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Evaluation of reproductive performance in indigenous endemic ornamental fish *Sahyadria denisonii* using hormones under captive environment

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Abstract

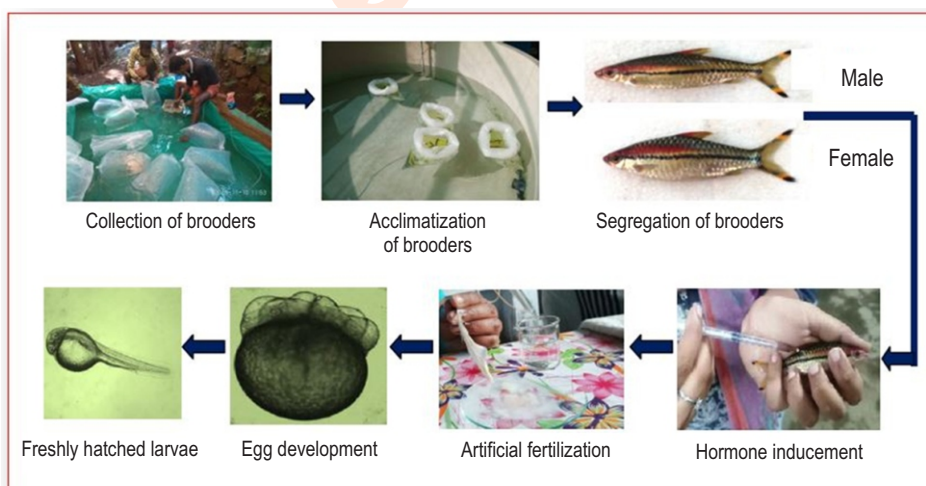
Aim: The present study aimed to develop the hatchery technology for production of indigenous ornamental fish *Sahyadria denisonii* to ensure year round seeds and for conservation of natural resources.

Methodology: In the present investigation, brooders were collected from wild, acclimatized to hatchery condition and enriched under captive environment. The mature brooders were injected with three different synthetic hormones namelyt. Human chorionic gonadotropin (HCG), Ovotide and WOVA-FH at different doses (0.3, 0.5, 0.7 and 0.9 ml/kg of female and half a dose to male).

Results: Fishes did not release their gametes naturally after induction. Hence, artificial fertilization was carried out after 16 hrs of injection. Among the synthetic hormones used WOVA-FH @ 0.5 ml kg⁻¹ of female and half a dose to male showed better breeding performance in terms of number of spawned eggs, fertilization rate (75.3%) and hatching rate (83.0%).

Interpretation: Synthetic hormone WOVA-FH, human chorionic gonadotropin (HCG) and ovotide to female and male through artificial fertilization can be adopted for captive breeding of Indigenous endemic ornamental fish *Sahyadria denisonii*

Key words: Captive environment, Hormones, Indigenous fish, Reproductive performance, *Sahyadria denisonii*



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Introduction

Sahyadria denisonii (Miss Kerala) is a much sought fish in the international ornamental fish trade. *S. denisonii* is endemic to Western Ghats where it occurs in fragmented population in the states of Kerala and Karnataka (Prasad et al., 2008; Raghavan et al., 2010). It has been reported from the Chandragiri Valapatnam, Karyangod, Chaliyar, Bharatapuzha, Kuppam, iritti, Anjarakandipuzha, Bhavani river, Chalakudi, Periyar, Manimala, Thejaswini, Achenkovil and Pampa rivers (Shaji et al., 2000; Kurup et al., 2004; Mercy, 2010). This species is characterized by a torpedo shaped body with silver scales, a horizontal red line running from the snout through eye about half way down the body and below the red line, black line that runs throughout the length of fish from snout to caudal peduncle. This slender barb has a forked tail with black or yellow spots. At maturation, they develop a distinct greenish blue marking on top of the head. The fish grows to a maximum of 15 cm and has longevity of 5-8 years (Menon et al., 1999). It received fame after this beautiful fish won an award in the "New Species" category at Aquarama exhibition of Singapore in 1991 (Suhair and Santhosh, 2015).

S. denisonii is an active schooling fish, thrives well in subtropical climatic condition and is more active at dawn and dusk than day time. It prefers water with a pH range of 6.5-7.3, hardness of 100-400 mg l⁻¹, oxygen level of 5 mg l⁻¹ and above and temperature range of 18-26°C (Raghavan et al., 2009). *S. denisonii* is omnivore in nature feeding on worms, insects, crustaceans, plants material and organic debris and formulated flakes, pellets and another processed feeds. Red pigmentation can be intensified by a diet rich in carotenoids (Suhair and Santhosh, 2015). Miss Kerala (*Sahyadria denisonii*) constituted about 60-65% of the total live ornamental fish exported from India worth US\$ 1.54 million in the year 2008-2010. For last few decades, wild population of *S. denisonii* has been found to decline due to various reasons. Due to over exploitation, International Union for Conservation of Nature and Natural resources (IUCN) has categorized this fish as vulnerable species in 2001, and recently it is listed under endangered because of intense exploitation, deterioration of habitat and decline in number of adult individuals due to high demand. Development of captive breeding technology is a possible way to conserve the natural biodiversity without affecting the ornamental fish trade.

Advancement in breeding technology has added a new dimension in the ornamental fish trade with species diversification and varieties being introduced (Silas et al., 2011). Induced breeding under captive condition is important tool in seed production, which ensures to get pure and required fish seed (Seema, 2013). Some attempts have been done by the researchers to induce breed the popular indigenous ornamental fishes such as *Puntius titteya* (Sundarabharathy et al., 2004), *Puntius shalynius* and *Puntius sophore* (Mahapatra et al., 2010), *Puntius sarana* (Basavaraja et al., 2014 and Udit et al., 2014), *Gonoproktopterus curmuca* (Padmakumar et al., 2014), *Barbus gonionotus* (Siddhwartha Kumar et al., 2014) and *Puntius*

manipurensis (Motilan et al., 2014). Captive breeding technology has been developed successfully for prioritized Western Ghats ornamental fishes such as *Puntius pookodensis*, *Puntius melanostigma*, *Puntius melanampyx*, *Garra mullya*, *Danio malabaricus*, *Chela fasciata*, *Nemacheilus triangularis*, *Nemacheilus semiarmatus* and *Pristolepis marginata* (Mercy et al., 2009). Except for few studies on biology and breeding aspects conducted in its natural habitat (Mercy et al., 2015), captive breeding of *S. denisonii* have not yielded promising results and technology has not yet reached the farmers for commercialization. Hence, the present study was undertaken to develop the captive breeding technique for production of endemic ornamental fish, *S. denisonii*.

Materials and Methods

Brooders of *S. denisonii* were collected from the streams of Tejaswini River, Kasargod district of Kerala. Selected healthy brooders were packed in oxygen filled polyethylene bags and transported to Aquatic Rainbow Technology Park (ARTP), Chennai. Brood fishes (9.0-11.0 g) were stocked in Re-circulated Aquaculture system (RAS) facilitated 4 ton capacity Fibre Reinforced Plastic (FRP) tank containing water (pH: 6.8-7.0; TDS: 50-100 mg l⁻¹) provided with continuous aeration to mimic natural water source of Western Ghats river. Fishes were acclimatized to captive condition by feeding with plankton and small quantity of supplementary feed. After acclimatization, male and female fishes were segregated based on sexual dimorphism. Brood stock of male and female were enriched for three months with high quality protein rich (42%) pellet feed at 5% of body weight and intermittently fed with blood worms to induce maturation. Mature brooders were selected and injected intramuscularly between the anterior region of dorsal fin and lateral line with inducing agents. Selected brooders were induced with synthetic hormones namely, Human chorionic gonadotropin (HCG), Ovotide and WOVA-FH at different doses (0.3, 0.5, 0.7 and 0.9 ml kg⁻¹ b.wt. of female and half dose to male).

The experiment was designed with twelve treatments T1 (HCG at 0.3 ml kg⁻¹), T2 (HCG at 0.5 ml kg⁻¹), T3 (HCG at 0.7 ml kg⁻¹), T4 (HCG at 0.9 ml kg⁻¹), T5 (Ovotide at 0.3 ml kg⁻¹), T6 (Ovotide at 0.5 ml kg⁻¹), T7 (Ovotide at 0.7 ml kg⁻¹), T8 (Ovotide at 0.9 ml kg⁻¹), T9 (WOVA-FH at 0.3 ml kg⁻¹), T10 (WOVA-FH at 0.5 ml kg⁻¹), T11 (WOVA-FH at 0.7 ml kg⁻¹), T12 (WOVA-FH at 0.9 ml kg⁻¹) and control in triplicate. Females were induced with two split doses, first dose was administrated at 6.00 p.m. and second dose at 12.00 p.m. Males were induced once with half dose, while administrating the second dose to female. Induced fishes, i.e., males and females were released into the hapa fixed inside the aquarium tanks in 2:1 ratio. The fishes of control group were maintained without hormonal. *S. denisonii* did not release the eggs naturally in control and in the treatments, hence, artificial fertilization was carried out after 14-16 hrs upon hormonal injection. For artificial fertilization, the induced brooders were anesthetized was 2-phenoxy ethanol. Initially, the females were stripped on a clean watch glass by applying gentle pressure over

the abdomen. Following that without much delay within 45-60 sec males were also stripped and milt was poured over the eggs. The egg and milt collected on the watch glass was mixed thoroughly using fine fur brush. Fertilized eggs were washed with distilled water to clear the excess milt and other debris. The fertilized eggs were then transferred into the aerated glass tank. Fertilized eggs were collected and placed on the petri-dish along with water collected from hatching tank and observed under a microscope. Each developmental stage of egg and embryo (till hatching) was recorded as image with a trinocular microscope attached with a camera and a monitor display.

The efficiency of hormone-inducement was estimated based on parameters like spawned eggs, fertilization rate and hatching rate. Spawned eggs were estimated by random sampling and counting. The fertilization rate of eggs was determined by observing and counting fertilized eggs having intact nucleus of the total eggs released. Hatching rate was estimated by comparing the number of larvae released and fertilized eggs. Fertilization rate and hatching rate was calibrated.

Statistical analyses: The results were compared by analysis of variance (ANOVA) using SPSS package, VERSION 20. Duncan's Multiple Range Test was used for post-hoc comparison of mean ($P < 0.05$) between different groups. All the data presented in the text, figures and tables were expressed as mean \pm standard error. Statistical significance for the test was set at $P < 0.05$.

Results and Discussion

In the present study, *S. denisonii* was successfully bred under captivity by adopting induced breeding technique. Control group fishes did not show positive response of breeding. Among the treatments, artificial fertilization was carried out by stripping the males and females after 14-16 hrs of inducement. Since,

natural release of gametes was not observed in induced fishes, the latency period was not considered to evaluate the hormone efficiency. Among the treatments, 0.3 ml kg^{-1} body weight of female and half dose to male (T1, T5 and T9) none of the hormone treatment showed positive response to artificial fertilization.

Breeding response varies with the mode of action of the hormone which may be attributed to varied levels of dopamine activity in different fishes (Billiard *et al.*, 1984; Peter *et al.*, 1986) this may be the reason for the significantly varied breeding response among the hormones and doses in the present study. Significant difference ($P < 0.05$) was observed in the quantity of spawned eggs among the treatments. The interactive effect of hormone and dose revealed that combination of WOVA-FH @ 0.5 ml kg^{-1} resulted in high number of eggs, *i.e.*, 556.66 nos 13 g^{-1} female. Further, T4, T8 and T12 treatments were considered poor, followed by T1, T5 and T9 among the treatments, since these treatment groups released low quantity of eggs by stripping. Similarly at low and high doses, Das *et al.* (2016), Sundarabharathy *et al.* (2004) and Motilan *et al.* (2014) reported poor breeding success in *Osteobrama belangeri*, *Puntius titteya* and *Pethia manipurensis*.

In the present investigation, it was observed that fertilization rate varied significantly ($P < 0.05$) at different doses and hormone. The interaction effect (Table 1) revealed that T10 yielded the highest fertilization rate (75.3%) and poor fertilization was observed at T1, T5 and T9 followed by T4, T8 and T12 dose. Usually, inducement of fish at higher and lower dose causes poor fertilization that might be due to early and late inducement in brooders (Routray *et al.*, 2001). But, in case of artificial fertilization, low fertilization at higher dose might be attributed to its dopamine activity at different doses (Das *et al.*, 2016). The highest percent of hatching rate (83%) was observed in T10 treatment. Similar to the present study, WOVA-FH was reported

Table 1: Breeding performance of indigenous ornamental fish *S. denisonii* with, different hormones and dose interaction

Treatments	Spawned eggs (Nos/female)	Fertilization rate (%)	Hatching rate (%)	Breeding performance
T1 (HCG at 0.3 ml)	0.0	0.0	0.0	Poor
T2 (HCG at 0.5 ml)	240 ^a	47.66 ^b	55.00 ^a	Average
T3 (HCG at 0.7 ml)	343.33 ^c	58.00 ^d	68.66 ^c	Good
T4 (HCG at 0.9 ml)	310.0 ^b	41.00 ^b	60.333 ^b	Average
T5 (Ovatide at 0.3 ml)	0.0	0.0	0.0	Poor
T6 (Ovatide at 0.5 ml)	253 ^a	50.00 ^c	69.00 ^c	Good
T7 (Ovatide at 0.7 ml)	306.6 ^b	42.661 ^b	71.33 ^c	Good
T8 (Ovatide at 0.9 ml)	371.6 ^c	38.33 ^a	62.661 ^b	Average
T9 (WOVA-FH at 0.3 ml)	0.0	0.0	0.0	Poor
T10 (WOVA-FH at 0.5 ml)	556.6 ^e	75.33 ^f	83.00 ^e	Better
T11 (WOVA-FH at 0.7 ml)	449.3 ^d	64.33 ^e	79.33 ^d	Good
T12 (WOVA-FH at 0.9 ml)	0.0	0.0	0.0	Poor
SEM	9.484	1.440	1.330	-
P value	0.00	0.00	0.00	-

(*Values with different superscript within the column differ significantly.)

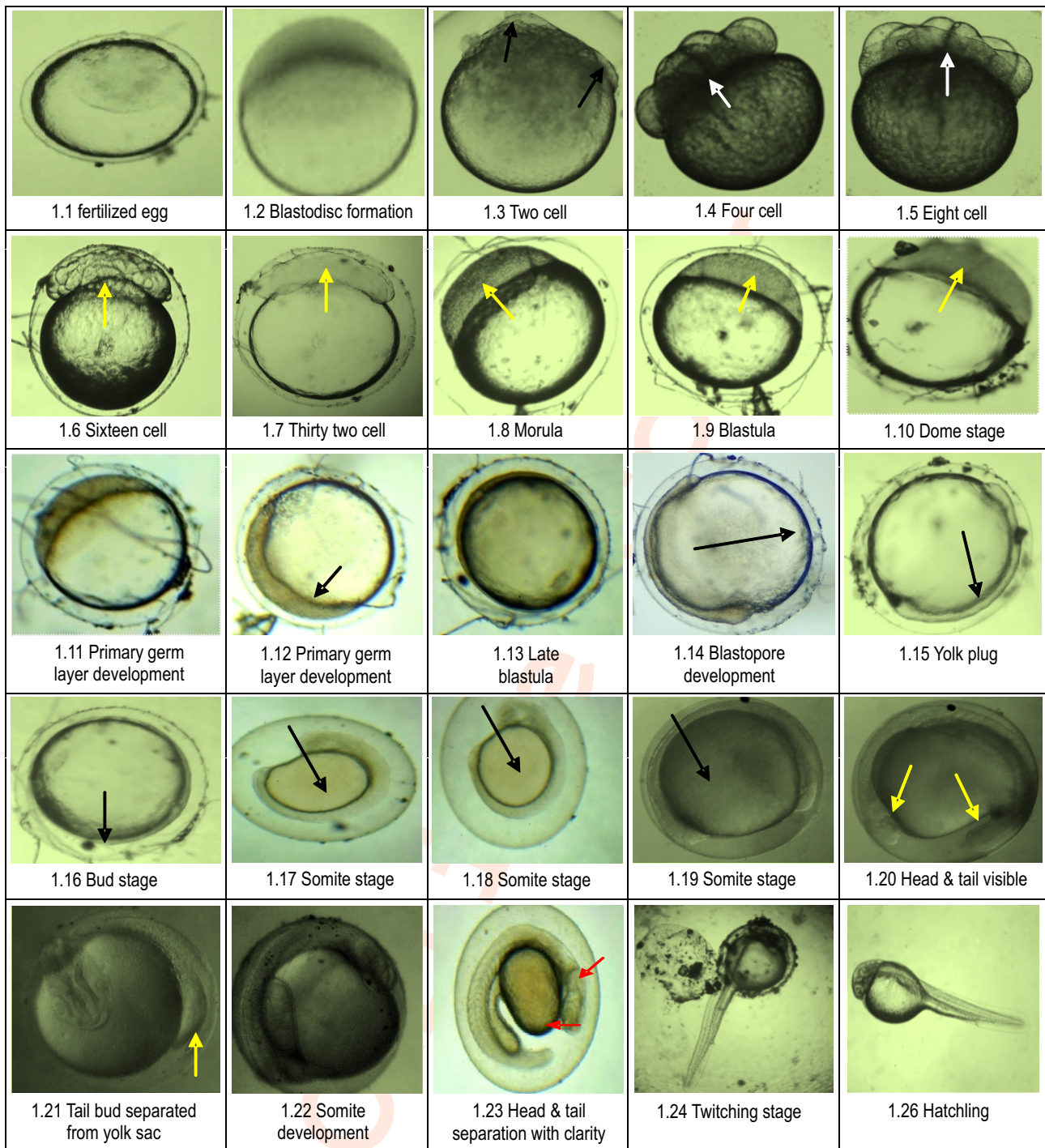


Fig. 1: Egg and embryonic developmental stages of *S. denisonii* observed under trinocular microscope. The developmental stages were observed from 0.10 hrs (fig. 1.1) to 34.00 hrs (fig. 1.26) till hatching.

to be an ideal hormone for inducing breeding in captivity for majority of fresh water teleosts (Sundarabharathy *et al.*, 2004; Behera *et al.*, 2010; Motilan *et al.*, 2014). Higher spawning and hatching rate in Indian major carps with administration of

WOVA–FH at 0.4–0.5 ml kg⁻¹ for female body weight was reported by Rath *et al.* (2001). Likewise, Behera *et al.* (2010) and Motilan *et al.* (2014) reported the highest spawning and hatching rate in captive breeding of *O. belangeri* and *P. manipurensis* when

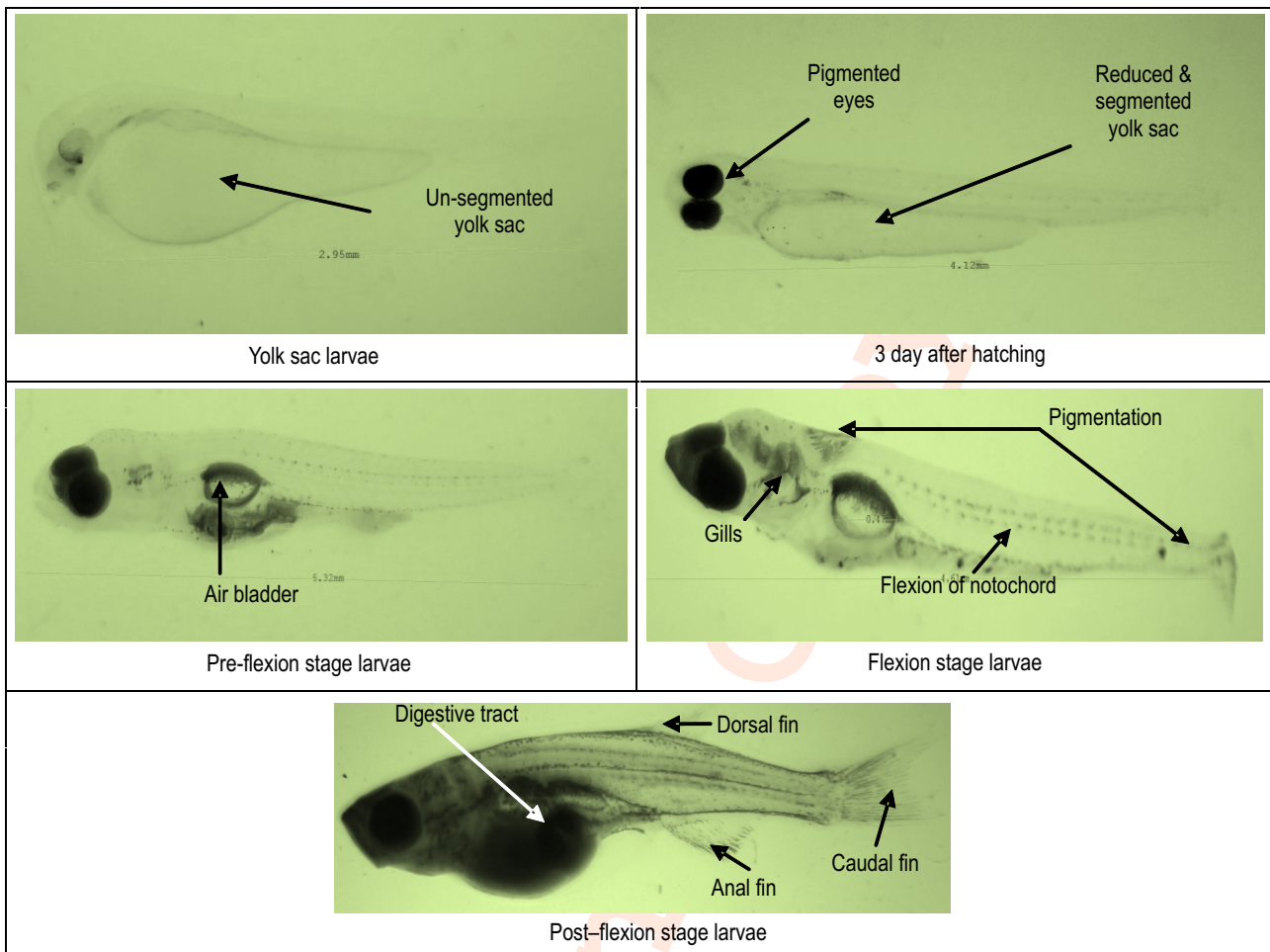


Fig. 2: Larval developmental stages: Yolk sac, pre-flexion, flexion and post-flexion larval stages of *S. denisonii* observed under the trinocular microscope.

induced with 0.5 ml kg^{-1} WOVA-FH in females. In case of, *S. denisonii* better artificial fertilization and hatching rate was obtained at 0.5 ml kg^{-1} for female and half dose for males with all the hormones which can be compared with the results reported by Mercy *et al.* (2015) in which breeding was successful using Ovaprim @ 0.4 ml kg^{-1} b.wt.

Egg and embryonic development of *S. denisonii* recorded by trinocular microscope is presented in detail in Fig. 1. The embryonic period was estimated from the time when as eggs were fertilized and ended until the eggs hatched out as larvae (Mollah *et al.*, 2011). The eggs of *S. denisonii* were small, bead like having the size of 0.89 mm in diameter. The fertilised eggs (Fig. 1.1) were transparent with orange brown colour, whereas unfertilized eggs were opaque in pigmentation. The eggs released were sticky in nature and attached to the bottom of hatching tank. Development initiated immediately after fertilisation and activated the cytoplasmic movements. As yolk free cytoplasm moves towards the animal pole, blastodisc separates it from the vegetal pole 10 min after fertilization (Fig.

1.2). Cell division was initiated by the formation of two blastomeres from the blastodisc (Fig. 1.3) after 20 min of fertilization followed by four (Fig. 1.4), eight (Fig. 1.5), sixteen (Fig. 1.6) and thirty two cell (Fig. 1.1) stage by cleavage at 0.45, 1.05, 1.25 and 2.00 hrs of post fertilization. Cell division continued rapidly, morula (Fig. 1.8) and blastula (Fig. 1.9) stage occurred 3.10 and 3.55 hr after the fertilization. After 5.30 hr of fertilization, development of blastoderm was observed. Dome shape was observed after 5.30 hr of fertilization (Fig. 1.10). At gastrula stage, three primary germ layers were formed due to extensive cell movements (Fig. 1.11, 1.12). Blastopore (Fig. 1.14) and yolk plug stage (Fig. 1.15) development were observed after 8.25 hr and 9.10 hr of fertilization. Development of somites initiated after 12 hr of fertilization and continued till hatching (Fig. 1.11 to 1.25). Head and tail clearly were visible after 14.15 hr (Fig. 1.20) of fertilization. Lens formation initiated and tail bud started separating from the yolk sac at 20.42 hr (Fig. 1.21) post fertilization. Exactly 24.00 hr after fertilization, heartbeat in the developing embryo began. Head and tail of the embryo differentiation was clearly noted after 25.50 hrs (Fig. 1.23) post fertilization. Twitching movement of the

embryo initiated after 23 hrs but vigorous twitching of the embryo was observed after 28 hr (Fig. 1.24) post fertilization, At this phase, tail bud became longer and rudiments of primary organs became visible. Following this, after 1 h circulation began at 29.10 hr post fertilization. Embryo started to twist or rotate completely inside the egg membrane at the period, 30.30 hr post fertilization.

At 31.00 hr post fertilization twitching and lashing of the embryo inside the egg capsule became rapid. Embryo wriggled out of the egg shell (Fig. 1.25) and hatched into hatchling, 34 hrs after fertilization. The results were found slightly faster than previous report by Mercy *et al.* (2015). The present study used the larger size and healthy brood stock which might be the reason for early hatching. Optimum water quality parameters are conducive for the hatching process (Laurila *et al.*, 1981). Water quality parameters such as temperature (23 - 26°C), pH (6.8-7.0), TDS (40-5. mg l⁻¹), alkalinity (50-55 mg l⁻¹), hardness (80-100 mg l⁻¹), and dissolved oxygen (5-6.5 mg l⁻¹) were observed in optimum range and acted favourable for hatching process.

Newly hatched *S. denisonii* larva was 1.19 mm long (Fig. 2). Larvae were transparent with un-segmented yolk sac. Larvae head was pointed forward and distinct from the yolk. At hatching, the digestive tract appeared as a straight undifferentiated tube attached dorsally to the yolk sac. Three days after hatching, yolk sac was reduced and development dorsal fin was initiated. Dark pigment was observed in the eyes. Gradually, dark pigmentation in the eyes increased. After 4 days of hatching, opening of mouth was initiated. During this period, notochord was observed to be straight. After 4 days of hatching, (Lecito-exotrophic period) complete absorption of yolk sac was observed. Eyes were pigmented and functional. Complete opening of mouth took place during this period.

Larvae were ready for exogenous feeding. Gill rudimentary buds development initiated. During pre-flexion larva (exo-trophic period) stage, the swim bladder was developing and filled with air 5 days after hatching. Thirteen days after hatching caudal fin rays appeared. Head, body and fins were heavily pigmented over the body. Gills developed with the gill filaments. Twenty one days after hatching in the flexion larva, small pelvic fins were visible along with the well developed digestive system with accessory organs and circulatory system with gill arches. These results corroborates with the results of Mercy *et al.* (2015) in *S. denisonii*. During post-flexion stage, 22-32 days after hatching, caudal and dorsal fins were separated and developed prominently. All fin rays were formed. Well developed circulatory, digestive and initiation of reproductive organ development was observed. The egg, embryonic and larval development were similar to other teleosts (Van Snik *et al.*, 1997; Gisbert, 1999; Gisbert *et al.*, 2002). The present study successfully developed the breeding technology for endemic ornamental fish *Sahyadria denisonii* which would help to introduce hatchery-bred seeds of valuable indigenous fish in the ornamental fish trade, reduce the exploitation pressure on natural resources to a considerable level and contribute in the

conservation of natural resources.

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References

- Basavaraja, N., N.N. Biswal and K. Paubiaklun: Captive brood-stock development, induced breeding, histology and nursery rearing of olive barb, *Puntius sarana* (hamilton). *J. Aquacult. Tropics*, **29**, 155-169 (2014).
- Behera, B.K., P. Das, N.S. Singh and A.K. Sahu: Captive breeding of an endemic medium carp Pengba, *Osteobrama belangeri* (Val.) with Wova-FH in Manipur. *J. Aquac.*, **18**, 23–29 (2010).
- Billiard, R., K. Bieniarz, R.E. Peter, M. Sokolowska and C.Weil: Effect of LHRH and LHRH-a on plasma GtH levels and maturation/ovulation in common carp, *Cyprinus carpio* kept under various environmental conditions. *Aquacul.*, **41**, 245–254 (1984).
- Das, P., B.K. Beherab, D.K. Meenaa, S.K. Singh, S.C. Mandal, S. Sahoo Das, A.K. Yadav and B.K. Bhattacharjya: Comparative efficacy of different inducing agents on breeding performance of a near threatened cyprinid *Osteobrama belangeri* in captivity. *Aquaculture Reports*, **4**, 118–182 (2016).
- Gisbert, E.: Early development and allometric growth patterns in Siberian sturgeon and their ecological significance. *J. Fish Biol.*, **54**, 852-862 (1999).
- Gisbert, E., G. Merino, J.B. Muguet, D. Bush, R.H. Piedrahita and D.E. Conklin: Morphological development and allometric growth patterns in hatchery-reared California halibut larvae. *J. Fish Biol.*, **61**, 1217-1229 (2002).
- Kurup, B.M., K.V. Radhakrishnan and T.G. Manojkumar: Biodiversity status of fishes inhabiting rivers of Kerala (S. India) with special reference to endemism, threats and conservation measures. In: Proceedings of 2nd Large Rivers Symposium (LARS2) (Eds.: R.L. Welcome & T. Petr). Mekong River Commission and Food and Agricultural Organization, pp. 163-182 (2004).
- Laurila, S., J. Piironen and I.J. Holopainen: Notes on egg development and larval and juvenile growth of crucian carp *Carassius auratus*. *Ann. Zool. Fennici.*, **24**, 315-321 (1981).
- Mahapatra, B.K.: Breeding and rearing of indigenous ornamental barbs *Puntius shanynius* and *Puntius sophore* of NE India. In: Proceeding of Golden jubilee National Seminar on Diversification of Aquaculture Through Locally Available Fish Species, pp. 92-94 (2010).
- Menon, A.G.K., K. Rema devi and M.P. Thobias: *Puntius chalakkudiensis*, a new colourful species of *Puntius* (Family: Cyprinidae) fish from Kerala, South India. *Records of the Zoological Survey of India*, **91**, 61-64 (1999).
- Mercy, T.V.A., V. Malika and S. Sajan: Breakthrough in breeding of *Puntius denisonii*. *INFOFISH*, **3**, 13-11 (2010)
- Mercy, T.V., S. Sajan and V. Malika: Captive breeding and developmental biology of *Sahyadria denisonii* (Day 1865) (Cyprinidae), an endangered fish of the Western Ghats, India. *Indian J. Fish.*, **62**, 19-28 (2015).
- Mercy, T.V.A., V. Malika, S. Sajan and S. Sahayak: Distribution, reproductive biology and development of captive breeding technology of *Puntius denisonii*- an indigenous ornamental fish of

- the Western Ghats of India. Presented in the International Conference on Ind-Aquaria, Marine Products Export Development Authority at Calcutta, West Bengal (2009).
- Mollah, M.F.A., K. Taslima, H. Rashid, Z. Hossain, M.N. Sarowar and M.R.K. Khan: Embryonic and larval development of critically endangered riverine catfish *Rita rita*. *Eurasia J. Biosci.*, **5**, 110-118 (2011).
- Motilan, Y., Y. Bedajit and W. Sunitibala: Captive breeding of endangered Barbs *Pethia manipurensis* by oral delivery of gonadotropic signaling molecular analogue WOVA-FH. *IJSRP*, **4**, 1-4 (2014).
- Padmakumar, K.G., L. Bindu, P.S. Sreerekha, J. Nitta, A. Krishnan and P.S. Manu: First report on captive breeding of endemic red tailed silver shark *Gonoproktopterus curmuca* (Cyprinidae: Hamilton-Buchanan 1807). *IJFAS*, **4**, 156-160 (2014).
- Peter, R.E., R. Sokolowska and C.S. Nahorniak: Comparison of (D-Arg 6, Trap 1, Leu 8, Pro 9 Net) luteinising hormone (LHRA) in combination with pimoziide, instimulating gonadotropin release and ovulation in the gold fish *Carrassius auratus*. *Canadian J. Zool.*, **65**, 981-991 (1986).
- Prasad, G., A. Ali and K.R. Raghavan: Threatened fishes of the world. *Puntius denisonii* Day (Cyprinidae). *Environ. Biol. Fish.*, **83**, 189-190 (2008).
- Raghavan, R., G. Prasad, A. Ali, B. Pereira and L. Sujarittanonta: Damsel in distress, The tale of Miss Kerala, *Puntius denisonii* (Day), an endemic and endangered cyprinid of Western Ghats biodiversity hotspot (South India). *Aquatic Conservation: Marine and Freshwater Ecosystems*, **19**, 67-74 (2009).
- Raghavan, R.: Fish Conservation in Kerala. (Eds. A. Benziger and L. Sonnenschein). World Aquariums and Oceans Federation, St. Louis, USA (2010).
- Rath, S.C., S.K. Sarkar, S.D. Gupta and N. Sarangi: Comparative account of induced breeding of Indian major carps with ovaprim, ovatide, wova- FH and carp pituitary extract. *Indian J. Ani. Sci.*, **11**, 1051-1060 (2001).
- Routray, P., D.K. Verma, S.K. Sarkar and N. Sarangi: Recent advances in carp seed production and milt cryopreservation. *Fish. Physiol. Biochem.*, **33**, 413-421 (2001).
- Seema, L., R.K. Krishan and S. Jyoti: Effect of ovatide hormone in oocyte maturation in sexually immature *Puntius sophore* a freshwater cyprinid. *IJSRP*, **3**, 1-4 (2013).
- Shaji, C.P., P.S. Easa and A. Gopalakrishnan: Freshwater fish diversity of Western Ghats. In: Endemic Fish Diversity of Western Ghats. NBFGR-NATP Publication (Eds. A.G. Ponniah and A. Gopalakrishnan). National Bureau of Fish Genetic Resources, Lucknow, India, 341pp (2000).
- Siddhartha, K.B., N. Gupta, M.M. Haque and R. Amin: Embryonic and larval development of silver barb (*Barbodes gonionotus*) in a mobile hatchery under laboratory condition. *ESJ*, Special Edition, **3**, 258-270 (2014).
- Silas, E.G., A. Gopalakrishnan, A. Ramachandran, A. Mercy and T.V. Kripan Sarkar, Pushpangathan, K.R. Anil, R.P. Kumar, M.K. Mohan and K.K. Anikuttan: Guidelines for Green Certification of Freshwater Ornamental Fish. The Marine Products Development Authority, Kochi (2011).
- Suhair, K. and S. Kumar.: A Hand Book on Induced Breeding Technology and larval Rearing of Ornamental fish 'Miss Kerala' *Sahyadria denisonii* (Day, 1865). Department of Fisheries, Kerala (2015).
- Sundarabarathy, T.V., U. Edirisinghe and C.M.B. Dematawewa: Captive breeding and rearing of fry and juveniles of cherry barb (*Puntius tittैया Deraniyagala*), a highly threatened endemic fish species in Sri Lanka. *Trop. Agricul. Res.*, **16**, 137-149 (2004).
- Udit, U.K., A.K. Reddy, P. Kumar, M.A. Rather, R. Das and D.K. Singh: Induced breeding, embryonic and larval development of critically endangered fish *Puntius sarana* (Hamilton, 1822) under captive condition. *J. Anim. Plant Sci.*, **24**, 159-166 (2014).
- Van Snik, G.M.J., J.G.M. Van den Boogaart and J.W.M. Osse: Larval growth patterns in *Cyprinus carpio* and *Clarias gariepinus* with attention to the fanfold. *J. Fish Biol.*, **50**, 1339-1352 (1997).