaculture. The aim of the present study was to determine the efficiency of synthetic hormone Wova-FH on reproduction and breeding behaviour of *A. testudineus* and to standardize the doses for conservation and aquaculture.

Materials and methods

Broodstock collection and maintenance

The captive breeding experiments were conducted at Roy Fish Farm, located at Beldanga (24:57.1390 N; 88:20.4770 E and altitude 152 m), District Maldah, West Bengal. The brood fishes of both male and female ($n = 30$) were collected from the river Punarbhava, located in Maldah district, West Bengal during December–February 2003. They were carried in aluminium hundi (60 liters) from the river site and kept in a plastic pool installed in the vehicle and transported. The fishes were maintained in earthen small sized ponds (0.05 ha, average depth 70–80 cm) at Roy Fish Farm, Beldanga, Maldah, West Bengal. The fishes were fed small sized live prawns and provided at 5% of body weight per day. No mortality was noticed during the stocking period. Both male and female found to be gravid and both male and female was easily distinguishable. Adult fish showed sexual dimorphism. Sexes are apart by girth, as that of the female is larger (particularly when in spawning condition). Males are darker in colour and have more of a knife-edged anal fin than females. Among other features 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 slight pressure on the abdomen. In case of female pectoral fin was smooth and genital papilla was found swollen and slight pink in colour, the abdomen was bulging and soft in appearance.

Captive breeding

Spawners were selected for induced spawning experiment in the month of June 2003. The brood stocks were collected from the earthen pond by repeated drag netting followed by dewatering, segregated and transferred into nylon hapa (1.5 × 2.5 × 3.0 m) for acclimatization. The experiment was carried out on eight females and the body weight varying from 65 to 67 g. Three sets of experiments were conducted for three different doses (Table 1) in separate nylon hapa. A control was also maintained where no hormonal injections were done. Healthy and sexually mature brooders were selected. Free oozing males and ripe female were taken in the ratio of 2:1, respectively, for breeding. All female were injected 'Wova-FH' intramuscularly while partial injection was given to male depending upon their maturity status. Wova-FH is a preparation containing synthetic gonadotropin-releasing hormone analogue (SGnRH) (a product of Biostadt Agrisciences, Wockhardt Life Science, Mumbai, India) and water-soluble. Immediately after administering the hormone, the brooders were released into the spawning hapa, provided with *Hydrilla verticillata* for hiding purposes.

After spawning, effective fecundity of each female was determined by randomly taking a representative sample of eggs in a 10 mL graduated measuring tube from the total eggs released by the female. The total

Table 1 Results of captive breeding experiments of *Anabas testudineus* by Wova-FH

Size of female (g)	Wova-FH dosage to female (mL per kg body weight)	Average size of male (g)	Wova-FH dosage to male (mL per kg body weight)	Latency period (h)	Egg output/female	Fertilization (%)	Hatching (%)	Remark
67.0 ($n = 2$)	0.3	63.99 ± 4.51	0.3	8	130 000	98.50 ± 3.5	90.5 ± 3.65	Complete spawning
65.0 ($n = 2$)	0.2	62.49 ± 4.31	0.2	9	104 000	62.15 ± 4.66	70.5 ± 4.56	Complete spawning
65.3 ($n = 2$)	0.1	63.59 ± 3.61	0.1	13	52 000	36.24 ± 5.85	59.5 ± 4.5.5	Partial spawning
65.0 ($n = 2$)	Control	62.68 ± 4.51	Control	–	No breeding	Nil	Nil	No spawning

number of eggs in 1 mL were counted and multiplied with total volume of eggs released. The fertilization rate of eggs determined by randomly taking a sample of approximately 100 eggs from the total eggs in a Petri dish. Fertilized eggs having intact nucleus were only considered for calculating percentage of fertilization. The ova diameter was measured by keeping approximately 20 eggs in a row along the measuring scale under a dissecting microscope. The total length of eggs was divided by number of eggs to obtain mean diameter of each egg. The 1-day-old hatchlings were maintained plastic troughs. Aeration was provided in plastic troughs and water was exchanged daily. The water quality parameters of brood stock pond and spawning hapa were analysed as per APHA, AWWA, WPCF (1998). The physicochemical parameters of broodstock pond were; air temperature (30 ± 1.1 °C), water temperature (31 ± 2.2 °C), pH 7.5 ± 0.23, dissolved oxygen (8.0 ± 2.3 ppm), free CO_2 (2.3 ± 0.5 ppm) and turbidity (2.5 ± 11 cm). The values of physicochemical parameters of spawning hapa were; air temperature 30 ± 1.0 °C, water temperature $28.5.0 \pm 2.2$ °C, pH 7.5 ± 0.2, dissolved oxygen 8.0 ± 1.3 ppm, free CO_2 2.3 ± 0.5 ppm, turbidity $3.5.0 \pm 0.5$ cm, alkalinity $35.8.0 \pm 5.0$ ppm and water hardness $60.6 \pm 4.6.0$ ppm. We analysed significance of the effects of hormone on the investigating traits using analysis of variance (ANOVA) using a statistical software package SPSS version 11.5. The traits taken in to consideration were the latency period, egg output per female, fertilization and hatching rate. The significance of the effects on the investigating traits was checked using *F*-test. A probability level of 0.05 was utilized to account for the statistical significance.

Results

Breeding season, behaviour and percentage of female spawned and survival of spawners

Brooders of *A. testudineus* were found to be mature enough during June 2003. We found a varied degree of response of inducing agent in all intensity level. However, the difference in fertilization, latency period, egg output and hatching rate was observed in the present experiment (Table 1). In the present study, out of eight female selected for induced breeding in three experimental sets, six responded positively and produced viable eggs. One female injected at the dose of 0.1 mL kg^{-1} of body weight Wova-FH was not responded. In control set no breeding behaviour was observed. The females maintained immobile in the hapa throughout the inducing process but, shortly before ovulation, started erratic swimming repeatedly. After breeding all brooders were survived.

Brooders showed chasing behaviour after 8–9 h of injection of Wova-FH at the dose of 0.2 and 0.3 mL kg^{-1} body weight. However, in the fishes administered at the dose 0.1 mL kg^{-1} body weight showed breeding behaviour after 13 h of injection. 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 the other male was found passive and idle in the corner of the breeding hapa. Mating was preceded by elaborate courtship. It was observed that male rubbed its body with female and released its milt and the eggs were fertilized externally. Parental care was not noticed in this species. The fertilization rate estimated in the present experiment varied from 36.24%, 62.15% to 98.5% in the hormonal doses of 0.1, 0.2 and 0.3 mL kg^{-1} of body weight respectively.

Effect of Wova-FH on latency period, fertilization, egg output and hatching

Analysis of variance showed significant effect ($P \leq 0.05$) of hormonal doses on latency period ($P \leq 0.02$), egg output ($P \leq 0.04$) and hatching rate ($P \leq 0.01$). However, it was non-significant for fertilization rate ($P \leq 0.06$). The correlations were high between the doses of Wova-FH with egg output (+0.98), fertilization (+0.99), hatching rate (+0.98) whereas correlation was negative (-0.94) for latency period.

Egg production

Eggs are bright golden in colour, clear pearl like in appearance and free floating in nature. Freshly fertilized eggs were 0.72 ± 0.05 mm in diameter, non-adhesive and rise to the water surface. Few eggs were attached with water hyacinth roots kept in the spawning hapa. The fertilization rate was varied from 36.24% to 98.5% (Table 1) and ova diameter was ranged from 0.60 to 0.84 mm. Fertilized eggs were hatched out after 9–11 h of spawning. Different developmental stages were observed under microscope. No nest building activity was observed in the spawning hapa and bubble nests or care for their eggs, which float at the surface.

Hatchling maintenance and survival

The 1-day-old hatchlings were maintained in nylon hapa and plastic troughs simultaneously. Movement of hatchlings were very fast, air bladder was prominently visible with regular fanning of pectorals fins. The survival of the 1-day hatchlings varies from 50% to 60% and show schooling behaviour. After 2–3 days, mouth was slightly developed and they started feeding external feed. The yolk sac completely absorbed after third to fourth day head developed. And after fifth day, postlarvae are developed (7.2 mm) that were reared in plastic troughs with providing aquatic macrophytes as hiding cover. After one month of rearing in fibreglass tank fed with carp eggs at the rate of 7% of total body weight, fish attained an average size of 22.76 mm per 0.365 g.

Discussion

The observations indicate that normal spawning of *A. testudineus* occurred at the doses of 0.2 and 0.3 mL kg^{-1} body weight of female and the dose of the hormone affected the percentage of fertilization, egg output, hatching rate and hatchling production respectively. The dosage of other synthetic hormones like Ovaprim in tropical air breathing fishes has been experimented by several authors. The doses of Ovaprim selected for induced spawning of murrels (*Channa* spp.) ranged from 0.3 to 0.6 mL kg^{-1} body weight (Haniffa, Merlin & Shaik Mohamed 2000). Singh, Ram and Singh (2002) reported significant increase in ovulated eggs per fish in *Heteropneustes fossilis* injected at the dose of 0.2 mL kg^{-1} of body weight after ovaprim treatment. However, no reports are available in standardizing the doses of Wova-FH in most of the freshwater food fishes.

In our study, differences in latency period was noticed. Higher latency period in Wova-FH at the dose of 0.1 mL kg^{-1} of body weight indicates difference in the mode of action of the hormone. Similar observation was reported by Habibi, Marchant, Nathorniak, Van der Loo, Peter, River and Vale (1989) in *Carassius auratus*. Longer latency period in low dose of synthetic hormone Ovatide was reported by Pandey, Koteeswaran and Singh (2002). The latency period of Ovaprim induced air breathing fishes are 18 h for *Channa punctatus* and *H. fossilis* (Haniffa *et al.* 2000). Pandey and colleagues (2002) reported varied interspawning period between 8 and 15 h in *H. fossilis* injected in the doses of 0.3–1.0 mL kg^{-1} of body weight of synthetic hormone Ovatide (M/S Hemmo Pharma, Mumbai, India). According to Billard, Bieniarz, Peter, Sokolowska, Weil and Crim (1984) and Peter, Sokolowska and Nahorniak (1986), differences in dose requirement may be attributed to varied level of dopamine activity in different species of fish. In our observation no nest building activity and parental care was observed by the brooders. Similar observation is reported by Sakurai, Sakamoto and Mori (1992).

In the present observation, number of eggs released by the female were ranged from 52 000 to 130 000 numbers indicating high fecundity. The present data of fecundity seems high as compared with other reports available in India. Khan and Mukhopadhyay (1972) observed fecundity ranging from 10 002 to 36 477 in size range of 99–169 mm. However, Benerjee and Prasad (1974) reported the fecundity of 4588–34 993 in Bihar region in the fish size range 73–182 mm per 8.4–100.2 g. The fecundity data recorded at the Assam centre is 3812–28 490 eggs in the fish size range of 74–138 mm per 7–57 g (Central Inland Fisheries Research Institute workshop Report (CIFRI 1982)). Chanchal, Pandey and Nath (1978) reported minimum 3481 to maximum 42 564 in the fish weight range of 9.0–53.1 g. Banerjee and Thakur (1981) reported sheding of 2000–13 000 eggs in seven sets of induced bred *A. testudineus* (24.8–40.1 g) in glass aquaria.

In our study, water temperature of breeding pool recorded in the experiments were 28.5 $\pm$ 2.2 °C indicating quite favourable for breeding. In the present study, high rate of hatching may be attributed to the optimum range of physiochemical parameters of water (viz., pH, temperature and dissolved oxygen). Moitra, Pandey, Ghosk and Munshi (1979) recommended the optimum water temperature as 28.6 $\pm$ 10 °C for breeding of *A. testudineus* under laboratory conditions. Many instances of the natural breeding are reported to have been occurred in captivity by simple collecting the migratory spawning pairs and keeping them together in freshly accumulated rain/tap water.

The objective of the present study was fulfilled and maximum hatching rate was observed in the dose of 0.3 mL kg^{-1} of body weight. Based on the present experiments the Wova-FH dose of 0.3 mL kg^{-1} body weight for female can be recommended. In conclusion, it is recommended that the seed of *A. testudineus* could be produced in captivity through scientific management of eggs, larvae and hatchlings. Since the breeding protocols does not require higher investment this can be taken up by small farmers for seed

production. Evidently, the protocols emerged can be utilized for species restoration and conservation strategies could be adopted.

Acknowledgments

We express our sincere thanks to Project Implementation Unit (PIU), National Agricultural Technology Project (NATP), Indian Council of Agricultural Research, New Delhi for financial assistance. We are also thankful to Miss Archana Srivastava for collection of literatures and typing the MS. The authors would like to thank Mr Dipak Roy, Progressive Fish Farmer for providing farm and hatchery facilities to carry out the experiment.

References

APHA, AWWA, WPCF (American Public Health Association, American Water Works Association and Water Pollution Control Federation) (1998) *Standard Methods for the Examination of Water and Waste Water*, 15th edn. American Public Health Association, Washington, DC, USA, 1134pp.

Ayyappan S., Raizada S. & Reddy A.K. (2001) Captive breeding and culture of new species of aquaculture. In: *Captive Breeding for Aquaculture and Fish Germplasm Conservation, Publication 3* (ed. by A.G. Ponniah, K.K. Lal & V.S. Basheer), pp. 1–20. National Bureau of Fish Genetic Resources (NBFGR) – National Agricultural Technology Project (NATP), Lucknow, India.

Banerjee S.R. & Prasad D. (1974) Observations on reproduction and survival of *Anabas testudineus* (Bloch) in Bihar region. *Journal of Inland Fisheries Society of India* **6**, 6–17.

Banerjee S.R. & Thakur N.K. (1981) Observations on the spawning behaviour of *Anabas testudineus* (Bloch). *Indian Journal of Animal Sciences* **51**, 651–654.

Billard R., Bieniarz K., Peter R.E., Sokolowska M., Weil C. & Crim L.W. (1984) Effects of LHRH and LHRH-a on plasma GtH levels and maturation/ovulation in common carp, *Cyprinus carpio* kept under various environmental conditions. *Aquaculture* **41**, 245–254.

Chanchal A.K., Pandey B.N. & Nath S. (1978) Studies on some aspects of the biology of *Anabas testudineus* (Teleostei: Anabantidae). *Matsya* **4**, 15–19.

CIFRI (1982) *All India coordinated research project on air-breathing fish culture*. Central Inland Fisheries Research Institute (CIFRI) 6th Workshop Report, December 27–28, pp. 20–21.

Habibi H.R., Marchant T.A., Nathorniak C.S., Van der Loo H., Peter R.E., River J.E. & Vale W.W. (1989) Functional relationship between receptor binding and biological activity for analogues of mammalian and salmon gonadotrophin-releasing hormones in the pituitary of gold fish (*Carassius auratus*). *Biological Report* **40**, 1152–1161.

Haniffa M.A., Merlin R.T. & Shaik Mohamed J. (2000) Induced spawning of the stripped murrel *Channa striatus* using pituitary extract, HCG, LHRH-a and ovaprim. *Acta Ichthyology – Piscatoria* **30**, 53–60.

Khan H.A. (1972) Induced breeding of air breathing fishes. *Indian Farming* **22**, 44–45.

Khan H.A. & Mukhopadhyay S.K. (1972) On the fecundity of climbing perch, *Anabas testudineus* (Bloch). *Journal of Inland Fisheries Society of India* **4**, 212–213.

Khan H.A. & Mukhopadhyay S.K. (1975) Production of stocking material of some air breathing fishes by hypophysation. *Journal of Inland Fisheries Society of India* **7**, 156–161.

Moitra A., Pandey A., Ghosk T.K. & Munshi J.S.D. (1979) Breeding and rearing of *Anabas testudineus* (Bloch). Abstract. In: *Symposium on Inland Aquaculture*, pp. 2–3. Central Inland Fisheries Research Institute, Barrackpore, West Bengal.

Pandey A.K., Koteeswaran R. & Singh B.N. (2002) Breeding of fishes with synthetic hormone drug ovatide for mass scale seed production. *Aquaculture* **3**, 137–142.

Peter R.E., Sokolowska M. & Nahorniak C.S. (1986) Comparison of (D-Arg^{6} Trap^{7}, Leu^{8}, Pro^{9} Net) luteinising hormone (LHRA) in combination with pimozide, in stimulating gonadotropin release and ovulation in the gold fish, *Carrassius auratus*. *Canadian Journal of Zoology* **65**, 987–991.

Ponniah A.G. & Sarkar U.K. (2000) Evaluation of North East Indian fishes for their potential as cultivable, sport and ornamental fishes along with their conservation and endemic status. In: *Fish Biodiversity of North-East India, Publication 3* (ed. by A.G. Ponniah & U.K. Sarkar), pp. 11–30. National Bureau of Fish Genetic Resources (NBFGR) – National Agricultural Technology Project (NATP), Lucknow, India.

Sakurai A., Sakamoto Y. & Mori F. (1992) *Aquarium Fishes of the World*. Chronicle Books, San Francisco, CA, USA, 288pp.

Singh D.V., Ram R.N. & Singh I.J. (2002) Evaluation of dose of ovaprim for inducing ovarian and ovulatory response in the catfish, *Heteropneustes fossilis*. *Indian Journal of Fisheries* **49**, 1–12.

Talwar P.K. & Jhingran A.G. (1991) *Inland Fishes of India and Adjacent Countries*, Vol. I. Oxford and IBH Publishing, New Delhi, India, 1158pp.