Int.J.Curr.Microbiol.App.Sci (2018) *7*(4): 652-658

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 7 Number 04 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.704.074

Induction of Spontaneous Captive Spawning, Embryonic Development and Larval Rearing in *Mystus cavasius*

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Keywords

Bagridae, Catfish, Seed production, Embryonic development

Article Info

Accepted:
07 March 2018
Available Online:
10 April 2018

A B S T R A C T

We report induction of spontaneous spawning in *Mystus cavasius* in glass aquaria provided with water hyacinth for egg attachment. The brooders (20 to 25g) were injected intramuscularly with Ovatide™ at the rate of 0.2 millilitre/Kilogram body weight and introduced into the spawning tanks in the evening. Maximum spawning response was obtained when two pairs of brooders were housed in one tank. Courtship was initiated 3 hours post injection. Batch spawning over a span of 6 to7 hours occurred 7 hours post induction. The first hatchling emerged 14 hours post fertilization and yolk sac absorption was completed 48 hours post hatching at temperature of 26±2^0 C. Fertilization and hatching rates of 62% and 60% respectively were obtained in spontaneous spawning as against 48.9% and 43.2% in artificial fertilization. Immediately after yolk sac absorption, the fry exhibited obligatory air breathing behaviour that lasted upto 18-20 days post hatching. Maintaining water column at 10 centimetre and stocking density at 50 to 60 numbers/litre for the first 18 days, and providing a combination of feeds improved fry survivability. Our study holds special interest in the development of field friendly seed production protocols for the species.

Introduction

*Mystus cavasius*is a Bagrid catfish, highly prized as food fish and found naturally in a variety of lentic and lotic freshwater environments of the Indian subcontinent (www.indiabiodiversity.org). *M. cavasius* is omnivorous with a dietary preference for macro invertebrates (Chaturvedi and Parihar, 2012). A specific growth rate of around 2.7% per day is reported for the species at stocking densities of 2 lakhs per ha (Rahman *et al.*, 2013) which is comparable to that reported for *Heteroclarias* (Okunsebor and Ayuma, 2011) and *Clarias gariepinus* (Marimuthu *et al.*, 2010). Since they attain table size in a short

duration, this species can be of special relevance in places where the production units are largely seasonal, isolated small backyard ponds such as the North Eastern states of India (Pandey and De, 2014). However, despite its high market preference and hardy nature, commercial aquaculture is not widely taken up for this species due to unavailability of seed.

Seed production in most catfishes rely on the artificial external fertilization ofeggs stripped from a female using ground testes dissected from a male.

Pilot scale propagation by artificial fertilization has been reported for a few Bagrid catfishes such as *M. gulio* (Alam *et al.,* 2006), *M. punctatus* (Ramanathan *et al.,* 1985) and *Rita rita* (Mollah *et al.,* 2008).

However this technique is skill intensive and the success rates depend on several factors (Zonneveld *et al.,* 1988; Manickam and Joy, 1989; Sahoo *et al.,* 2008; Srivastava *et al.,* 2012; Agbebi *et al.,* 2013; Dhara and Saha, 2013).

This study reports the successful induction of spontaneous spawning in captivity and completion of larval rearing of *M. cavasius*in order to develop a farmer friendly seed production technology. We also documented the embryonic development and several behavioural aspects of the *M. cavasius* larvae to enable future refinement of hatchery protocols.

Significance statement

We present the embryonic development, larval behaviour and the detailed methodology for spontaneous induction of captive spawning in *Mystus cavasius* using a simple, inexpensive and field friendly set up. Our methodology can be employed for eco- friendly seed production of this commercially important Bagrid catfish.

Materials and Methods

The study was conducted at the ICAR RC for NEHR, Tripura during the natural breeding season of the species (June to September). Live, healthy and mature specimens of *Mystus cavasius* (20 to 25g) procured from a live- fish market were maintained in constantly aerated water in 1 tonne FRP tanks for 15 days. The animals were fed *ad libitum* with boiled chicken liver, fish, mollusc meat and freeze dried blood worms. The tanks were provided with sufficient hide-outs and 50% water exchange was performed every alternate day.

The breeding trials were conducted in rectangular glass aquaria of size 90cm x 45cm x 45cmcovered on all sides with opaque plastic sheets. Constant aeration was provided. Water depth was maintained at 30cm and *Eichhornia crassipes* (water hyacinth) was provided in the aquarium to cover 2/3rd of the water surface area.

Optimal water quality (temperature: 25-27°C; dissolved oxygen: 5-6ppm; pH: 7.5-8.0) was maintained throughout the experiment. A muslin cloth was spread across the tank bottom to capture the spawned fertile eggs that fall off.

This also facilitated the removal of adherent empty egg shells that are potential sources of fungal growth. Brooders were given intramuscular Ovatide^{TM} injections at the rate of0.2millilitre per kilogrambody weight in the evening and two pairs of injected brooders were introduced per aquarium.

Fecundity was estimated gravimetrically (Hunter *et al.,* 1989) from 12 females. Fifteen fertilized eggs were used to document the chronology of major development milestones using a microscope (Zeiss (AXIO), Germany) and its attached camera (Jenoptik ProgResCT3, Germany).

Results and Discussion

Brooder behaviour

The adults were highly covert and shied away from the slightest perception of human activity. Courtship was initiated with a latency period of 2 to 3 h and spawning occurred 6 to 7 h post injection. The spawning response in terms of latency period for induction, fertilization and hatching rates was highest when two males and two females were placed together in one tank. While the parents neither exhibited parental care nor attempted to eat the spawned eggs, the hatchlings were actively hunted.

The mean relative fecundity was 3081.22±120 eggs / gram weight of ovary. The fertilization and hatching rates were estimated to be 48.9% and 43.2% respectively from artificially fertilized eggs. However in the naturally spawned eggs, fertilization and hatching rates of 62% and 60% respectively was obtained.

Embryonic development

The major milestones of embryonic development were imaged in our study (Fig. 1). Immediately after fertilization perivitelline space formation began with the yolk shrinking away from the perivitelline membrane followed by the formation of blastodisc. The first cell division was observed 50 minutes after fertilization. A series of cell divisions resulted in the formation of morula as a compact ball of cells. Blastula was formed about 3: 20 hours post fertilization (hpf).The characteristic dome shaped cell mass of the blastula increasingly enveloped the yolk mass to form the gastrulation ring. Gastrulation started with the initiation of epiboly, 6:01 hpf. The elongated embryo enveloping the yolk had the first embryo-like appearance at 10:00 hpf with clearly visible optic cups and development of somite blocks. The first hatchling emerged 14hpf and measured 2.0 mm in total length; hatching was completed in 6 hours.

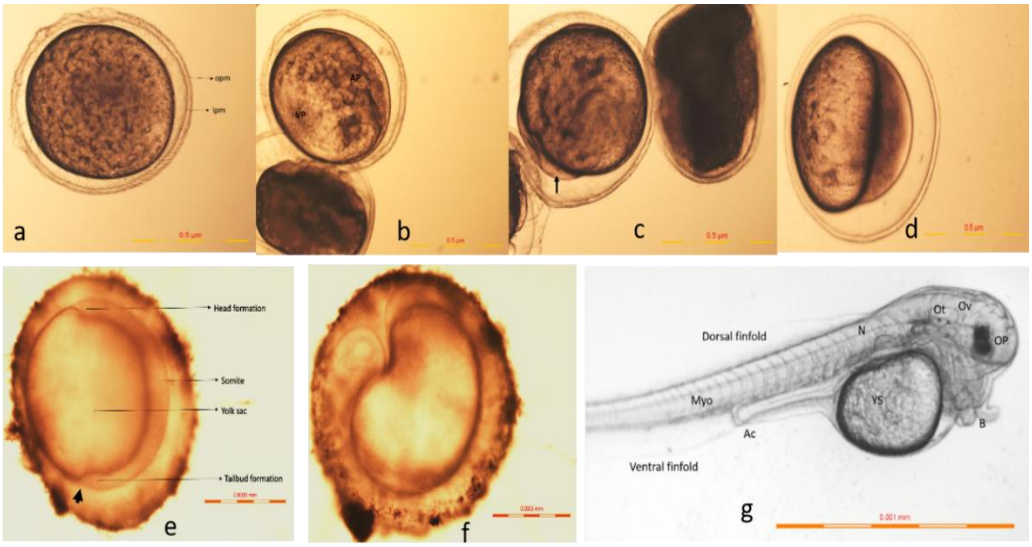
Larval rearing

Larval rearing was completed in 20 days with 63% survival. The yolk sac larvae initially crawled on the tank bottom but began to exhibit dart like swimming patterns within 6 hours of hatching. Unlike adults, larvae did not exhibit photophobia and swarmed near the water surface. Complete absorption of yolk sac occurred in 48 h post hatching in our study. Major developmental changes were also associated with this shift from endogenous to exogenous feeding. Two day old fry had well differentiated mouth parts, barbels, fins and a short alimentary canal (Fig. 2a, b) and exhibited obligatory air breathing behaviour which continued upto 18days.

Differential growth and cannibalism were pronounced in the larvae from the fifth day post hatching in our study. Hence, a daily feeding regimen of a combination diet of infusoria, plankton and egg custard to suit all stages of larvae, coupled with a larval stocking density of 50-60 numbers /Litre were adopted to reduce cannibalism in the larvae. Water hyacinth was provided in the larval rearing tanks to provide refuge to the smaller individuals. Cannibalistic behaviour was negligible in the fishes 18 days and older.

At 20 days of age the fry measured 1.0±0.3cm (Fig. 3), accepted adult feed, and exhibited adult like characteristics such as avoidance of light and seeking of benthic hideouts. This study reports the successful induction of spontaneous spawning in captivity and larval development in *M. cavasius*. The earlier report of captive propagation in the species (Karmakar, 2014) describes a downscaled version of eco-hatchery employing circular water currents and no aquatic vegetation.

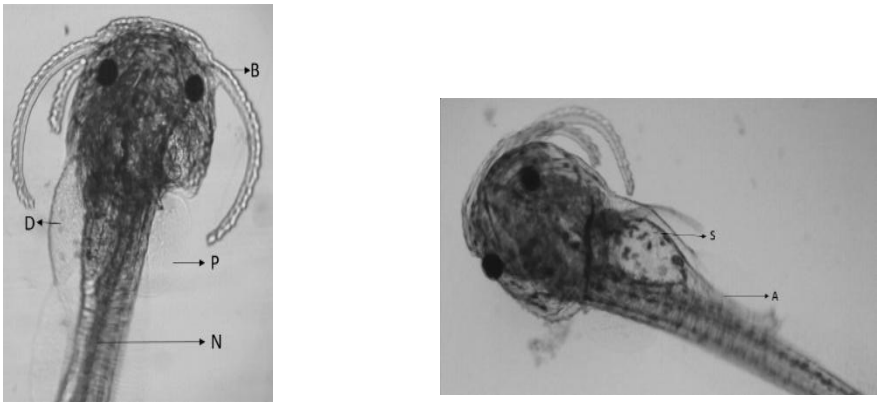
Fig.1 Embryonic development in *Mystus cavasius*



a: Perivitelline space formation immediately after fertilization; b: Blastodisc formation in the fertilized egg characterized by the yolk mass concentrating to the vegetal pole and the granular cytoplasm accumulating at the animal pole; c: One cell Stage showing a prominent bulge at the animal pole due to accumulation of protoplasm (indicated by arrow); d: Blastula stage characterized by the cell mass forming a dome like structure over the cell mass. e: Head and tail bud differentiation in 8 hour old embryo. Somite block formation is also initiated along the back of the embryo. f: The first discernible embryo with clearly separate head and tail region observed at 10 hours. The prominent optic cups are visible at the head region. Movement of the embryo was prominent at this stage. g: Hatchling

opm: Outer perivitelline membrane; ipm: Inner perivitelline membrane. AP: Animal Pole; VP: Vegetal Pole. Ac: Alimentary canal; B: Barbel; N: notochord; Myo: Myotomes; Ot: Otolith; OV: Otic vesicle; OP: YS: Yolk sac

Fig.2 *Mystus cavasius* larval development: 48 hour old fry

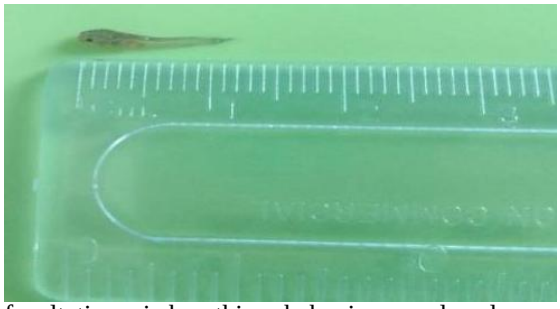


a b

a. Well differentiated mouthparts, fins and barbels can be seen. b: The same fry pictured in a different angle showing the pouch like stomach and short developing alimentary canal. The eyes have become well pigmented and melanophores can be seen all over body.

B: Barbel; D: Dorsal fin N: notochord; P: pectoral fin; S: stomach; A: alimentary canal

Fig.3 Twenty day old fry of *Mystus cavasius*



Fry at 20 day stage exhibits facultative air-breathing behaviour, reduced cannibalism and general adult-like behaviour. The fry can be stocked for grow out at this stage

Interestingly, the author does not mention the sticky nature of the fertilised eggs of the species which get damaged upon dislodging from the substratum. Thus the reported idea of employing circulating water currents without damaging the eggs during incubation appears intriguing.

The fertilized eggs were round, opaque, golden brown and adhesive. Radial striations were observed in the chorionic membranes of fertilized water hardened eggs but not unfertilized eggs. Treatment with sodium sulphite (15 gram/litre) to remove stickiness of eggs (Hazzaa and Hussein, 2003) also resulted in the loss of these micro striation structures indicating their role in egg adhesion. Our observations on embryonic development are well in agreement with existing reports on *M. cavassius* (Rahman *et al.,* 2004). Minor deviations could be the effect of water temperature or genetic differences, as these factors are known to have profound effects on embryonic durations (Pankhurst and Munday, 2011).

Reduction of water column to 8 to 10 cm to prevent fatal exhaustion of the fry was done immediately after spawning as recommended for air breathing catfish nursery rearing (Potongkam and Miller, 2006).Successful nursery rearing in earthen ponds was reported for 12 day old *M. cavasius* for two months (Rahman *et al.,* 2013). However we observed that fry were better equipped survive in deeper waters at 18 days age when they became facultative air breathers. Moreover fry younger than 18 days age were significantly cannibalistic. Cannibalism, considered to be caused due to a variety of factors (Hecht and Appelbaum 1988; Sogard and Ollah, 1994; Saether *et al.,* 2015), is considered a major bottleneck in larval rearing of farmed catfishes (Mukai *et al.,* 2013).Therefore, in view of the better adaptability at around 18 to 20 days of age, we recommend this age for stocking in earthen ponds.

Our study reports aneasy, inexpensive and farmer friendly method for captive spawning and larval rearing of *Mystus cavasius*. Further studies for refinement of larval nutrition and grow out practices are warranted for profitable aquaculture of this highly versatile species.

Acknowledgement

The authors gratefully acknowledge the Director, ICAR Research Complex for North Eastern Hills Region, for his kind support in the conduct of this study. We also acknowledge the funding from the Indian Council of Agricultural Research for the conduct of this experiment under the Institutional Project IXX12229. The authors declare that they have no conflict of interests.

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How to cite this article:

Rekha Das, Himanshu Priyadarshi, Satya Prakash, Chandan Debnath, Lopamudra Sahoo, Abhijit Singha, Ch. Basudha Devi and Das, S.K. 2018. Induction of Spontaneous Captive Spawning, Embryonic Development and Larval Rearing in *Mystus cavasius*. *Int.J.Curr.Microbiol.App.Sci.* 7(04): 652-658. doi: https://doi.org/10.20546/ijcmas.2018.704.074