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Captive Breeding, Fecundity, and Early Larval Development of Snow Trout (*Schizothorax richardsonii*) in a Flow-Through Hatchery System

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Abstract

The snow trout (*Schizothorax richardsonii*), an ecologically and economically important coldwater fish species endemic to the Himalayan and sub-Himalayan regions, is facing declining populations due to overfishing and environmental stressors. This study aimed to establish preliminary data on broodstock development, reproductive performance, and larval rearing of *S. richardsonii* under captive conditions using a flow-through hatchery system at ICAR-CICFR, Bhimtal. Broodstock were collected at monthly intervals to assess gonadal maturation and spawning behaviour, confirming peak breeding seasons during March–April and September–October. Successful artificial breeding was achieved through hormonal induction using Ovatide at a dose of 0.5 mL/kg body weight for females and 0.3 mL/kg for males, yielding a fertilization rate of 95% and a peak hatching rate of 90% in September. The absolute fecundity ranged from 2000 to 3000 eggs per female, with egg diameter ranging from 2.2 to 2.5 mm. Larval growth exhibited distinct developmental phases, with total length increasing from 8.1 ± 0.15 mm at hatching to 18.3 ± 1.22 mm at 40 days post-hatching. The mean daily growth rate was 0.4 mm/day in early stages, followed by a shift to proportional increase in length and weight after yolk absorption. The cumulative larval survival was 65–70% at 40 days post-hatching under controlled conditions, with water temperature maintained at 20–22 °C, pH 7.2–7.5, and dissolved oxygen ranging between 7.0–8.0 mg/L. Seasonal variations in reproductive indices aligned with previous reports, identifying March and September as the peak spawning periods. This study underscores the potential of induced spawning and highlights the importance of captive breeding and optimized larval rearing techniques for the sustainable propagation of this vulnerable species.

Keywords: Snow trout, flow-through hatchery, breeding, larval rearing, conservation

Introduction

Schizothorax richardsonii (Gray, 1832), an important fish species of the Schizothoracinae subfamily, is primarily found in streams and rivers of the Himalayan and sub-Himalayan regions (Joshi, 2004). This species, locally known as 'Asela' in the Central Himalayas, holds great ecological and economic value, serving as a crucial component of fisheries and a primary subsistence food source in hilly areas with numerous cold-water rivers and streams (Sarma, Akhtar, & Singh, 2018). The populations of *S. richardsonii* have been declining despite their importance due to overfishing, harmful fishing methods, erratic climate conditions, and growing human pressures (Sharma, 1989; Das & Joshi, 1993; Sehgal, 1999). This decline has intensified over the last ten years; as a result, it has been listed as Vulnerable under the IUCN Red List (Vishwanath, 2021). The species exhibits two distinct breeding seasons, from March to April and from September to October, with peak breeding activity occurring between late September and early October (Joshi,

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2004; Rayal, Saher, Bahuguna, & Negi, 2020; Ciji et al., 2021; Sharma, Bisht, Pandey, & Vishwakarma, 2022). Coldwater fisheries, particularly in hilly regions, are crucial for food security and local livelihoods. In these areas, *S. richardsonii* is preferred and has strong potential as a candidate species for aquaculture (Sharma et al., 2022). However, due to its inherently slow growth rate and difficulties in achieving gonadal maturation in captivity, breeding and seed production efforts have remained challenging. Understanding the reproductive biology of the species, including fecundity, larval rearing, and nutritional needs, is essential for addressing these constraints. Although the breeding biology and reproductive physiology of *S. richardsonii* have been extensively studied over the past few decades (Bisht & Joshi, 1975; Qadri, Mir, & Yousuf, 1983; Joshi., Das, Khan, Pathak, & Sarkar, 2016), significant knowledge gaps remain, particularly in captive breeding and larval rearing. This study aims to provide preliminary data on broodstock development, fecundity, and larval rearing of *S. richardsonii* in a flow-through hatchery system. These findings aim to inform conservation efforts and facilitate the long-term management of *S. richardsonii* populations in upland areas facing environmental changes and anthropogenic pressures (Sarma et al., 2024). Overall, the findings of the study will help address the need for targeted conservation strategies to prevent further population decline and ensure the long-term survival of this important cold-water fish species.

Materials and Methods

Brooders of *S. richardsonii* were collected from the Shipra River near Kainchi, Nainital, Uttarakhand (29.4223° N, 79.5125° E) during January, March, May, July, September, and November using a cast net. In each sampling event, 30 individuals (15 males and 15 females) weighing between 30 g and 95 g were collected. Of these, 10 males and 10 females were sacrificed for the assessment of gonadal development, while the remaining 10 fish (5 males and 5 females) were maintained in rearing tanks for subsequent rearing and breeding trials. The collected fish were transported to the ICAR-CICFR hatchery in oxygenated polyethylene bags containing 20% water and 80% pure oxygen, ensuring adequate aeration during transit.

A tank measuring 2.1 m × 2.1 m × 0.5 m was prepared specifically for the brooders, designed to create an optimal environment for the fish growth and maturation. The tank was filled with a 20 cm layer of gravel, composed of a mixture of sand, pebbles, and grey limestone in a 1:2:1 ratio. This substrate provided a natural habitat for the brooders and also served as a base for the bio-filtration system. To create an effective bio-filter, a network of slitted polyvinyl chloride (PVC) pipes was installed over the gravel layer and connected to two aquarium pump heads, each with a capacity of 2800 litres per hour, ensuring continuous water circulation. The tank was conditioned for a week before the fish was introduced, allowing sufficient time for the development of beneficial nitrifying bacteria essential for maintaining water quality by breaking down harmful substances. The fish were reared in this controlled environment, and regular monitoring was conducted to detect any signs of disease or abnormal behaviour, ensuring their well-being as they approached maturity. The state of sexual ripeness of the brooders was assessed twice weekly during the breeding months through physical observation, focusing on indicators of breeding readiness.

Following a specific period of environmental conditioning, broodstock of *S. richardsonii* that had attained reproductive competence were identified, segregated, and prepared for spawning induction. The brooders were separated from the main stock and placed in another tank, maintaining a male-to-female ratio of 2:1. Selection of mature brooders was based on secondary sexual characteristics. Males were identified by tubercles on the snout, rough pectoral fins, and the release of milt on gentle press, while females displayed rounded bellies and re-

Table 1. Breeding performance of *S. richardsonii* brooders induced with Ovatide hormone

Breeding Set Date	No of Brooder	Responded females injected	No of Eggs Released	Ova diameter (mm)	Fertilization Rate	Hatching Rate	Larvae Survival rate
04/04/2023	2F 4M	1	~3000	2.0-2.5	80%	70%	50%
20/09/2023	3F 6M	2	~4500	2.0-2.5	95%	90%	70%

leased eggs under gentle pressure, indicating readiness for spawning. To calm the brooders for handling, they were anesthetized using clove oil at a concentration of 0.05 mL in 500 mL of water. Broodstock were hormonally induced using Ovatide (Hemmo Pharmaceuticals Pvt. Ltd. Thane, India) via 1 mL intramuscular injections at the base of dorsal fin following Fernandez-Palacios et al. (2014). The dosage was 0.5 mL/kg body weight for females and 0.2 mL/kg body weight for males (Najar et al., 2014). After hormonal administration, the breeding pairs were transferred to a separate breeding tank (2.4 m×1.5 m×0.5 m), the bottom of which was fitted with a hapa net to facilitate the collection of eggs released during spawning. Approximately 24 hours after the latency period, the females were stripped for eggs, and the eggs were collected in a clean, dry plastic bowl (approximately 500 mL capacity) to prevent contamination and ensure easy mixing with milt during fertilization. During the breeding trail in the month of April out of two only one female responded (90 grams) to the hormone and laid an approx. of 3000 eggs and in September out of three two females (95 and 120 grams) laid ~4,500 eggs. The fecundity of female weighing 95 g had a fecundity of ~ 2000 eggs/kg body weight and the other female had ~2500 eggs/kg body weight (Table 1). A dry fertilization method, as outlined by Sharma et al. (2022), was employed. In this process, milt from the males was gently drizzled over the freshly collected eggs and carefully mixed using a bird's feather to ensure uniform fertilization. The eggs were left to fertilize for about 10 minutes, after which they were rinsed under a gentle flow of tap water to remove any excess milt. The eggs were then left in the same container for water hardening for about 20 minutes, after which they were transferred into the incubation trays.

The fertilized eggs were characterized by their adhesive, spherical, translucent, and demersal nature, with a diameter of 2.2–2.5 mm. These eggs were carefully placed in an incubation tray (45 cm × 45 cm × 40 cm) in a single layer to prevent clumping, which could hinder proper development. The water temperature in the incubation setup was maintained at an ambient of 20–22 °C using a 1000-watt thermostat, while a steady flow of fresh water was introduced at a rate of 250–500 mL per minute. The inlet water temperature was 18–19 °C. The physicochemical parameters of the water used for larval rearing were closely monitored and maintained within optimal ranges to ensure successful development and production throughout the entire larval stage. The pH of the water was also consistent, ranging between 7.2 and 7.5, which is optimal for embryo development. The water pH was maintained through daily monitoring using a digital pH meter, aided by regular partial water exchange (20–30%) and aeration. Dissolved oxygen levels were also recorded daily in the morning and evening and it ranged between 6.8 and 7.5 mg/L throughout the experimental period. To protect the eggs from direct sunlight, a plastic cover was placed over the incubation tray, and they were left undisturbed until hatching occurred. During incubation of the developing eggs, constant monitoring was conducted to remove non-viable eggs, identified by their characteristic white opacity, in order to prevent any fungal infection to the live ones. The embryonic and morphological development of the developing embryos and larvae was studied using a stereozoom microscope (Leica S9i). A total of six random samples of both egg and larvae were taken carefully with ample water in a concave glass slide, and were kept in time-lapse photography till the completion of hatching.

The hatching rate was calculated by the formula:

Hatching rate (%) =

$$\frac{\text{Number of hatchlings}}{\text{Total number of fertilized eggs kept}} \times 100$$

For monthly GSI estimation, ten males and ten females were randomly selected, dissected, and their gonads were carefully removed and weighed to the nearest 0.01 g.

The gonadosomatic index (GSI) was calculated using the formula:

$$GSI = \frac{Gonad\ weight\ (g)}{Totay\ body\ weiht\ (g)} \times 100$$

Larval rearing in fish is a crucial stage in aquaculture and research, bridging the gap between fertilization and juvenile development. In this experiment, the larvae were successfully reared under controlled conditions from hatching (0 days post-hatching, or dph) up to 40 days. After proper fertilization and incubation, eggs hatched at 120 hours at a temperature of 22 ± 0.5 °C, with a recorded hatching rate of 70% in April and 90% in September. The hatched larvae were transparent with a slight yellowish yolk and tended to gather at the sides of the incubation tray. They remained in the yolk sac stage for the first 8 days of rearing. Larvae were fed with *Artemia nauplii* (OSI Pro 80™ cysts, USA) during 8–15 dph, mentioned as mixed feeding period (when remains of yolk were present) thereafter from 15-21dph larvae were acclimatized to commercial larval (Growel Feeds Ltd., India; 52% crude protein, 12% lipid; particle size 200–300 μm) crumbled feed and from 21 dph onward, the larvae were fed exclusively with this diet throughout rearing (Fig. 5). Water quality parameters were closely monitored and maintained within optimal ranges during the period. Water temperature was consistently maintained at 23 ± 0.8 °C, dissolved oxygen levels ranged between 7 and 8 mg/L, and the pH varied from 7.5 to 8.1. Additionally, alkalinity was measured at 135 ± 6 mg/L, hardness at 40.5 ± 4.8 mg/L, and total ammonia nitrogen (TAN) levels were maintained below 0.002 mg/L. Water quality measurements, including dissolved oxygen, pH, and temperature, were conducted using a multiparameter water quality probe (YSI ProDSS, USA), while

Table 2. Embryological development of *S. richardsonii*

Time Post hatch	Temperature	Dissolved oxygen	Embryological Observations
0 h	21.4	6.65	Eggs were translucent, and the chorion was observed to be separated from the vitelline membrane, forming a perivitelline space.
1 h	21.6	6.48	The onset of meroblastic cleavage later divided the zygote into two blastomeres.
2 h	21.6	6.55	The successive cleavage division divided the zygote into a four-celled stage, seen by four distinct, symmetrically arranged blastomeres at the animal pole.
3 h	21.6	6.61	The third cleavage produced eight cells
4 h	21.4	6.63	The cells appeared compact, rounded, and uniformly sized in the sixteen-celled stage, indicating precise and synchronous cleavage
5 h	21.8	6.31	A 32-cell stage was observed, showing progressive cellular differentia tion, which is typically seen in early teleost fish development.
8 h	21.6	6.74	The blastomere appeared in the shape of a ball and was well defined in the sixty-four-cell stage.
12 h	21.5	6.46	This was the "hight" stage. The yolk syncytial layer was observed on top of the yolk cell
17 h	21.8	6.51	The onset of epiboly was observed, marked by the gradual thinning and spreading of the blastodisc over the yolk, indicating the beginning of gastrulation.
30 h	22.0	6.53	The epiboly continued with the spreading and covered about 50% of the yolk sphere
40 h	21.8	6.44	The embryo reached about 75% epiboly.
50 h	21.5	6.50	A beak-like structure appeared at the anterior part of the embryo, i.e., the ploster
70 h	21.7	6.18	The twenty-five-somite stage was observed, and the yolk sac was seen to be kidney bean-shaped
100 h	21.3	6.34	The caudal region of the embryo broke the chorion casing, and the larvae were seen struggling to break through the casing
120 h	21.6	6.41	The newly hatched larvae were observed; the larvae were cylindrical and slightly tapered at the posterior end. Pectoral fins were also seen during this stage

TAN levels were assessed with a portable photometer (YSI EcoSense 9500, USA). Alkalinity and hardness were determined using titration kits (Himedia, India).

Cumulative survival rate of the larvae from hatchlings to swim-up fry was 65-70%, estimated by calculating the initial number of fertilized eggs and the final count of swim-up fry. The survival rate was calculated by using the following formula:

$$\text{Survival rate (\%)} = \frac{\text{Number of live larvae}}{\text{Total number of hatchlings kept}} \times 100$$

The variation in monthly GSI was analyzed using ANOVA, followed by Tukey's post-hoc test for multiple comparisons (Midway, Robertson, Flinn, & Kaller, 2020). Additionally, the relationship between body weight and ovarian weight for each month was analyzed.

Results and Discussion

In this study, it was observed that under captive conditions, snow trout eggs were successfully fertilized at a high rate of 95%, with a hatching rate of 90%. Most eggs hatched within 96–120 hours after fertilization at temperatures ranging from 20–23 °C. Similar water quality parameters were also reported during the breeding of *S. richardsonii* under captive conditions by Pandey et al. (2010) and Sharma et al. (2022). Water temperature averaged at 23.14 ± 0.14 °C, dissolved oxygen levels remained consistently around 6.05 ± 0.33 mg/L, pH was maintained at a slightly alkaline level of 7.23 ± 0.54, and carbon dioxide (CO_2) levels were relatively low at 1.70 ± 1.44 mg/L. Statistical analysis showed significant differences in gonad weight (GW) across the months (Fig. 2). Specifically, GW was higher in March and September compared to other months. The months with no significant differences in GW (November, January, May, and July) were grouped, and the gonadosomatic index (GSI) of *S. richardsonii* showed noticeable seasonal variation throughout the study period. The highest mean GSI was recorded in September (4.34 ± 3.34), indicating peak gonadal maturation and reproductive readiness during this month. Conversely, the lowest mean GSI was

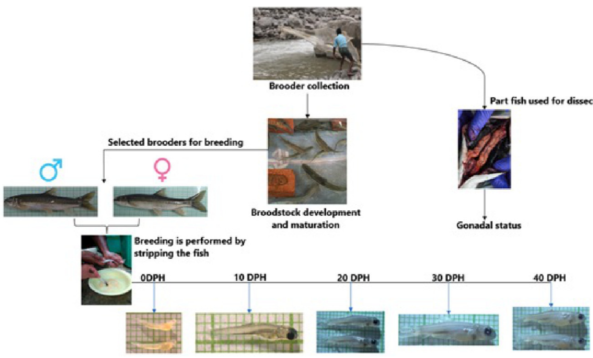


Fig. 1. Schematic representation of *S. richardsonii* from brooder collection to larval rearing

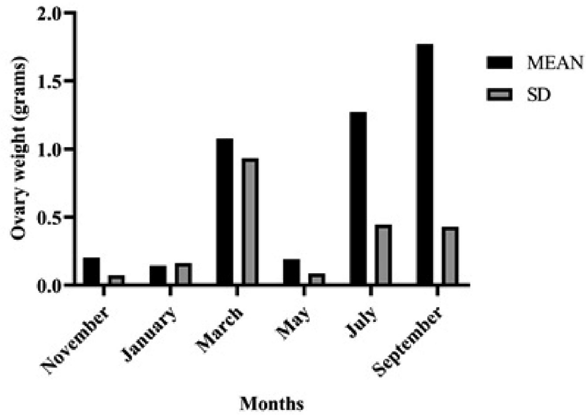


Fig. 2. Gonad weight of *S. richardsonii* female across different seasons, presented as mean ± SD, with n = 10.

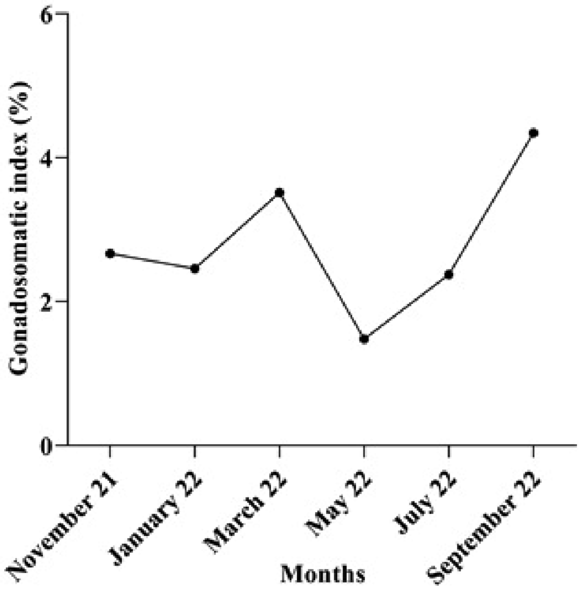


Fig. 3. Seasonal variation in the Gonadosomatic Index (GSI, %) of female *S. richardsonii*. Data expressed as Mean ± SD, n = 10.

observed in May (1.47 ± 1.37), reflecting a post-spawning or resting phase in the reproductive cycle. This trend confirms the occurrence of a bimodal spawning pattern in *S. richardsonii*, with reproductive peaks usually happening during the pre-monsoon (March–April) and post-monsoon (September–October) periods (Fig. 3). In this study, under captive conditions, maturing and spawning stages were highly prevalent in March and September, indicating the preferred spawning season for the fish. The results align with previous research on the reproductive biology of *S. richardsonii*. The seasonal variation in GSI and the timing of spawning observed here are consistent with findings from Agarwal and Singh (2009), Wagle (2014), and Joshi et al. (2016). The pronounced decline in GSI values following March and September indicates that *S. richardsonii* undergoes spawning during these periods. This finding further implies that, although the gonads attain full maturity, they remain in a quiescent state throughout the cold winter months (Joshi et al., 2016). This study also highlights that identifying key factors influencing spawning behaviour, such as water temperature and photoperiod, can be used to manipulate reproductive cycles and induce spawning at specific times of the year. Moreover, photothermal manipulation offers a promising strategy for inducing spawning at planned times, thereby improving hatching efficiency (Mylonas, Fostier, & Zanuy, 2010). The successful captive breeding of snow trout has significant implications for aquaculture and conservation. Producing large amounts of high-quality seed stock can reduce pressure on wild populations and promote sustainable aquaculture. In captivity, embryonic development of *S. richardsonii* was completed approximately 120 hours post-fertilization (hpf) at an average water temperature of 21.6 °C. The development followed the typical teleost pattern, including stages of cleavage, blastula, gastrulation, organogenesis, and hatching. Fertile eggs were transparent at 0 hpf, with a well-developed perivitelline space. Cleavage began at 1 hpf and advanced through the 2-, 4-, 8-, and 16-cell stages by 4 hpf. By 8 hpf, embryos reached the 64-cell stage, with blastomeres forming a dense mass. The high stage appeared at 12 hours post-fertilization (hpf), confirmed by the yolk syncytial layer, while epiboly started at 17 hpf. Epiboly progressed to 50% at 30 hpf and 75% at 40 hpf, indicating active gastrulation. By 50 hpf, a noticeable anterior protrusion (ploster) was visible, and at 70 hpf, the embryo was at the 25-somite stage, with a kidney-shaped yolk sac. Hatching took place at 100 hpf with chorion rupture, and free larvae emerged at 120 hpf (Table 2). The newly born larvae were cylindrical, with a tapered posterior and visible pectoral fins. This developmental timeline aligns with previous studies on cyprinids, though slight variations in timing reflect species-specific and temperature-dependent differences (Akhtar et al., 2015; Ngasainao, Sharma, Chandra, & Chakrabarti, 2024). The post-hatch development of *S. richardsonii* larvae was observed over a 40-day period, revealing gradual morphological and behavioural changes necessary for survival. At 0 days post hatch (dph), the larvae were unpigmented, with no obvious eye pigmentation or redness, indicating an early developmental

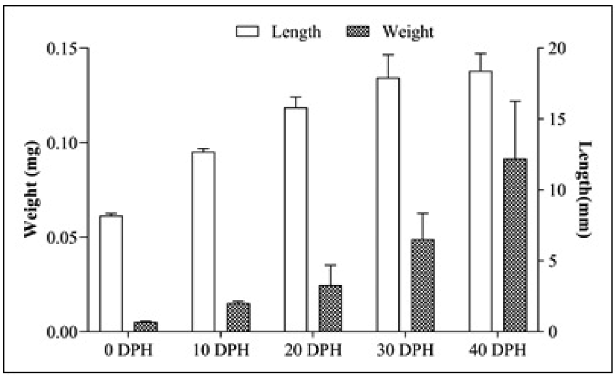


Fig. 4. Weight and length of *S. richardsonii* larvae at different dph, presented as mean ± SD, with n = 10.

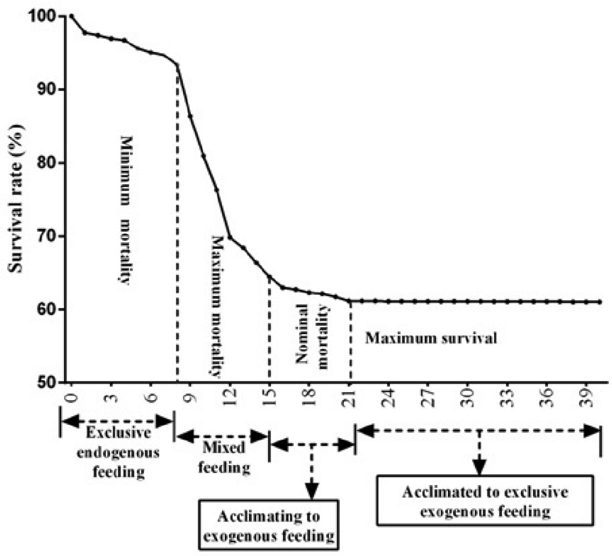
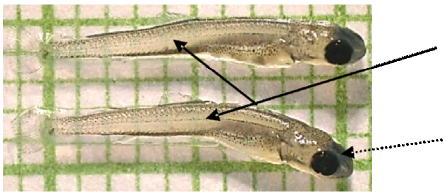
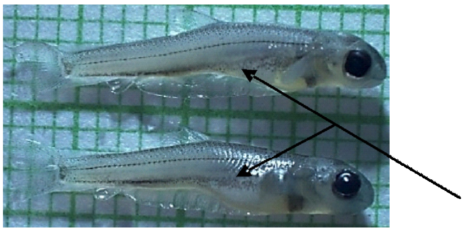
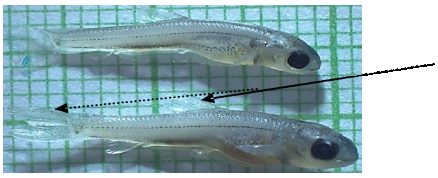


Fig. 5. Larval survival trend of *S. richardsonii* from 0 to 40 Days post-hatch

Table 3. Early morphological development of *S. richardsonii* larvae

Time (Days Post hatch)	Developmental Events	Morphological Observations
0		• The initial hatching event occurred, and no indications of redness were observed during this stage. • No eye pigmentation at this stage.
10		• The lateral line system is more developed, improving environmental awareness • Well-developed eyes and sensory structures, optimizing prey detection and movement
20		• The larval body is elongated, showing increased differentiation of anatomical struc tures • Increased pigmentation with melanophores developing along the body
30		• The vertebral column and fin rays appear more pronounced, suggesting progressive ossification and strengthening of the skeletal system
40		• The larvae exhibit substantial somatic growth • At this stage, the larvae exhibit improved swimming and feeding efficiency, which is essential for their survival in their natural habitat.

stage. By 10 dph, the mouth had opened, the rayed fins were visible, and the lateral line and eyes were fully formed, allowing for active foraging and environmental sensing. At 20 dph, fin fold resorption and body elongation signified the shift to juvenile form, which was accompanied by increasing pigmentation from developing melanophores. By 30 days post-hatching (dph), skeletal elements such as the vertebral column and fin rays were more defined. At 40 dph, larvae demonstrated improved swimming, eating efficiency, and hiding behaviour, indicating adaptive features for real stream habitats. These developmental milestones are consistent with trends observed in other hill-stream cyprinids (Table 3). The growth pattern shows that while larvae steadily gain length from 0 to 40 dph, measuring 8.1880±0.15007 mm at 0 dph; 12.696±0.20206 mm at 10 dph; 15.820±0.73621 mm at 20 dph; 17.920±1.61724 mm at 30 dph; 18.398±1.22265 mm at 40 dph, their weight increases slightly behind, i.e., 0.0052±0.00038 mg at 0 dph; 0.0152±0.00084 mg at 10 dph; 0.0246±0.01062 mg at 20 dph; 0.0490±0.01364 mg at 30 dph; 0.0916±0.03038 mg at 40 dph. This growth pattern, depicted in Fig. 4, provides valuable insights into the developmental biology of this fish species, highlighting the dynamic nature of larval growth and the changing emphasis on length and weight over time. The newly hatched fry was small, with a delicate cream-yellow colour and a prominent yolk sac that extended significantly. The initial post-hatching days were characterized by sluggish behaviour and tendencies to cluster, as also reported by Joshi (2004). The overall survival rate from hatching to 40 days post-hatch was around 65–70% (Fig. 5). The controlled conditions created an optimal environment for early development, allowing high survival during the critical initial stages. The growth trajectory over 40 days showed a distinct pattern, with early stages around 10 dph marked by rapid length increase and slower weight gain. This suggests that early in development, the larvae focus on linear growth to enhance swimming and foraging. As they mature (20–40 dph), growth becomes more balanced, with both length and weight increasing steadily, indicating a shift in developmental priorities. These patterns align with growth trends observed in other cold-water species, where early stages emphasize morphological adaptations that improve survival and feeding efficiency (Akhtar et al., 2015; Ngasainao et al., 2024; Simasiku et al., 2024). Overall, this study provides valuable insights into the growth and development biology of *S. richardsonii*, highlighting the variable nature of larval growth where focus shifts over time, an adaptive strategy common among fish in changing environments, supporting longer spawning periods and increasing reproductive success amid fluctuating conditions (Chandra & Ganie, 2023).

This study offers valuable insights into the reproductive biology and early development of snow trout. By addressing the challenges of asynchronous breeding and improving rearing conditions, it is possible to establish sustainable aquaculture practices for this important species. Additional research is necessary to refine breeding methods and improve feeding strategies, thereby increasing the survival rates of snow trout larvae. Our findings can be useful for future researchers to generate essential baseline data for hatchery operations and conservation efforts for this coldwater species.

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Ethical statement

In this work, we strictly adhered to the guidelines of the Institutional Animal Care and Use Committee of ICAR-Directorate of Coldwater Fisheries Research (currently ICAR-CICFR) and ARRIVE (Animal Research: Reporting of In Vivo Experiments) for the care and maintenance of the experimental animals.

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