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Note

Observations on captive breeding of the threatened freshwater shark *Wallago attu* (Bloch & Schneider, 1801)

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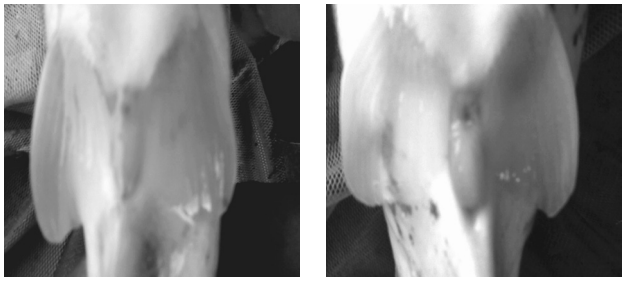
ABSTRACT

Juveniles of the threatened freshwater shark *Wallago attu* procured from a local village pond were successfully reared to brood size under captive conditions in an earthen pond of 0.09 ha, wherein they attained full maturity. The farm reared brooders were successfully induced bred by sGnRH analogue and dopamine antagonist in circular spawning tank of carp hatchery, provided with artificial substratum, wherein spawning took place naturally. Fecundity estimated was 40,000 eggs kg^{-1}. Fertilisation rate was 85±5% and the hatching rate was 90%. Eggs hatched out in 14 - 18 h at 28±1°C. The yolk-sac larvae exhibited severe cannibalism even from one day post-hatch. The larvae were reared for 10 days in FRP tanks on composite diets of plankton, egg custard and carp spawn with 15% survival.

Keywords: Cannibalism, Captive breeding, sGnRH, *Wallago attu*

The freshwater shark *Wallago attu* (family Siluridae) is one of the important catfish species of Indian sub-continent and South-east Asia (Coad, 1981; Ahmad and Niazi, 1988; Pethiyagoda, 1991; Talwar and Jhingran, 1991). It is commonly found in tropical and sub-tropical areas including tidal rivers of India (Nath and Day, 1989; Chondar, 1999; Acharya and Iftekhar, 2000) and grows to 244 cm in length (Pethiyagoda, 1991) and weight above 40 kg (Day, 1889 and Misra, 1959). The species has been known to constitute major fishery of the rivers Yamuna and Ganga as well as in Hirakund Reservoir in the past and is in great demand in many states particularly the north Indian states. However, the population of this species has plummeted considerably in the natural water bodies in the last 2-3 decades due to anthropogenic activities and has been categorised as a threatened fish in India and Bangladesh (Kurup, 1992; Arunachalam *et al*., 2000; Dahanukar *et al*., 2004; Hossain *et al.*, 2008; Ng, 2010). Considering the importance of this species as a food commodity and its current declining population structure (Patra *et al*., 2005; Mishra *et al*., 2009), there is urgent need to sustain their population through aquaculture with establishment of standard breeding and larval rearing protocols. Though some successful attempts of breeding and larval rearing have been taken up in the past in India (Ahmad, 1944; CIFRI, 1985; Gupta *et al*., 1992; Sahoo *et al*., 2002; 2006), the captive breeding and larval rearing protocols have not been standardised so far.

In the present study, four healthy juveniles of *W. attu* weighing 600-1100 g were procured from a local fish pond in February 2011 and reared at the farm of ICAR-National Bureau of Fish Genetic Resources (ICAR-NBFGR), Lucknow in an earthen pond of size 0.09 ha (water depth 4 feet) along with Indian major carps (IMC), for 5 months. The IMC were fed mixture of mustard oil cake and rice polish (1:1 w/w) daily, whereas no specific feed was given to *W. attu* as the pond had sufficient availability of trash fishes. The fishes were observed to be in fully mature condition during second half of July 2011, showing distinct secondary sexual characters in males and females (Fig. 1 a, b). Fully mature pair of *W. attu* comprising one male (750 g) and one female (1250 g) were selected from the rearing pond for induced breeding and the brood fishes were acclimatised to hatchery water conditions of a circular carp hatchery for 5 h prior to hormonal injection. Injection of salmon gonadotropin releasing hormone (sGnRH) anologue and dopamine antagonist @ 0.6 ml kg^{-1} body weight to female and 0.2 ml kg^{-1} body weight to male was given intramuscularly in the evening hours for induced spawning. Spawning was observed on 6 August 2011 in the 4.5 m dia spawning tank of the circular carp hatchery with a water flow rate of 2 l sec^{-1}. Since



(a) (b)

Fig. 1. Secondary sexual characters in *W. attu.* (a): male, (b): Female

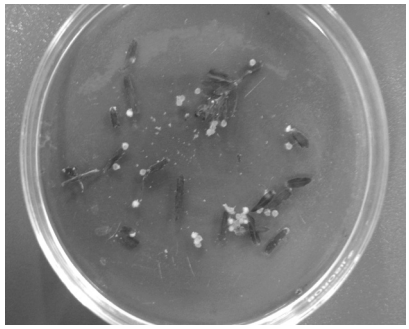


Fig. 3. Fertilised eggs of *W. attu*

the ova of *W. attu* are very small (dia 1.2-1.8 mm) and highly adhesive, egg collectors in the form of wooden logs wrapped with *Hydrilla reticulata* were placed in the spawning tank (Fig. 2). Spawning impulse was observed in the fishes after 8 h of hormonal injection and the female fish laid eggs in batches over the submerged substrata comprising of wooden logs and *H. reticulata*, which were

Fig. 2. Wooden logs wrapped with *H. reticulata,* used as egg collectors in the spawning tank

immediately fertilised by the chasing male. The eggs were found strongly sticking to the submerged weeds, wooden logs and also on the wall and bottom of the spawning tank. On an average 85±5% fertilisation was observed in the samples of eggs collected from egg collectors and from the wall and bottom of the tank. The fertilised eggs were almost spherical and pale yellow in colour having 1.2-1.8 mm dia (Fig. 3).

The brooders were removed with hand nets 8 h after breeding and the female was found to have shrunken belly indicating complete spawning. The eggs hatched out within 14-18 h (temperature 28±1°C) after spawning. The fry were collected on day 4post-hatch (dph) when yolk-sac was completely absorbed, by draining water in the spawning tank in a bolting silk hapa (2 x 1 x 1 m) and stocked in two FRP tanks (8x4x4). Larvae were fed with plankton collected from the farm ponds and finely mashed egg custard. About 1000 spawn of IMC were introduced in each tank to observe predation behaviour of the larvae. The larvae were reared for 10 days in the tanks and survival was recorded (Fig. 4). The fry were then released in an earthen pond of size 0.03 ha for further rearing and growth was recorded after 60 days of rearing.

Fig. 4. Ten days old fry of *W. attu* produced in captivity

The developmental stages of eggs as well as the incubation and hatching periods were observed to be in agreement with the findings of Ahmad (1944). The just hatched larvae were 6.5±0.4 mm in length and 3.0±0.2 mg in weight. They settled at the bottom of the tank owing to the presence of round bulky yolk-sac. The total number of larvae estimated was around 40,000. The larvae showed strong cannibalism from second day onwards. They were noticed to consume their siblings, even though both prey and predator larvae had large yolk-sac. Similar observations were also reported by Sahoo *et al*. (2002; 2006). The fry reared in the FRP tanks for 10 days showed great amount of size variation ranging from 13-27 mm total length. The fry grown in the pond for 60 days were also observed to have variable growth rates with mean total length of 140±20.0 mm (Fig. 5). The water quality parameters of both spawning and rearing tanks were analysed (APHA, 1985) and

were found to be in the normal range (water temperature 28±1°C; pH 7.4±0.2; total alkalinity 170±10 mg l^{-1}; dissolved oxygen 6.2+0.8 mg l^{-1} and free carbon dioxide nil).

Fig. 5. Hatchery produced *W. attu* grown in pond for 60 days

The breeding biology of *W. attu* has been described by several authors and according to them, the species breeds in running waters during monsoon season from May-October in India, Pakistan, Bangladesh, Nepal, Thailand and Cambodia (Ahmad, 1944; Ahmad and Niazi, 1988; Rahman, 1989; Shrestha, 1990; Goswami and Devraj, 1992). According to the above workers, maturity first starts in the eastern parts of the country and subsequently in the west. Rearing of juveniles of *W. attu* in pond condition to fully mature stage has been demonstrated by Gupta *et al.* (1992) and Sahoo *et al*. (2002; 2006) at Bhubneshwar, Odisha (eastern part of India) where this species was found to mature fully during June. These studies have opened prospects of developing broodstock of *W. attu* under captive condition in a short span of time when juveniles/adults of this species could be collected from wild water bodies/wetlands and reared in ponds for getting mature brooders. The full maturity attained (in both sexes) and the high fertilisation rate (90%) observed durings July-August in the present study, indicate that the breeding season of the species under captive condition in Uttar Pradesh is July-August.

Captive spawning of *W. attu* has been attempted in India and Bangladesh. Ahmad (1944) was the first to successfully achieve artificial spawning of this species by mixing gametes of the instantly caught fully mature female and male fish in a bowl with 80% fertilisation. Induced breeding of *W. attu* was first time achieved by ICAR-Central Inland Fisheries Reseach Institute (CIFRI, 1985) with injection of pituitary extract and LHRH formulation, “Hoe 766 vet” and progesterone to both male and female brooders in captivity that resulted in 23-51% fertilisation and 90% hatching. Parameswaran *et al*. (1988) demonstrated successful breeding with LHRH analogue buserelin acetate. Gupta *et al*. (1992) and Sahoo *et al*. (2002; 2006) conducted successful spawning trials in the recent past using pituitary gland extract and Ovaprim® (Syndel Laboratories Ltd., Canada) respectively, following stripping method for artificial mixing of gametes. However in the present study, single dose of sGnRH led to successfull natural spawning. The fertilisation and hatching rates were also found very high as compared to the report of Gupta *et al.* (1992). The higher rate of hatching (90%) obtained could be attributed to natural spawning and flow through water system in the spawning tank.

Though spawning has been successfully achieved in the present as well as past studies, the major problem faced was low larval survival rates as the larvae exhibited strong cannibalism (Gupta *et al*., 1992; Sahoo *et al.*, 2002). The severity lies in the fact that large scale cannibalism was noticed even in one-day old sac-fry larvae as also reported by Gupta *et al*. (1992). Therefore, the major challenge is to stop or at least minimise cannibalism. Several studies have demonstrated that although cannibalism in fishes is difficult to stop even after feeding the fish to satiation. However, it can be reduced considerably by increasing availability of natural food (Fox, 1975; Hecht and Pienaar, 1993) or combination of both live and artificial diets (Qin and Fast, 1998; Giri *et al*., 2002; Sahoo *et al*., 2006); by exposing the larvae to red-light (Sahoo *et al*., 2006); and also by weaning the fish to accept formulated feed just at the time when cannibalism is likely to begin in the larvae (Qin and Fast, 1996). Attempts to feed 4 day old larvae with pond plankton, egg custard and carp spawn in the present study, were partly successful as only smaller larvae consumed plankton and egg custard whereas, larvae that developed cannibalistic behaviour were observed to regularly prey upon their siblings which were slow in movement and did not prey upon the carp spawn due to its fast swimming behaviour. In case of predatory fishes, it has been observed that once cannibalism starts, they do not take artificial diets subsequently. Therefore, the larvae need to be initially weaned on live and/or formulated feed and graded at regular intervals to minimise cannibalism as suggested/practised in case of many predatory fishes like seabass, groupers, murrel and red drum (Quin and Fast 1998; Liao *et al.*, 2001; Jinn-Rong *et al*., 2003; Appelbaum and Arokiaraj, 2010; Raizada *et al*., 2012).

It is therefore, concluded that the broodstock of *W. attu* can be easily reared to full maturity in captive conditions. Spawning tanks of circular carp hatchery could be suitably used for natural spawning of *W. attu* under controlled conditions using sGnRH anologue and dopamine antagonist. However, strategies to stop/reduce cannibalism needs to be developed particularly when sac-fry is just one-day old.

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