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Captive breeding and developmental biology of *Sahyadria denisonii* (Day 1865) (Cyprinidae), an endangered fish of the Western Ghats, India

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ABSTRACT

Over the past few decades wild population of *Sahyadria denisonii* (Day 1865), an endemic ornamental barb of the Western Ghats of India has been overexploited for aquarium trade and is presently listed under endangered (EN) category in the IUCN Red List. The present study communicates the first ever success of captive breeding and early developmental studies of *S. denisonii*. Life history phases of *S. denisonii* were classified into embryonic, larval, juvenile, subadult and adult stages. Spawning season was from November to March in wild and fecundity varied depending on the size and age of breeding pairs. Eggs were obtained through induced breeding using ovaprim hormone at 0.4 ml per kg body weight. Fertilised eggs were adhesive, demersal and attached to any substratum having a diameter of 1184-1312 µm. Hatching took place 36 h after fertilisation at a water temperature of 27.5±0.5°C. At hatching, mean larval length was 3.5±0.2 mm with high amount of yolk and the yolk sac remained up to 3-4 days. Organogenesis of larvae was completed 15-20 days after hatching. In this paper full developmental sequence from egg to adult stages of *S. denisonii* in controlled condition is described.

Keywords: Captive breeding, Embryonic development, Miss Kerala, *Sahyadria denisonii*, Western Ghats

Introduction

The Western Ghats of India is one of the biodiversity hotspots of the world (Myers, 2000) and its range of hills running along India's west coast (08º 19'08'' - 21º 16'24''N to 72º 56'24''- 78º 19'40''E) is one of the richest regions in terms of its biological diversity. The Western Ghats extends 1490 km from north to south with a minimum width of 48 km and maximum of 210 km, covering a total area of 136,800 km^2 (Molur *et al.,* 2011). The area is drained by 38 east flowing and 27 west flowing major rivers with running water and lacustrine habitats (Abell *et al.,* 2008). Ragahvan and Dahanukar (2013), listed 320 species of freshwater fishes belonging to 11 orders, 35 families and 112 genera including certain secondary freshwater species, which can also live in brackishwater and marine habitats. The Kerala State on the south - western corner of the Indian peninsula is crisscrossed by 44 rivers arising from the Western Ghats (41 west flowing and 3 east flowing), having an immensely rich ichthyofauna of well over 300 species, of which about 50% have ornamental and recreational value (Mercy, 2009).

With the high demand and pricing of many ornamental fish, they are being harvested at greater volumes and sold at higher rates, threatening the viability and sustainability of the resources (Chao, 2001; Vagelli and Erdmann, 2002; Cato and Brown, 2003; Lunn and Moreau, 2004). In India, most wild caught aquarium fish are from the Eastern Himalayas and Western Ghats which are ecological hotspots, known for their amazing freshwater biodiversity and endemism (Allen *et al.,* 2010; Molur *et al.,* 2011). Raghavan *et al*. (2013) reported that around two dozen fishes are regularly being exported from Western Ghats of Kerala, while Liya and Ramachandran (2013) listed *Tetraodon travancoricus*, *Dario dario*, *Sahyadria denisonii*, *Botia striata* and *Carinotetraodon imitator* as the major species of ornamental fishes traded from India for the years 2005-2010. Of India's total live ornamental fish exports to the tune of US $ 1.54 million during 2007-08, *S. denisonii* accounted for almost 60-65%. Raghavan *et al.* (2013) recorded that 310,791 numbers of *S. denisonii* were exported from India during 2005-2012 and the main markets were Singapore (48.63%), Hong Kong (30.52%) and Malaysia (18.4%), with negligible quantities being exported to Germany, United Kingdom and Japan.

Among the native ornamental fishes of Western Ghats region, no species has received global fame and hobbyist attention as that of the Redline torpedo fish, *S. denisonii*. The distribution of *S. denisonii* is restricted to the southern regions (Kerala and south Karnataka) of the Western Ghats hotspot of India and their population appears to be extremely fragmented in fourteen rivers namely Chalakudy, Periyar, Achencovil, Pampa, Valapattanam, Chaliyar, Kallar, Chandragiri, Bharathapuzha, Bhavani, Karingode, Kuppam, Anjarkandipusha, Kuttiyadi

(Biju *et al.,* 2000; Shaji and Easa, 2001; Shaji *et al.,* 2000; Raghavan *et al*., 2010; Mercy *et al*., 2010 b; 2013 b).

Raghavan *et al.* (2010) and Silas *et al.* (2011) observed that over-exploitation of freshwater fishes from the wild for aquarium trade is the main reason for severe depletion of their population. The existing export business of freshwater ornamental fishes from India is not sustainable since the present trade is completely dependent on wild collection (Dahanukar *et al.,* 2004; Raghavan *et al.,* 2013). Over the last few decades, wild population of *S. denisonii* has declined due to various reasons (Dahanukar *et al.,* 2004; Mercy *et al.,* 2010a; Raghavan *et al*., 2013) and IUCN's Freshwater Biodiversity Assessment of the Western Ghats has categorised this species as Endangered (Ali *et al*., 2010). In a bid to ensure long term availability of *S. denisonii*, the government of Kerala is currently in the process of finalising various measures aimed at checking the uncontrolled, unorganised, unscientific nature of wild collection and export of this species (Raghavan *et al*., 2010).

Captive breeding is a conservation strategy that is widely used for the recovery and reintroduction of endangered species (Kelley *et al.,* 2006). Efforts were taken to develop captive breeding technology for indigenous fish species from Western Ghats of India (Ogale, 2002; Padmakumar *et al.,* 2004; Swain *et al.,* 2008; Mercy, 2009). It is envisaged that if *S. denisonii* can be produced in captivity at a commercial level which will definitely boost the share of the species in the trade to a large extent and will naturally lead to the conservation of its germplasm. In this context, captive breeding technology for *S. denisonii* was successfully developed for the first time and the early embryonic as well as larval development stages were documented.

Materials and methods

Development of broodstock under simulated natural condition

A water flowing habitat was constructed near the Iruvanchipuzha, tributary of River Chaliyar, in such a way that water from the same river is pumped to overhead storage tank (10x10x2 m). Water was allowed to flow continuously through the artificial habitat (10x3x2 m) by gravitational force. All the materials used in the artificial habitat, such as sand, bottom sediments, rocks and plants were brought from the same river from where the fishes were collected. A diagrammatic representation of the habitat is given in Fig. 1. Fifty juveniles of *S. denisonii* in the size range 6-8 cm were collected from the Iruvanjipuzha Tributary of River Chaliyar in Calicut District of southern Kerala during September 2008. The collected fishes were transported in oxygen filled polythene bags and stocked in running water habitat. They were fed with protein rich artificial food, bloodworms and earthworms *ad libitum*.

Captive breeding

Twenty five males and 15 females of *S. denisonii* collected from the wild were segregated and reared in glass tanks (3x2x2 ft.). As there is no distinct sexual dimorphism in this species; the only dimorphic character observed was that female has a slightly wider body than the male when sexually mature. In a fully ripe female (size 10 cm and above), eggs are seen extruded under slight pressure and male fish (size 9 cm and above) is identified by the flow of milt on applying gentle pressure on the abdominal region towards the genital openings (Mercy *et al.,* 2013b). Maturity of the ripe eggs was determined by observing the position of migratory nucleus of the eggs under a microscope (Rottmann *et al.,* 1991).

Induced breeding experiments were conducted during December 2009 to March 2010, coinciding with the natural spawning of this species in nature. From the life history parameters, it was evident that the fish bred during November-April months (Mercy *et al*., 2010a, 2013a, Solomon *et al.,* 2011). Since the broodfishes are very sensitive and handling stress can lead to their mortality, the fish were anaesthetised before handling using clove oil (30 mg l^{-1}) (Sajan *et al*., 2012). The anaesthetised male (10.4±1.6 cm, 15.5±2.7 g) and female (12.1±1.5 cm, 21.5±3.4 g) broodfishes were injected intramuscularly between the anterior region of dorsal fin and lateral line using a 1ml syringe with single dose of ovaprim (SGnRH+ Domperidone- Syndel laboratory, Canada) at a rate of 0.4 ml kg^{-1} body weight by late evening (around 18.00 hrs). Breeding set comprising male and female in 2:1 ratio were injected and were kept together in glass tanks (150 l) provided with continuous aeration. It was observed that induced broodfishes did not release eggs or milt by their own. After 10-12 h of latency period, fish were inspected for readiness for ovulation. Both males and females were stripped, and the eggs were dry fertilised using milt collected (Harvey and Carolsfeld, 1993). Fertilised eggs were transferred to a glass tank (10 l) and aerated gently. Ten trials were made successfully during the season. Water quality parameters were monitored at weekly intervals (Table 1). Embryonic and larval developments were documented and photographed with binocular stereo microscope (LABOMED) with digital camera (Canon Power Shot-A570).

The hatchery produced juveniles (F_1 generation) were reared in the hatchery at Thiruvambady and hundred numbers of juveniles were transported live to the hatchery at the College of Fisheries, Ernakulam during June 2010. They were acclimatised and reared in outdoor rectangular cement tanks (10 t capacity) and were fed with the same food as before. Water quality parameters were maintained similar to the wild (Table 1). During January 2012, three

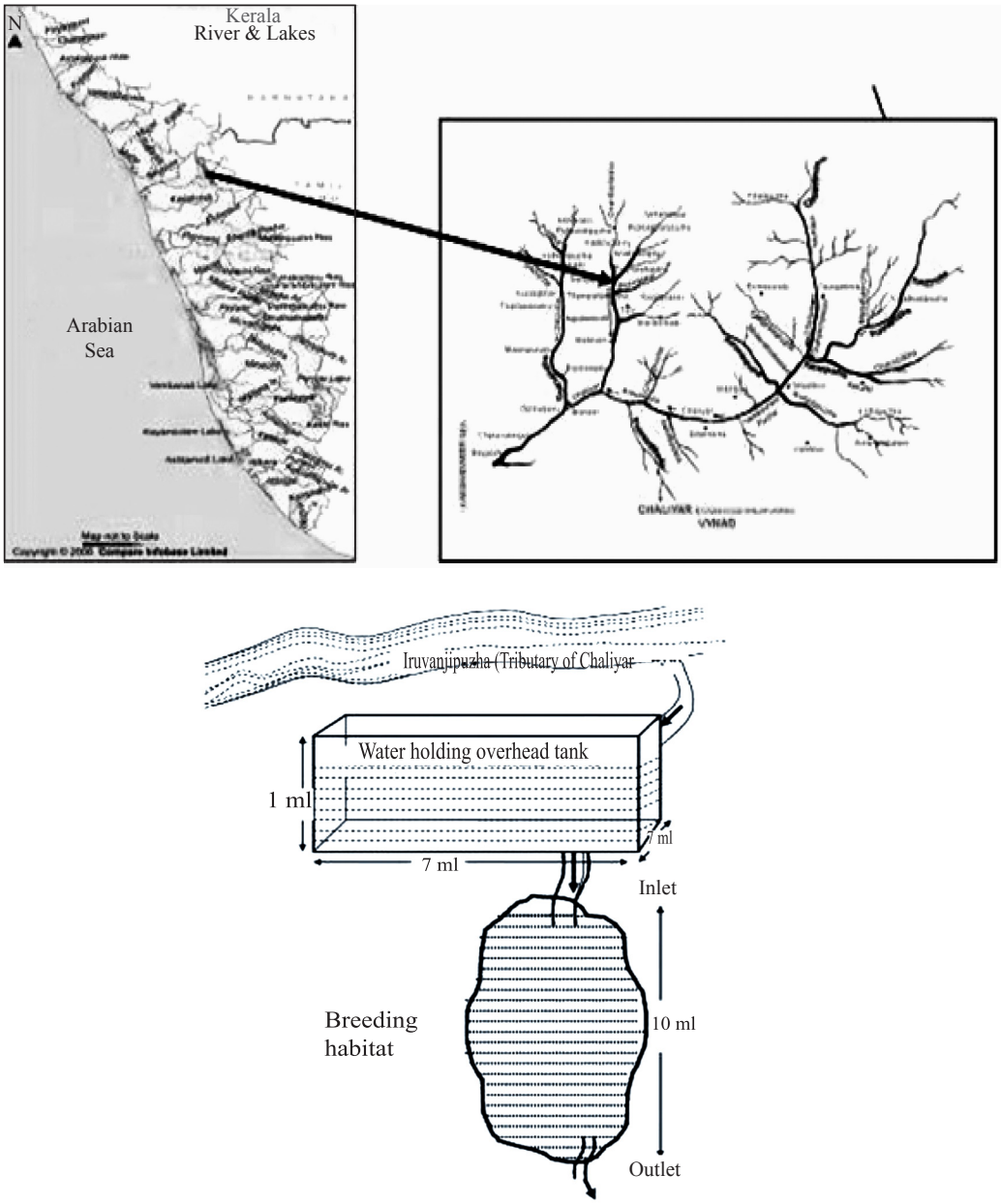

Fig. 1. Schematic representation of the modification of habitat for captive breeding of *Sahyadria denisonii*

Table 1. Water quality parameters of the wild habitat and broodstock rearing tank

Parameters	Range
Water temperature (°C)	26 - 28
pH	7.0 -7.5
Dissolved oxygen (mg l^{-1})	5.0- 6.8
Total alkalinity (mg l^{-1})	20 - 25
Hardness (mg l^{-1})	20 - 25
Ammonia (mg l^{-1})	<0.01
Nitrate (mg l^{-1})	<0.01

pairs of *S. denisonii* of F_1 generation were successfully bred following the same protocol as above. Instead of clove oil, MS-222 (150 mg l^{-1}) was used as anaesthetic agent (Mercy *et al*., 2013a)

Results and discussion

The results of this study gave a better understanding about the breeding protocol of *S. denisonii.* In hatchery, broodstock management is one of the major aspects for successful induced breeding of any fish species. Proper care of broodstock is very important for assuring the production of eggs, fry and fingerlings (Siddik *et al*., 2013). In the present study, *S. denisonii* was successfully bred under captivity by artificial fertilisation, using ovaprim as inducing agent. Ovaprim was used as an inducing agent for *Labeo dussumieri* (Kurup, 1994); *Channa striatus* (Haniffa *et al.,* 2000); *Systomus*

sarana (Chakraborty *et al.,* 2003); *Mystus montanus* (Arockiaraj *et al.,* 2003), *Schizothorax richardsonii* (Agarwal *et al*., 2007); *Garra surendranathanii* (Thamby, 2009) and *Dwakinsia filamentosus* (Mercy, 2009). Ovaprim has been accepted as the most superior inducing agent for carps with high spawning success, percentage of fertilisation and hatching rate (Nandeesha *et al.*, 1990). Fertilised eggs (Fig. 2.2) of *S. denisonii* were heavily yolked, transparent, spherical and yellow in colour. The eggs were sticky and seen stuck to the glass surface similar to *Puntius* species. Egg incubation and hatching is better performed in glass tanks under slight continuous aeration. According to Balon (1975), *S. denisonii* is a lithophil (morphotype), open substratum spawner and falls under ethological class of non-guarders as per the eco-morphological classification. According to Mercy *et al.* (2013b) absolute fecundity of *S. denisonii* ranged from 293 to 967ova per female, while Solomon *et al.* (2011) recorded it as 376 to 1098.

The embryonic period started just after fertilisation and ended when the embryo acquired all the organ systems as in other fishes (Table 2; Fig. 2). In the present breeding trial, latency period was observed as 12.78±0.83 h, fertilisation rate as 86.11±5.23% and hatching rate as 85.89±2.98%. The higher rates of artificial fertilisation during the present investigation may be related to the dry method of fertilisation where the viability of the spermatozoa remains high. However, the percentage of fertilisation is also related to the maturity and weight of fish (Haque and Ahmed, 1991; Agarwal *et al*., 2007). In the present study, breeding was performed at an ambient temperature of 26.0-28.0°C. This range of temperature is suitable for breeding of most indigenous small fishes (Islam and Chowdhury, 1976; Akhteruzzaman *et al*., 1992; Siddik *et al*., 2013).

Embryonic phases

Formation of embryo : The fertilised eggs (Fig. 2.2) were spherical, orange brown in colour and adhesive with a size of 1184-1312 µm dia. Fertilised eggs were sticky due to the sticky secondary membrane from the follicular envelope. Egg development started immediately after fertilisation and it activated the cytoplasmic movements. Yolk free cytoplasm begins to stream towards the animal pole gradually segregating the blastodisc from the vegetal cytoplasm. Within 20 min, the streaming movement of the protoplasm towards the lower pole is completed and a blastodisc is formed (Fig. 2.4). The first cleavage commenced 20 min after fertilisation when the blastodisc was divided into two blastomeres (Fig. 2.5) followed by second cleavage at the 40th min after fertilisation (Fig. 2.6). The sixteen cell stage (Fig. 2.8) was noticed within 80 min and 32 cell stage at 100-110 min post-fertilisation. Cell division continued rapidly after the eight blastomere stage (Fig. 2.7). Morula (Fig. 2.9) and blastula (Fig. 2.10) stage occurred at 180th and 210th min respectively.

Cell division took place more or less synchronously during the early stages of the blastula period. Blastoderm

Table 2. Embryonic developmental stages of *Sahyadria denisonii*

Developmental stages	Duration	Figure	Features
Eggs freely ooze out	00:00 h.	2.01	Sticky and orange in colour
Fertilized eggs	00:00 h	2.02	Spherical, orange coloured, demersal and adhesive
		2.03	
Blastodisc stage	00:10 h	2.04	Cytoplasm streams toward animal pole
Two cell stage	00:20 h	2.05	First cleavage
Four cell stage	00:40 h	2.06	Second cleavage
Eight cell stage	00:60 h	2.07	Third cleavage
Sixteen cell stage	01:20 h	2.08	Fourth cleavage
Morula stage	03:00 h	2.09	Blastodermal cells formed after cleavage, arranged in group in the animal pole
Blastula stage	03:30 h	2.10	Crown like blastodern spread over yolk at animal pole
Dome stage	06:00 h	2.11	Bblastoderm expanded to the equator of the yolk sphere; pre-gastrula stage
Late blastula stage	08:00 h	2.12	Blastoderm extended towards the vegetal pole
Blastopore stage	08:30 h	2.13	Large yolk plug protrudes from the blastopore.
Yolk plug stage	09:00 h	2.14	Yolk invasion completed by gradual spreading over the germ layer. Rudimentary head and tail appeared and became differentiated.
Head - tail bud stage	17:00 h	2.16	Head and tail rudiment visible
Tail free stage	18:00 h	2.17	Yolk at the tail end elevated to inside to free tail from yolk mass.
Eye vesicle stage	19:00 h	2.18	Eye vesicle observed
Twitching stage	30:00 h	2.19	Vigorous movements before hatching.
Just before hatching	34:00 h	2.20	Continuous beating of the caudal region especially around middle region of the body
Hatching	36:00 h	5.21	The egg shell breaks up and the tail emerges out first, followed by the head; 3.54 ± 0.21 mm in total length
		2.22	
		2.23	
Just hatched larvae	36:00 h	2.24	Larvae hatch out from egg capsule
Hatchling 3.54 ± 0.21 mm TL	36:00 h.	2.25	Newly hatched larvae non-pigmented and had an average total length of 3.54 ± 0.21 mm and yolk sac of 1 mm dia.

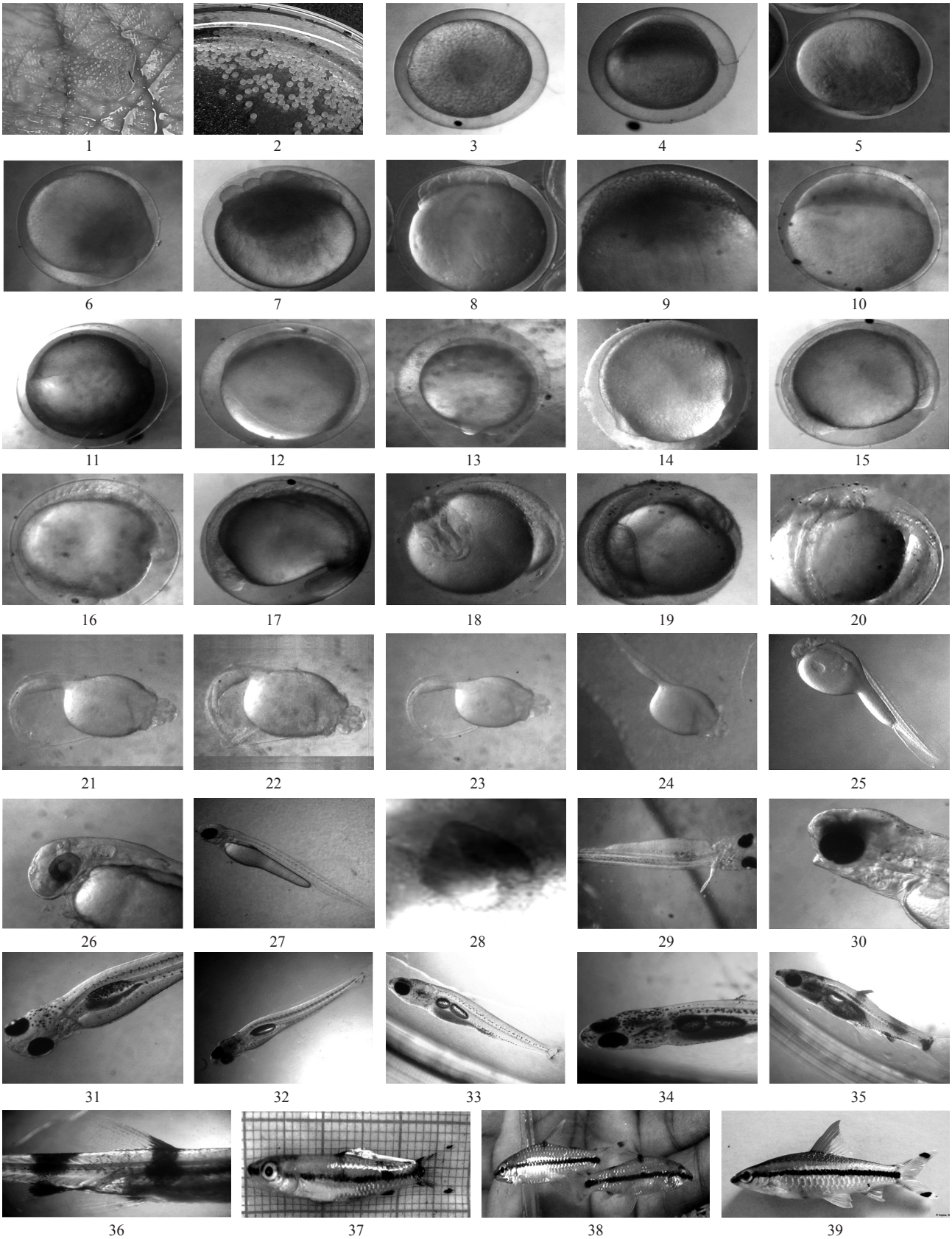

Fig. 2. Embryonic and larval development stages of *Sahyadria denisonii*

was observed at 5 h post-fertilisation, (hpf) followed by dome stage (Fig. 2.11) at 1 h. At dome stage, the blastoderm formed a dome-like shape due to bulging of the yolk towards the animal pole and the epiboly continued during the gastrulation period. In the gastrula period, extensive cell movements were observed, including involution, convergence and extension, producing the three primary germ layers and the embryonic axis. Gastrulation began with cell involution at around 50% epiboly. At 75% epiboly stage (Fig. 2.12), the embryonic shield became less distinctive, as compared to shield stage and its cells got repacked to elongate the shield along the animal pole axis. At 90% epiboly stage, the yolk plug was clearly seen in the vegetal pole (Fig. 2.13 and 2.14) and was noticed at 9 hpf. The segmentation period was observed at 15 h which was characterised by the sequential formation of the somites, and this period lasted up to hatching. During this period, the embryo elongated along the animal pole axis, the tail bud became longer and rudiments of the primary organs became visible (Fig. 2.15).

Differentiation of embryo: The head and tail bud of the embryo (Fig. 2.16) became clearly distinguishable after 17 h. In this stage, the optic primordium had a prominent horizontal crease and tail bud became more prominent. At 19 hpf, the eye vesicle was observed, the embryo increased in size and eighteen myotomes were clearly visible (Fig. 2.17). Tail bud started to separate from the yolk sac at 22 hpf (Fig. 2.18). The yolk extension got clearly delimited from the yolk ball as the tail straightened out. Twitching movements of the embryo started by 23 hpf. At 26 h, tail end was free from the rest of the yolk sac (Fig. 2.19) and at 30 h, embryo started to twist or rotate completely inside the egg membrane. After 34 h, movements of the embryo within the egg membrane were very frequent. Close to hatching, the twitching and lashing of the embryo inside the egg capsule became rapid (Fig. 2.20). The egg shell broke at 36 h due to rapid shaking movements of the body and the head emerged out first, followed by the tail (Fig. 2.21 to 2.24). Hatching of eggs continued up to 40 h, probably the temperature and oxygenation of the water played an important role in incubation as reported by Laurila *et al.* (1987) and Dwivedi and Zaidi (1983).

Larval phase

Larval phase can be further subdivided into yolk sac stage, pre-flexion stage, flexion stage and post-flexion stage (Fig. 2.25 to 2.39) according to the classification by Pena and Dumas (2009).

Yolk sac larva/eleuthero embryo: The newly hatched larvae (Fig. 2.25) were non-pigmented with an average total length of 3.5±0.2 mm and yolk sac of 1mm dia. Hatchlings had non- pigmented eyes and were devoid of distinct mouth and fins. Head seemed to be in contact with yolk sac and optic vesicles. The yolk sac is large and was found attached to the body along its ventral side. A narrow fin fold was found to be differentiated as a thin membrane, surrounding the caudal region and extended up to the yolk sac. The crawling movements of the hatchlings were detected immediately after hatching.

Two day old larva: On the second day, the larvae appeared to be more active; and inhabited the bottom of the tank as a group with average total length of about 4.1±0.2 mm (Fig. 2.28). The head portion seemed to be further developed. On closer observation, the heart was seen pulsating rhythmically. Pigmentation started in the eyes (Fig. 2.26). Normally in fish, the shape of the yolk sac undergoes significant changes during organogenesis (Winnicki *et al.,* 2001). In case of *S. denisonii* during embryogenesis, the yolk sac got divided into two parts, proximal portion with spherical shape and caudal part with cylindrical shape. Yolk sac became slightly reduced in size, with 3 mm in length towards the ventral side of the body (Fig. 2.27). Ventral and caudal fin folds were separated by the formation of anus. Formation of pectoral fin folds was also seen.

Three day old larva: Larvae on the third day, reached an average length of 4.5±0.2 mm. Yolk sac was seen further reduced. Mouth and anus became distinguishable. Pectoral fins with fin rays started to move vigorously (Fig. 2.29). Head became more prominent and free movements of the eye balls were also noticed Melanophores appeared around the head and snout (Fig. 2.30). Larvae exhibited vigorous movements upwards to the column water and finally sank to the bottom. All the active larvae got accumulated together into a lump-like ball showing strong thigmotactic behaviour.

Pre-flexion Stage : The pre-flexion stage began on day 4 when the larva started its first external feeding. Average length (TL) at this stage was 5.1±0.2 mm. Pre-flexion stage extended from a period of about 4 to 8 days; till it reached an average TL of 6.8±0.4 mm. Yolk sac got reduced and became tube like with 2.2 mm length and 0.5 mm width. At the onset of the pre-flexion stage the eye became fully pigmented and the mouth and anus opened. Alimentary canal was found to contain a yellowish fluid. Air bladder was noticed and occurrence of pigmentation on the air bladder started (Fig. 2.31 to 2.32). Opercular movements was also noticed. Caudal fin became modified with rudimentary fin rays. Pigments were found more concentrated on the anterior region of the body and also in caudal fin fold. Vigorous movements of the mouth was noticed as if the larva was in search of food. Eye balls exhibited rotating movements and larvae showed grouping behaviour when

all of them accumulated into a bundle towards the latter stage. Melanophores also appeared in the middle and in the notochord region. One week old larva was very active and started free swimming. Egg yolk was completely absorbed at this time. Average body length of the larvae increased to 7 mm and at this stage larvae fed entirely on exogenous food.

Flexion stage: The flexion stage started from day 08 (average TL 07.4±0.3 mm) and extended to day 17 (average TL 10.4±1.5 mm). The complement of the notochord flexion was characterised by the anterio-posterior orientation of the caudal rays. A key event was the development of flexion in the ventral side of the spinal cord in the notochord associated with the tail fin. On day 9 or 10, the single chambered air bladder (Fig. 2.32) was transformed into double chambered (Fig. 2.33). Furthermore, together with the disappearance of the larval fin fold and development of the fins a change in swimming style was also observed (Fig. 2.34). Similar observations were also reported in other teleost larvae (Van Snik *et al*., 1997; Gisbert, 1999; Gisbert *et al.,* 2002).

Post-flexion stage: The post-flexion stage was observed from day 17 (average TL 10.4±1.5 mm) and extended to day 26 (average TL 16.7±2.1 mm). After two weeks, the larvae completely metamorphosed; and the average body length increased to 11.2±1.5 mm. Changes during this stage included the development of dorsal fin having 6 - 7 fin rays. A vertical black band was formed over the body and an inferior mouth was clearly observed. Fin elements became evident in the dorsal, caudal, anal and pelvic fins during the post-flexion stage. Body pigmentation increased in the middle part of the body.

Larval feeding and weaning : The newly hatched larvae (3.5±0.2 mm) fed on its yolk content up to 3 to 4 days after hatching. Infusoria (pure culture of *Paramecium*) was given as first exogenous feed. The larvae were fed with freshly harvested paramecium at every 3 h interval. About 40-50% water exchange (30 l glass tanks) was done every day after exogenous feeding. After 10 days, the larvae were stocked in glass tanks (50 l) with an approximate stocking density of 10 larvae l^{-1} and were fed with live micro-worms (*Panagrellus* spp.) cultured using bread or oats medium. No substratum was provided during first two weeks of larval rearing and thereafter larval tanks were provided with 2 or 3 small pieces of clean rock as substratum for the larvae. Hatchlings were gradually weaned to artificial feeds (*Artemia* flakes), 12-14 days after hatching. Uneaten food was siphoned out from the bottom of each tank every morning before first feeding and dead fish were counted and preserved to analyse percentage of mortality.

Dissolved ammonia plays a vital role during larval rearing of *S. denisonii* and daily water quality assessment is necessary. In nature, post-larval stages of *S. denisonii* exhibit a bottom dwelling habit and they rely on bottom deposited diets such as detritus, algae and diatom that grows on pebbles and stones. Similar findings were also noticed by Costa and Fernando (1967). Feeding experiments suggested that artificial feed can be used for rearing post-larvae as well as juvenile stages of *S. denisonii* in aquaria (Mercy and Sajan, 2014) and it will be beneficial for the development of better larval rearing techniques for *S. denisonii* under hatchery condition.

Juvenile phase

One month old juveniles (average TL 29.8±2.3 mm) of *S. denisonii* showed vertical body banding pattern. Juveniles of *S. denisonii* possessed four vertical black cross band at nuchal, sub dorsal, supra anal and caudal positions (Fig. 2.35 to 2.36). Later, after disappearance of vertical cross bands, a lateral black bar started to appear from posterior margin of the eye region and extended towards the caudal peduncle. A transverse black and yellow band was observed in the tip of each lobe of the caudal fin. The anterior base of dorsal fin exhibited a black and red blotch (Fig. 2.36).

Sub-adult and adult phase

The juveniles (Fig. 2.37) reached an average TL of 35.8±5.3 mm size after rearing for three to four months in fiber tanks (200x100x50 cm). A red streak started to appear from the tip of the snout, parallel to the black stripe already formed posteriorly. It extended up to the middle of body below the dorsal fin. This sub-adult stage (Fig. 2.38) externally resembled adults in presence of these stripes. The four vertical cross bands disappeared and their body became greenish brown on the dorsal side and silvery in the ventral side (Fig. 2.38). Juveniles and sub-adults kept under fibre tank condition and in artificial habitat exhibited size variation during growth. This may be due to the availability of natural food and environmental conditions as has been reported by Hecht and Pienaar (1993) and Wankowski and Thorpe (1979) in *Salmo salar.* The fish became adult (Fig. 2.39) on reaching a size of TL 7.8±2.2 cm.

The results clearly demonstrated the possibility of using both clove oil and MS-222 as anaesthetics and synthetic fish breeding hormone ovaprim for effective induced breeding and seed production of *S. denisonii* under captive condition. This captive breeding technology has also been transferred to the ornamental fish breeders from different states by the Marine Product Export Development Authority, Kochi (Anon., 2014). The present work generated information on the early life history and developmental stages and also on commencement of first feeding time for larval rearing. The findings of the present

study can be used in induced breeding of *S. denisonii* in hatcheries and in conservation of this critically endangered valuable species.

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