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SHORT COMMUNICATION

Induced breeding and larval rearing of the vulnerable Shalyni barb, *Pethia shalynius* (Yazdani and Talukdar, 1975), in aquarium

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Captive breeding programmes are significant in reducing pressure on declining wild stocks, besides generating useful data on incubation period, hatching success, larval feeding, growth and weaning stages including free-swimming larval development stages (Celik & Cirik, 2020; De Silva, 2012; Diana, 2009; Fleming, 1994). *Pethia shalynius* is a freshwater barb endemic to the streams and rivers of the Khasi and Jaintia Hills, Meghalaya, northeast India (Manorama, & Ramanujam, 2016; Yazdani & Talukdar, 1975). It is characterized by the presence of a short compressed body, large bronze-to-gold cycloid scales, two black spots on either side of the caudal peduncle and a reddish-orange caudal peduncle and fins in males (Jayaram, 1999). Despite having a minor fisheries value (Froese & Pauly, 2019), wild populations of *P. shalynius* have drastically reduced primarily due to anthropogenic pressures such as coal mining and quarrying, and the species is currently categorized as vulnerable (Dahanukar, 2015).

The colourful *Pethia shalynius* is a potential candidate for the freshwater ornamental fish industry of India and abroad. However, no data on captive breeding and larval-rearing techniques of the species exist. Thus, the present study was undertaken to present a farmer-friendly procedure of brooder management, successfully induced spawning and larval rearing of *P. shalynius* in aquarium for the first time. A reliable culture and breeding practice of the species is needed to help reduce the pressure on wild stocks, besides boosting its demand as a valuable indigenous ornamental fish species of northeast India.

Live individuals of *Pethia shalynius* (N = 48; 22 males and 26 females) were collected from the Umkhohrah Stream, East Khasi Hills, Meghalaya, in November 2018, and transported to the Aquaculture and Biodiversity Centre, Gauhati University, Assam, in oxygenated plastic bags. Fish were acclimatized to the local temperature (~26°C) by dipping the poly bags in aquaria for 20–30 min. Prior to release in aquaria (121 cm × 45 cm × 45 cm), the fish were disinfected by dipping in 2–3% salt solution for 1–2 min. The aquaria were fitted with aerators, sponge filters (Sobo SB 1330) and thermostat (RS 300 W, 220–240 V) and supplied with de-chlorinated and RO-purified water. Water parameters were maintained at par with the species' natural habitat (i.e. pH, water temperature, conductivity and dissolved oxygen at 7.5 ± 0.5, 25 ± 0.8°C, 350–370 μS and 5.8–6.5 mg/L, respectively) and checked twice a week using a kit (YSI Pro DSS 82, USA). Fish were fed with zooplankton (*Cyclops* sp., *Daphnia* sp. and *Moina* sp.), *Tubifex* sp. and chopped earthworms twice daily at 10:00 hours (h) and 16:00 h of Indian Standard Time (IST). A photoperiod of 13-h light (L):11-h dark (D) was maintained.

After six months of rearing, the fish attained a maximum length of 6.1 ± 0.3 cm and weighed 3.2 ± 0.6 g. Sexually mature fish were selected based on the rapid oozing of ova/milt when gently pressed on the abdomen: (Figure 1a) shows the appearance of strong reddish-brown coloration on the caudal fin of mature males and (Figure 1b) indicates a deeper body with pale bronze scales of mature females. Brooders were anaesthetized in a 30 mg/L solution of clove oil (Sajan et al., 2012), and intraperitoneal injection of synthetic hormone (GONOPRO-FH, India) at 1 ml/kg body weight was administered in both sexes. They were released in smaller aquaria (61 cm × 45.7 cm × 45.7 cm) at a ratio of 1:1 (male/female), with the water temperature maintained at 24 ± 0.5°C (trials at higher temperatures, viz. 26–28°C, were unsuccessful), constant aeration and floating nylon mops. Following the successful spawning, the brooders

were removed, the water temperature of the aquaria was raised to 26°C, and 2–5 drops of 2 ppm methylene blue solution were added to prevent any fungal infection. All protocols were approved by the Institutional Animal Ethical Committee (IAEC) of Gauhati University, Assam, India.

Developmental stages post hatching were categorized as larva, juvenile and adult following Ahlstrom and Ball (1954). The larvae were maintained in constant aeration, thermostat at 26°C and a photoperiod of 13:11-h L/D cycle. pH, conductivity and D.O. content of the aquarium water were maintained at 7.5 ± 0.5, 350–370 µS and 5.9–6.5 mg/L respectively. The detail of feeding and water management protocols for the larval rearing of *P. shalynius* is summarized in Table 1. The total lengths (TL) (in cm) and body weights (BW) (in g) of the larvae were measured and expressed as mean ±standard deviation (SD) for 10 individuals at each corresponding stage of development.

FIGURE 1 Brooders of *Pethia shalynius* (Yazdani & Talukdar, 1975). (a) Male; (b) Female

Pethia shalynius was successfully bred three times (trial 1 (T1), trial 2 (T2) and trial 3 (T3)) (Table 2). After 10–12 h of hormone dosage, females laid 30–35 eggs and the males immediately fertilized them. Fertilized eggs were transparent, spherical, non-adhesive, unpigmented and 0.75–0.80 mm in diameter. Unfertilized eggs were opaque. The larvae hatched between 26 and 27 hours post fertilization (hpf) at 26 ± 0.5°C. Newly hatched larvae were transparent, unpigmented, bearing a large yolk sac and measured 2.3–2.5 mm in TL (Figure 2a,b). At 2 days post hatching (dph), the yolk sac reduced with gradual differentiation of the notochord and caudal-fin bud (cfb) (Figure 2c). Scattered melanophores appeared towards the anterior half of the body, and eye and anus development commenced (Figure 2d). At 3 dph, free-swimming larvae, measuring 2.7 ± 0.5 mm, were observed and they started to feed on *Paramecium*. Active and voracious feeding by the larvae was observed on provision of live feed. However, offering artificial feed (e.g. pellet/powdered feed) did not induce any active feeding behaviour. At 15 dph, the jaws were well-formed and melanophore aggregation was apparent on the body (Figure 3a). At 30 dph, the larvae measured 1.3 ± 0.1 cm

TABLE 1 Standardized feeding protocol and water quality management developed for larval rearing of *Pethia shalynius* (Yazdani & Talukdar, 1975) in aquarium

Days post hatching (dph)	0	3	5	10	15	20	25	30	45	60	90	120	150	180	210	240	270
Feeding protocol																	
Paramecium (100 ind/ml)[a]		+	+	+													
Microworm (15 ind/ml)			+	+	+												
Artemia (5–6 ind/ml)				+	+	+	+	+	+	+							
Tubifex (2–3 ind/ml)							+	+	+	+	+	+	+	+	+	+	+
Earthworm (2–3% of BW[b])											+	+	+	+	+	+	+
Water quality management																	
Water exchange @ 10%	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Water addition @ 5%					+	+	+	+	+	+	+	+					
Water addition @ 10%												+	+	+	+	+	+

Note: +: feeding and water quality checked at respective dph.

[a]ind: individual.

[b]BW: body weight.

TABLE 2 Summary of three successful breeding trials (trial 1 (T1), trial 2 (T2) and trial 3 (T3)) of *Pethia shalynius* (Yazdani & Talukdar, 1975) in aquarium (temperature of spawning tank: 24 ± 0.5°C; temperature of larval-rearing tank: 26 ± 0.5°C)

Trials	T1		T2		T3		Mean ± SD[a]	
Brooders	Male	Female	Male	Female	Male	Female	Male	Female
Total length (cm)	6.3	5.8	6.2	6.1	6.3	6.2	6.3 ± 0.1	6.0 ± 0.2
Body weight (g)	3.8	3.9	3.8	3.8	3.7	3.9	3.8 ± 0.1	3.9 ± 0.1
Hormone dosage (ml/kg body weight)	1	1	1	1	1	1	1	
Latency period (hours: minutes)	12:00		10:15		11:30		11:15 ± 0.03	
No. of released eggs	35		25		30		30 ± 5.00	
No. of fertilized eggs	30		21		27		26 ± 4.6	
No. of hatched larvae	27		19		24		23.3 ± 4.04	
No. of surviving adults	24		17		21		20.7 ± 3.5	
Fertilization rate (%)	85.7		84.0		90.0		86.6 ± 3.1	
Hatching rate (%)	90.0		90.4		88.9		89.8 ± 0.8	
Survival rate (%)	88.9		89.4		87.5		88.6 ± 0.9	

[a]SD: standard deviation.

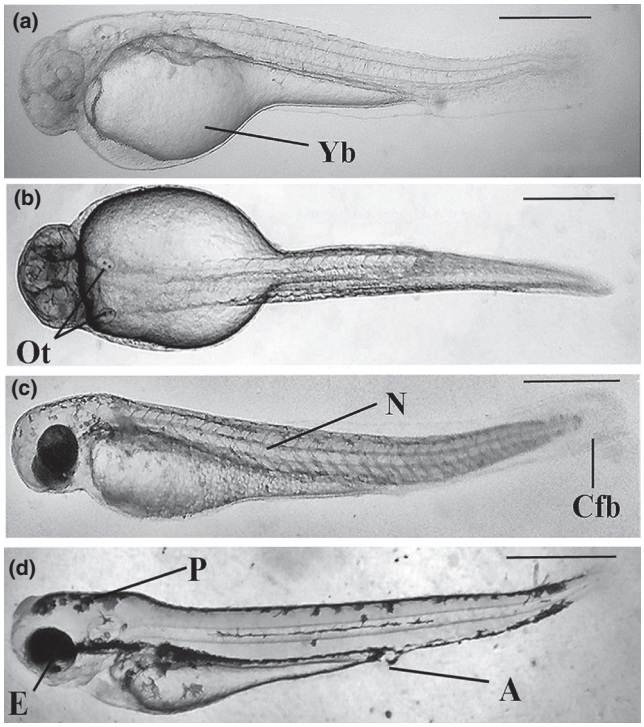

FIGURE 2 Early larval stages of *Pethia shalynius* (Yazdani & Talukdar, 1975). (a) Newly hatched larva, with complete yolk sac; (b) dorsal view of newly hatched larva with developed otolith; (c) 2-day-old larva, yolk sac still present, notochord visible, caudal-fin bud developed; (d) 3-day-old larva, end of yolk reserve (Yb: yolk ball; Ot: otoliths; N: notochord; Cfb: caudal-fin bud; E: eye; A: anus; P: pigmentation; scale bars of (a–d) = 500 μm)

and weighed 0.24 ± 0.02 g, and body pigmentation peaked and scales appeared (Figure 3b). At 60 dph, the larvae attained the juvenile stage, characterized by distinct dorsal, anal, caudal and pectoral fins supported by fin rays, horizontal bands at the base of the dorsal fin, aggregation of melanophores laterally on caudal peduncle to form two black spots and the near appearance of an adult barb-like body plan (Figure 3c,d). They measured 1.7 ± 0.14 cm in length and 0.4 ± 0.02 g in weight. At 120 dph, cycloid scales were distinct on the body (Figure 3e). At 180 dph, reddish-orange coloration appeared on the caudal peduncle, apparently brighter on males, and the fish measured 4.5 ± 0.1 cm in length and weighed 2.3 ± 0.1 g (Figure 3f). The average lengths and weights of the fish at 210 dph, 240 dph and 270 dph were 5.7 ± 0.1 cm, 2.5 ± 0.1 g (Figure 3g); 5.9 ± 0.01 cm, 3.1 ± 0.1 g (Figure 3h); and 6.1 ± 0.1 cm, 3.2 ± 0.1 g respectively (Figure 3i). The best fertilization rate was 90% (in T3), with an average rate of 86.6 ± 3.1% (for *N* = 3 spawnings). The best hatching rate was 90.4% (in T2), with an average of 89.8 ± 0.8% (for *N* = 3 spawnings). After 270 days, the best survival rate was 89.4% (T2), with an average of 88.6 ± 0.9% (for *N* = 3 spawnings).

Wild *Pethia shalynius* usually spawn once in a year between May to September, rarely in October, and have low fecundity as compared to other barb fish species (Manorama & Ramanujam, 2014, 2017a). Spawning took place at 24°C between May and June 2019. Despite the low fecundity (30–35 per female), the fertilization rates, hatching rates (90.4% (in T2)) and survival rates (89.4% (in T2)) were high with no occurrence of diseases. Manorama and Ramanujam (2017b) noted less feeding activity when recording decreased gastrosomatic indices in wild populations of *P. shalynius* during June and July. Adult *P. shalynius* in our study, too, refrained from feeding between April and June 2019. Partial abstinence from feeding can be correlated with fully developed mature gonads occupying the abdominal cavity and limiting adequate space for food intake, which is pronounced in females owing to larger size of the ovaries as compared to testes in males. A successful induced breeding programme primarily depends on the appropriate dosage of a specific hormone, condition of the broodstock and environment (Afroz et al., 2014; Miah et al., 2008). Exogenous feeding by free-swimming larvae followed soon after the yolk sac absorption at 3 dph. Success in larval rearing depends on the availability of adequate food that can be readily consumed,

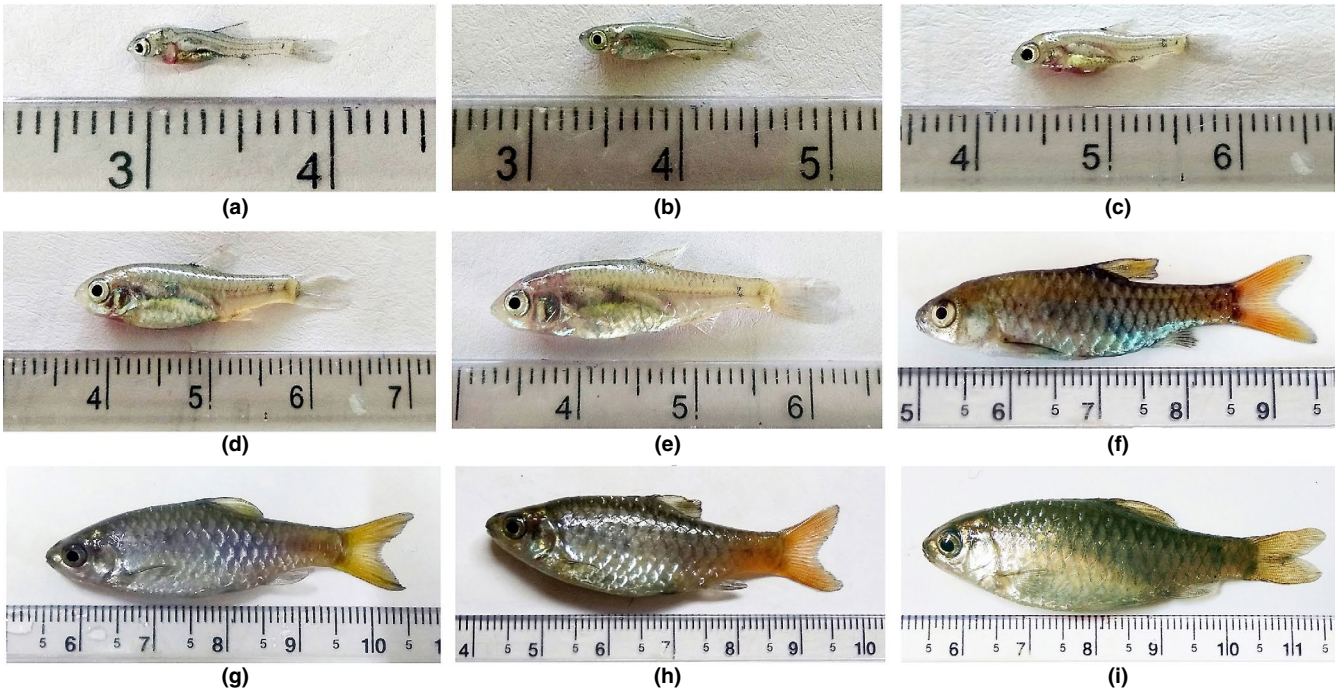

FIGURE 3 Late larval and juvenile stages of *Pethia shalynius* (Yazdani & Talukdar, 1975) (a) 15 dph larva, developing pelvic, pectoral, caudal fin; (b) 30 dph larva, darkening of the body, developed scales; (c) 60 dph juvenile, two black spot development at caudal peduncle; (d) 90 dph juvenile; (e) 120 dph adult; (f) 180 dph adult, reddish-orange coloration appeared on the caudal peduncle; (g) 210 dph adult; (h) 240-day-adult; (i) 270 dph adult (dph: days post hatching)

ably digested and comprises optimal nutrients. As live feed is constantly available along the water column for the larvae or juveniles to capture, it minimizes the costs of food deprivation, thereby improving growth rate in captivity and stimulating foraging behaviour (Montchowui et al., 2012; Nuñez et al., 2008). In our study, it was clearly observed that the larvae of *P. shalynius* readily accepted live feed, but lacked any preference for artificial feed.

In conclusion, the study showed that culture of *Pethia shalynius* is feasible in aquarium. Adequate availability of live feed is important for larval rearing of the species. Induced spawning was successful at 24℃ of water temperature and larviculture at 26℃. The colourful *P. shalynius* is a potential candidate for the freshwater ornamental fish trade from northeast India. Apart from the generation of livelihood of the people, developing a reliable culture of the species will eventually reduce its exploitation from the wild.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

The experiment was planned and critically examined by JKN, MK, HC, DS; the methodologies were carried out by JKN, MK, AS; and the manuscript was prepared and edited by JKN, HC, DS. All authors reviewed and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

All data generated in the present study are included in the manuscript and its figures and tables.

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