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Spawning and early development of the endemic and threatened Yellow Catfish *Horabagrus brachysoma* (Günther, 1864) (Teleostei: Bagridae)

L. Bindu [1] & K.G. Padmakumar [2]

[1,2] Kerala Agricultural University, Regional Agricultural Research Station, Kumarakom, Kerala 686566, India
[1] Present address: M.S.M. College, Kayamkulam, Alappuzha District, Kerala 690502, India
[1] bindukylm@gmail.com (corresponding author), [2] kgpadman@gmail.com

Abstract: The present study documents the embryonic and post embryonic development of the endemic and threatened bagrid catfish *Horabagrus brachysoma* under captive conditions. Ovaprim® induced fishes spawned after a latency period of 11.61±1.53 hr with 95.57±3.101 % fertilization and 68.6±30.96 % hatching rates. The fertilized eggs were non-adhesive and spherical with an average diameter of 1.61±0.05 mm. Hatching occurred in 24.9±1.75 hr after fertilization, producing larvae with a mean size of 3.94±0.112 mm. Mouth and barbels were prominent in two day old larvae. Yolk absorption was completed and the larvae attained a size of 5.9±0.307 mm by four day post hatch. The larvae accepted exogenous particulate feed from the fifth day onwards. The characteristic black humeral ocellus appeared in one week old larvae. The larvae achieved 58–65 mm in 85 days.

Keywords: Embryonic development, incubation period, larvae, Yellow Catfish

The Golden or the Yellow Catfish *Horabagrus brachysoma* (Günther, 1864), is an endemic species of the Western Ghats river systems in India (Dahanukar et al. 2011). The species is listed as 'Vulnerable' in the IUCN Red List of Threatened Species due to declining populations as a result of overfishing and habitat loss (Raghavan & Ali 2011). It enjoys a very high consumer preference in the local market as an edible fish, and high value in the international aquarium pet trade (Ali et al. 2007). Their utilization in the ornamental fish trade is however dependent on its capture from the wild. Although the fish could be domesticated and was observed to attain maturity in pond conditions (Padmakumar et al. 2011), there are no reports on its natural breeding in confined waters. Artificial breeding of this species was successfully carried out in the year 2000, by the Regional Agricultural Research Station (RARS), Kumarakom, Kerala under a network project in collaboration with the National Bureau of Fish Genetic Resources (NBFGR), Lucknow, India (Padmakumar et al. 2004). The RARS is engaged in a program for mass production of seeds of *H. brachysoma* with the goal of promoting larval rearing and aquaculture (Bindu 2006; Padmakumar et al. 2011) and the seeds produced as part of the Indian Council of Agricultural Research (ICAR) Mega Seed Project were utilized for stocking the Sasthamcotta Lake by the Kerala State Fisheries Department (Padmakumar et al. 2011). Previous research on *H. brachysoma* focused on its biology (Sreeraj et al. 2006; Padmakumar et al. 2010;

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Bindu et al. 2012), fishery (Sreeraj et al. 2007), breeding (Padmakumar et al. 2011), population genetics (Muneer et al. 2009, 2012) and aquaculture (Sahoo et al. 2010; Giri et al. 2011). However, there is only limited information on the embryonic development of this species under captive conditions (Padmakumar et al. 2004).

Factors like reproductive strategies, fecundity, ease of larviculture, economic acceptance and profitability are essential to determine the viability of a species for aquaculture (Wittenrich et al. 2007). Studies on early development of fish species are useful to improve the understanding of larval development and aquaculture practices. The present paper documents the embryonic and post embryonic development of *H. brachysoma* subjected to induced spawning.

Methods

Horabagrus brachysoma were collected from the riverine stretches of Vembanad Lake (09^{0}31'–09^{0}41'N & 76^{0}21'–76^{0}26'E), India and stocked in earthen ponds (250m^2) at RARS, for induced breeding experiments. Adult fishes of size 13–27 cm (19.26±3.33 cm) collected and farm-reared during 2003–2004 were utilized for the present study. Studies on the reproductive biology of the species revealed that the major spawning season in the wild is during the monsoon from June to July (Bindu et al. 2012) and is therefore categorized as a seasonal strategist spawner (Winemiller 1989). Based on this information, breeding trials were taken up during the monsoon months (June–September). Male and female fishes were selected based on their secondary sexual characters which were prominent during the breeding season (Padmakumar et al. 2011). Selected fishes were weighed and subjected to induced ovulation, intraperitonially, just below the pectoral fin, by using Ovaprim® (contains 20µg SGnRHa and 10mg Domperidone in 1ml) at the rate of 1ml kg^{-1} body weight. For studying the development, seven sets of experiments were carried out during 2004–2005. Since males were smaller than females in most cases, each breeding set comprised two males and one female, maintaining a sex ratio of 2:1. Injected fishes were kept in 1000 litre flat bottom Fibre Reinforced Plastics (FRP) tanks holding filtered freshwater up to a height of 20–30 cm. Spawning and fertilization occurred in this tank. Polyvinyl chloride (PVC) pipe was provided in each tank as hide outs.

The released eggs were collected and spread in glass tanks (123x49x47 cm) containing filtered freshwater (up to 10–15 cm) with gentle aeration, for hatching. Temperature, dissolved oxygen (DO) and pH of the water were monitored using a digital water quality analyzer (Eutech, Singapore). The spawning and hatching tanks were provided with well-aerated water (DO 5.0–5.6 mg l^{-1}, pH 6.5–7.8 and temperature 24–26 ^{0}C). Spawning fecundity (total eggs per spawning) was calculated by multiplying the eggs in one subsample with the total. Fertilization and hatching rates were calculated according to Lagler (1982). The diameter of the mature egg was determined to the nearest 0.01mm by averaging the measurements of at least 50 eggs. Random samples of 10–15 eggs were collected and observed periodically at 15 minute intervals, under a trinocular microscope (CETI, Belgium) at 10X magnification. All the embryonic and larval development stages were documented using Magnus Imaging System supported by PixelView software, with the aid of a microscope, connected to a computer. For this purpose, eggs were collected at the time of fertilization and the time was denoted as 0:0h. At each stage of development, dead eggs were removed from the spawning tank by siphoning from the bottom.

In the present study, early developmental stages of *H. brachysoma* were grouped into embryonic (from fertilized egg stage to the time when heart pulsation in the embryo became apparent), yolk-sac larvae period (from hatching to when the yolk of the larvae was absorbed), post yolk-sac larvae period (from complete absorption of yolk to the juvenile stage) and juvenile period (Liang et al. 2003). The embryonic and post embryonic stages were recorded as hours post fertilization (HPF) and days post hatching (DPH) respectively. After hatching, hatchlings were kept in 1000l FRP tanks. From four DPH they were fed with boiled egg yolk. Larval size up to five DPH was measured by taking the mean total length of 15 individuals on each day. One week old larvae were shifted to manured cement tanks (5x3x1.2 m) and hapa net (5x4x1 m) with 50% shade. The experiment (in duplicate) was conducted for 85 days, at the end of which the final length and weight of the juveniles were recorded. During this period, the larvae were fed twice a day with powdered commercial pellets Higashi Fresh (Higashimaru Limited, Chennai; crude protein 20%), containing fish meal as the major ingredient, at 10% of the tank biomass.

Results and Discussion

Spawning, embryonic development, hatching and larval development of *H. brachysoma* resemble other catfishes with slight variations in latency period, incubation time, egg size and larval size (Thakur 1980; Arockiaraj et al. 2003; Rahman et al. 2004; Islam 2005; Aneesh et al. 2013). Results of the selected breeding trials

are provided in Table 1. *Horabagrus brachysoma* was observed to be a broadcast spawner and the eggs were scattered on the bottom of the spawning tank. Spawning occurred 11.61±1.53 hr, after hormonal administration, at mean water temperature of 24.64 ±0.75 ^{0}C. Spawning fecundity in each trial (I–VI) varied between 13240 and 31960. The absolute fecundity (the total number of mature eggs in an ovary) of *H. brachysoma* is however higher (20472±20542) (Padmakumar et al. 2011; Bindu et al. 2012) than those reported for most catfishes (3000 to 20000) (Thakur & Das 1985; Szyper et al. 2001; Bindu et al. 2009), especially for species which do not exhibit parental care (Chaudhari & Singh 1984). The fertilized eggs of *H. brachysoma* were spherical, golden yellow in colour, with a diameter of 1.61±0.051 mm. These eggs are larger than that of *Clarias batrachus, Ompok pabda, Heteropneustes fossilis, Mystus cavasius* and *Clarias dussumieri* (Thakur & Das 1985; Rao et al. 1994; Kohinoor et al. 1997; Sreedhar & Haniffa 1999; Rahman et al. 2004; Aneesh et al. 2013). Fertilized eggs were non-adhesive, but free and demersal, and could be easily transferred to incubation tanks. This may be linked to the natural occurrence of bagrid catfishes in downstream slow flowing habitats in river systems (Arratia et al. 2003). More than 90% of fertilization and 23–95 % hatching was achieved in the experimental trials. In trial VII, conducted at the end of the breeding season (September), female fish was utilized for the second time for spawning in the very same season (utilized for the first time in June). Even though the spawning fecundity was lesser the same female produced viable eggs and larvae pointing out the possibility of multiple spawning in *H. brachysoma*.

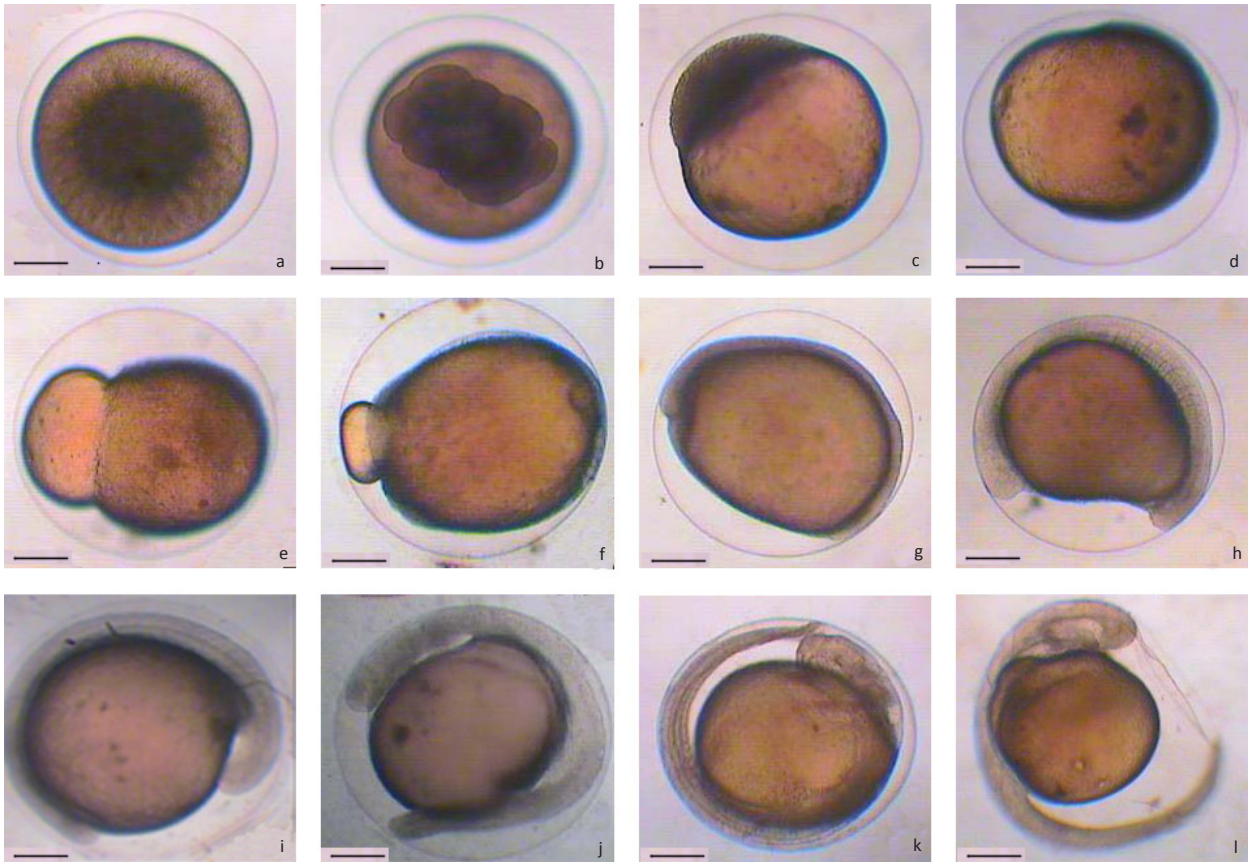
Table 1. Selected breeding trials of *Horabagrus brachysoma* carried out during 2004-2005 using Ovaprim @1ml $^{-1}$ as the inducing agent

Breeding parameters	Breeding trials						
	I	II	III	IV	V	VI	VII#
Fish weight (g) Male (2 individuals) Female (1 individual)	200 160	470 250	220* 190	535 220	400 225	330 230	370 250
Latency period (hours)	9	13	10	12	12.30	12	13
Fecundity	29450	13240	17280	29000	26208	31960	5170
Fertilization rate (%)	95	90	94	98	99	98	95
Incubation period (hours)	24.30	25	24	27	22	25	27
Hatching rate (%)	95	90	63	90	90	23	29
Water temperature (^{0}C)	24	25	24	25	24	26	24.5
Water pH	6.5	6.8	6.5	7.6	6.8	6.5	7.8

*- only one male was utilized; # - 2nd breeding of the female in the same season

Embryonic development

Stages of embryonic development are summarised in Table 2. At 1HPF, the chorion separated from the spherical yolk (Image 1a). Embryonic development commenced with the formation of the blastoderm at the animal pole region, leaving a large yolk at the vegetal pole. This was followed by cell division, reaching 16-celled stage after 30min (Image 1b). A clear blastocoel began to appear at about 3:30 HPF (Image 1c) and the blastula appeared as a cap of cells over the yolk. By 4 HPF, the diameter of the fertilized egg was 1.82±0.04 mm. During the late blastula stage (5:30 HPF) the blastoderm flattened and migrated over the yolk. An embryonic shield appeared in the gastrula stage (Image 1d). The germinal ring then migrated equidistantly around the yolk and constricted it. Gradually, epibolic germ layers spread to the equator of the spherical yolk surface, and at 7:30 HPF (Image 1e), the germinal ring accounted for three quarters of the total egg volume after which the yolk plug appeared. The formation of the embryonic shield (Image 1f) represents the early induction of the formation of embryo (Islam 2005). As the blastopore closed, the yolk plug become projected and the head rudiment is seen lifted up (Image 1g). The optic rudiment gradually became differentiated into a vesicle by 11 HPF. A head fold could be seen at the proximal end of the embryo and myomeres became clear. At 12 HPF (Image 1h) the tail bud appeared and the embryo was seen encircled over the yolk, reaching nearly three quarters of its circumference. The tail bud elongated in an antero-posterior direction by 14 HPF and the brain differentiation was evident. Distinct paired somites were observed at 15:30 HPF (Image 1i). Development of lenses and auditory placodes were observed at 18–19 HPF. The caudal fin fold became elongated and the embryo appeared 'C' shaped encircling the yolk. Twitching movements of the muscular somites at intervals were observed at this stage (Image 1j). By 20 HPF, the heart appeared anterior to the yolk and embryonic movement became rapid. At 21 HPF, tail became free and the embryo encircled almost 90% of the yolk mass. Gradually, the heart exhibited a rhythmic beat and the chorion became soft and wrinkled. As the development advanced the embryo appeared more and more elongated (Image 1k). Pectoral fin folds were formed and a pair of otoliths was observed in the auditory vesicle. The myomeres became prominent and the embryo at this stage started to twitch and roll within the chorion. The protruding embryo was seen to violently beat inside the eggshell, trying to rupture the shell within 1h of hatching. A similar movement has also been reported in other catfishes such as *H. fossilis* (Bindu



Images 1 a–l. Embryonic development of *Horabagrus brachysoma.* (© L. Bindu)
a - 1 HPF; b - 1.30 HPF; c - 3.30 HPF; d - 6 HPF; e - 7.30 HPF; f - 8 HPF; g - 8 HPF; h - 12 HPF; i - 15.30 HPF; j - 19.30 HPF; k - 23.30 HPF; l - 24 HPF (scale bar = 1mm)

et al. 2009), *C. batrachus* (Thakur 1980) and *Pangasius sutchi* (Islam 2005). Gradually the pulsation of the heart also became regular at 100–102 beats min^{-1}. The chorion ruptured and the tail emerged first, followed by the head. Hatching occurred at 24 HPF (Image 1l). The embryonic stages of *H. brachysoma* are similar to other tropical catfishes (Thakur & Das 1985; Arockiaraj et al. 2003; Islam 2005). Hatching time in catfishes vary widely between 16 HPF and 75 days (Thakur & Das 1985; Arratia et al. 2003; Padmakumar et al. 2004; Bindu et al. 2009; Aneesh et al. 2013). In the present experiment the hatching time varied between 22 and 27 hr at 24–26 ^{0}C. The hatching period of *P. sutchi* ranges from 24–36 hr at 22–32 ^{0}C (Islam 2005) and that of *C. gariepinus* 21–26 hr at 20–32 ^{0}C (Bruton 1979). A water pH of 6.5–7.0 (mean 6.93±0.55) was found to be suitable for hatching and larval rearing of *H. brachysoma* in the laboratory conditions. At a higher pH (7.6–7.8) hatching was delayed, all the developmental stages were arrested, while eggs succumbed at a pH higher than 8.5 (Bindu 2006).

Yolk-sac larvae period

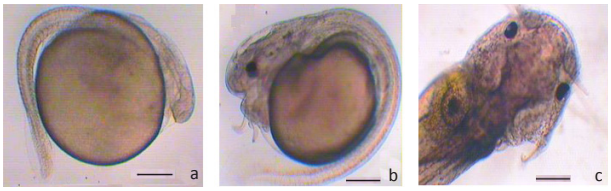
The newly hatched larvae (3.94±0.112 mm) retained the remnant yolk on the ventral side. The hatchlings were slender and transparent with unpigmented eyes, and without a distinct mouth (Image 2a). They were laterally compressed and curved around the circular yolk sac with the downward protrusion of the head. The notochord and myomeres were more prominent. The hatchlings gradually straightened with lashing tail movements and were congregated in the tank corners. Anal fin fold extended from the postero-dorsal end to the ventral region. Size of the hatchlings varied between 3.8 and 4.1 mm similar to *C. gariepinus* (3.4–4.0 mm), but larger than *Mystus montanus*, *M. cavasius, P. sutchi* and *H. fossilis* (Arockiaraj et al. 2003; Rahman et al. 2004; Islam 2005; Bindu et al. 2009).

Pigmented eyes and functional mouth parts formed in the 2 DPH larvae. Two pairs of barbels became visible and the auditory vesicles were more distinct (Image.2b). The pulsating, two chambered heart and the streaming of larval blood circulation to the caudal fin became perceptible at this stage. The body thickened and

Table 2. Stages of early development in *Horabagrus brachysoma*

HPF*(h:min.)	Developmental stages
00:00	Fertilization
01:00	Appearance of blastodisc
01:30	16 cell stage
02:00	32 cell stage
03:00	Early morula
04:00	Blastula
06:00	Early gastrula
07:30	Late gastrula
08:00	Neurula stage
08:30	Closure of blastopore
09:00	Appearance of optic rudiment
11:00	Appearance of myomeres
14:00	Tail region detach from the yolk
15:30	Tail buds projected out
18:00	Movement of the embryo started.
20:00	Appearance of heart
24:00	Hatching

* Hours post fertilization



Images 2 a–c. a - Newly hatched larva of *Horabagrus brachysoma*; b - at 2DPH; c - at 4DPH (scale bar = 1mm). (© L. Bindu)

increased in length. The length of the larvae ranged between 4.75 and 5.25 mm (5.08 ± 0.164 mm). At this time, the mean diameter of the yolk sac was 1.39±0.067 mm. At 3 DPH, the size of the larvae varied between 5 and 5.75 mm (5.52±0.571 mm). The absorption of the yolk sac started from the mouth region and by the end of 3 DPH, 90% of the yolk was absorbed and the yolk sac was 1.35±0.08 mm in diameter. The upper and lower jaws were well developed. Four pairs of barbels were clearly visible. Melanophores were observed along the head and dorsal surface of the body. The pelvic fin became visible, and the fin fold appeared to be continuous, extending dorsally from behind the head, up to the postero-ventral margin of the yolk. The larvae started to move actively in the water.

Post yolk-sac larvae period

The yolk was fully absorbed four days after the completion of embryonic development (Image 2c) and the diameter of the yolk sac reduced to 1.1±0.065 mm. the length of the larvae ranged from 5.5–6.3 mm (5.9±0.307 mm). The head appeared broad and well differentiated with denser pigmentation. Barbels became elongated; the maxillary pair was perceptibly longer than the others. Pectoral fin rays became well differentiated with their characteristic spines. In *H. brachysoma*, the hatchlings subsisted on endogenous nutrition from the yolk till 4 DPH, and accepted exogenous feeding from the 5 DPH at which stage they were fed on powdered yolk of boiled egg. The hatchlings could gradually be weaned to powdered pellet feed also. The gape size of the larvae became appropriate to feed on the live plankton in 4–5 DPH, and the jaw bones became stronger. At this time, the tip of the caudal fin bifurcated and caudal fin rays became clearly visible. The characteristic black humeral ocellus became more prominent, and the fry grew to an average size of 6.75±0.49 mm (range: 6.0–7.5 mm) in a week. They were subsequently transferred to fry nursing hapas and cement tanks for further rearing.

Juvenile Period

The fry attained a size of 26.2±2.39 mm in a month, when fed at the rate of 10% of biomass. Feed management, especially exogenous feeding of the newly hatched larvae, is considered to be a major constraint in fish larviculture (Sorgeloos & Leger 1992; Wittenrich et al. 2007). The observation that larvae of *H. brachysoma* accept and relish commercial feed at a very early stage, as is evident from 50–75 % survival in fry nursing, is of great significance. The larvae achieved 58–65 mm in 85 days (Table 3) (Image 3). In addition to the naturally available plankton, fish fry were also fed with rice bran and ground nut oil cake. Probably this mixed feeding behavior in the early life history stages of *H. brachysoma* appears to be a factor that favors smooth weaning of the early larvae to artificial diets.

Conclusion

Horabagrus brachysoma, is found to be a potential candidate for diversification of aquaculture due to its hardy nature, omnivorous feeding behaviour (Sreeraj et al. 2006; Padmakumar et al. 2009) as well as high fecundity (Bindu et al. 2012). Anthropogenic impacts including overfishing (Prasad et al. 2012), habitat loss and delayed monsoon could result in large scale depletion of wild populations of this threatened species. Mass seed production through induced breeding would therefore help in stock enhancement

Table 3. Growth parameters of *Horabagrus brachysoma* larvae reared in hapa nurseries and cement tanks (mean stocking size: 6.75±0.04 mm; rearing period: 85 days)

Trial No.		Final Length (mm)	Final Weight (mg)	*df*	*b*	*r*	Survival (%)
Tank	I	58.03±6.49	21.7±7.3	72	2.674	0.889	75
	II	58.24±6.42	21.8±7.2	71	2.630	0.871	70
Hapa	I	65.1±5.4	30.9±8.4	69	2.819	0.863	50
	II	64.9±4.4	31.8±7.4	68	2.758	0.843	55

df - degrees of freedom; *b* - exponent of length-weight relationship; *r* - coefficient of determination

and ranching in our natural water bodies facilitating the conservation and management of *H. brachysoma* wild populations. However significant improvements in larval rearing technology will need to be achieved for popularization of rearing and culture techniques. The results of the present study provide baseline information for further investigations regarding multiple spawning behavior, larval rearing and pond culture of this endemic and threatened species.

Image 3. Juveniles (85 days old) of *Horabagrus brachysoma*

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Errata

Babu, S. & S. Bhupathy (2013). Birds of Meghamalai Landscape, southern Western Ghats, India. *Journal of Threatened Taxa* 5(15): 4962–4972; http://dx.doi.org/10.11609/JoTT.o3594.4962-72

Page 4969 Table 1: The notation of Imae 2 to be moved from Malabar Whistling-thrush to Grey-breasted Laughing-thrush.

Page 4970: Image 2: Read caption as Gey-breasted Laughing-thrush instead of Malabar Whistling-thrush.