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Captive breeding and seed rearing of Assamese snakehead (*Channa stewartii*) for conservation and ornamental trade

S.K. Swain[1], S. K. Ail[2], S.K. Jena[1], M.K. Bairwa[1] and R. Kumar[1]*

[1]*ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar - 751 002, India*
[2]*ICAR-Central Marine Fisheries Research Institute, Kochi-682 018, India*

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***Corresponding Author Email:** rajeshfishco@yahoo.co.uk ***ORCiD:** https://orcid.org/0000-0002-4911-2981

Abstract

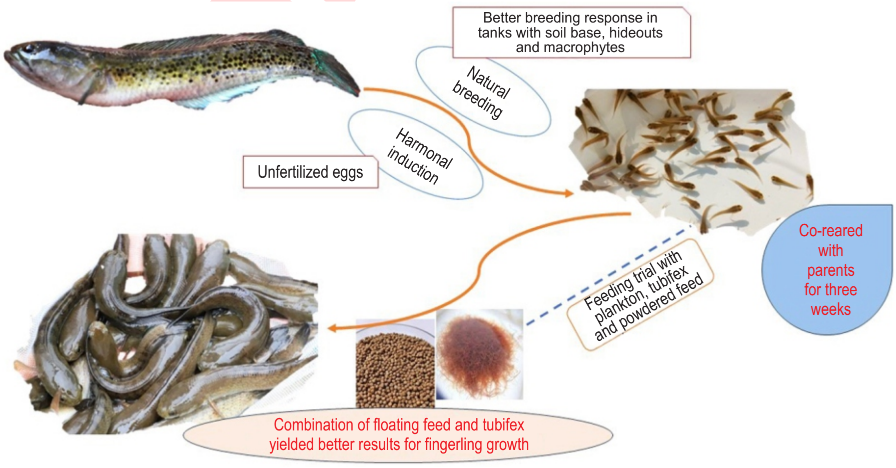
Aim: The indigenous *Channa stewartii,* an ornamental fish native to the Indian subcontinent, has faced significant population decline due to habitat loss and over exploitation for aquarium trade. This study aimed to develop broodstock, enable captive breeding, and optimize seed rearing for conservation and sustainable use.

Methodology: Wild adult *C. stewartii* specimens were collected from Assam, India, acclimatized, and bred in captivity. Despite unsuccessful attempts with hormonal induction, replicating natural habitat conditions yielded successful spontaneous breeding in RCC tanks. Three habitat setups were tested: T_1 (soil base, PVC hideouts), T_2 (soil base, PVC hideouts, floating macrophytes), and T_3 (soil-base only). Growth performance of fry and fingerlings was evaluated under different feeding regimes.

Results: Habitat set up in T_2 exhibited optimal breeding outcomes. Fry fed tubifex showed the highest growth parameters, while plankton-fed fry achieved the highest specific growth rate (SGR). In the second phase, juveniles were reared on mixed and sole feeding regimes. Juveniles fed a tubifex-floating feed combination exhibited the best growth (112.17 ± 8.84 mm, 12.13 ± 2.23 g), whereas those on plankton-feed combinations showed the least growth.

Interpretation: This study highlights the feasibility of breeding and rearing *C. stewartii* in captivity, emphasizing its potential for conservation and sustainable trade.

Key words: *Channa stewartii,* Gonadal development, Length-weight relationship, Natural breeding, Sexual dimorphism



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Introduction

The increasing demand for ornamental fishes has led to intensified exploitation of wild stocks, particularly from biodiversity-rich regions such as North-East India.The aquarium trade has targeted several beautiful fish species from the wild, while commercial culture has been developed for a handful of fish species. It is a fascinating industry where aesthetic value drives demand for precious natural fish stocks, posing significant conservation challenges. India is an abode to rich biodiversity hotspots, including Eastern Himalayan Region (North-Eastern Region) and the Western Ghats. North-Eastern biodiversity hotspot is the habitat of many diverse ornamental fish species which has good demand in the export market (Mahapatra *et al.,* 2007). Family Channidae belongs to the group of snakehead species that vary in morphological and morphometric traits depending upon the region of existence, and also have food as well as ornamental value (Kumar *et al.,* 2022; Damle, *et al.,* 2024).

Among the snakeheads, *Channa assamensis,* (Assam snakehead), *C. aurantimaculata* (Orange-spotted snakehead), *Channa barca* (Barca snakehead), *C. bleheri* (Rainbow snakehead), *C. bipuli* (Bipuli Snakehead) *C. gachua* (Dwarf snakehead), *C. nachi* (Meghalaya Snakehead), *C. stewartii* (Assamese snakehead), etc. are normally consumed as food by the local populations, but in the recent times these species have been targeted by fish collectors as aquarium fish for export. They are mostly endemic to the North-Eastern region of India mainly to beels in Assam (Kumar *et al.,* 2012). Several new species of *Channa* have been reported recently (Dey *et al.,* 2019; Praveenraj *et al.,* 2019, 2020). *Channa* spp. exhibit beautiful colours and are highly preferred by ornamental fish traders and aquarium hobbyists. As a preventive measure, to conserve biodiversity, seed production in captivity is highly essential for the promotion of the ornamental fish trade as well as restoration of the alarmingly depleted stock. Despite high ornamental and ecological value of endemic *Channa* species, scientific information on their biology, conservation status, and culture potential remains limited (Sharma *et al.,* 2022). Moreover, recent advances in sustainable aquaculture and conservation biology have not been adequately applied to *Channa* species. Therefore, more emphasis should be laid on developing captive breeding and culture technologies for indigenous ornamental fishes to reduce pressure on wild populations (Singh *et al.,* 2025).

Channa stewartii (Playfair, 1867), mainly found in the beels of North-Eastern states of India, has flaunting body colours to display for the aquarium. It is commonly known as Assamese snakehead in India. The continuous exploitation from the wild has resulted in a drastic decline in natural fish stocks of many ornamental snakehead species (Das and Manjhi, 2014). *Channa* spp. have high market value and are being exported mostly to Europe, Japan, USA, Singapore and other developed countries. The approximate export price of a few ornamental *Channa* species is $ 40-200 per fish. Limited information is available in the reproductive biology, food and feeding and artificial propagation of *C. stewartii.* Currently, *C. stewartii* is being over-exploited by the exporters/retailers from natural resources, hence, they are at a threat of extinction. Given the high ornamental and ecological importance of *C. stewartii,* there is an urgent need to develop effective conservation and sustainable utilization strategies. Increasing exploitation from the wild for the aquarium trade, coupled with the absence of standardized captive breeding protocols and limited information on larval rearing and seed production, pose a serious threat to its natural populations.

In view of this, the present study focused on domestication, broodstock development, captive breeding under controlled conditions, and rearing of offspring under different dietary regimes to improve growth and survival of *C. stewartii*. This approach aims to support stock restoration while also enhancing seed production for the export market.

Materials and Methods

Ethical statements: All the procedures were followed according to the guidelines set forth by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) for the responsible handling and use of experimental animals, and the current animal welfare law of India.

Broodstock collection from wild and its acclimatization: Broodstock of *Channa stewartii* (Mean weight: Male - 127 g, Female - 222.6 g) were collected from the beels of Assam, India namely Maguri, Motapong, Khamti Gohaliin Tinsukia District and Meribil, Lejai, Kalakhowa, Maijan, Kutuhain Dibrugarh District. Water samples were collected randomly from different beels and analysed as per standard procedure (APHA, 2018) to estimate the water quality of the natural habitat of *C. stewartii*. Around 200 numbers of *C. stewartii* of varying sizes (length 230-290 mm and weight 122-228g) were collected from these wetlands and transported to ICAR-Central Institute of Freshwater aquaculture (ICAR-CIFA), Bhubaneswar, Odisha during 2019.

Broodstock development in captivity (concrete tanks): The fish were transported by train in plastic bags with water and oxygen in 1:2 ratio, acclimatized and stocked in reinforced concrete tanks of 2500 l capacity with a stocking density of 10 fish per tank. Necessary shelter facility was created using cut PVC pipes of 3 inches diameter and 12 inches length. Since the fish prefer swampy areas,6-8 inches of soil base was provided to simulate natural environment. Nets were tightly secured at the top of the tanks to avoid jumping of fishes from the tank. Feeding was done with chopped fish meat (@ 3-5% of body weight) and tubifex worms (*Tubifex tubifex*). Behavioural characteristics in the captivity were studied and management measures were taken to confine them in indoor condition. After one month, the fish were segregated sex wise and kept in separate tanks for broodstock rearing for 3 months (Fig. 1). Feeding was shifted to squid meat gradually for early maturation and better gonadal development (Varghese *et al.,* 2009). The growth and survival were also estimated.

Assessment of gonadal development: Fish were anaesthetized with 2-Phenoxyethanol (200 ppm) for 2 min (Ranjan *et al.,* 2018; Kumar *et al.,* 2021a) for assessment of gonadal maturation. The gonadal biopsy was conducted using a flexible catheter (infant feeding tube, procured from the local medical shop, Bhubaneswar, India). The gonadal tissue was examined using a trinocular microscope (Olympus, CX 31, Japan) with a photographic attachment. The gonadal assessment of female fish was conducted during the pre-spawning and spawning season by assessing the diameter of the oocyte. For estimation of gonadosomatic index (GSI), two male and two female fish were sacrificed during breeding season (April) after anaesthetization (Hajirezaee *et al.,* 2012) with 100 ppm of MS222 (*Tricaine methanesulfonate*, Sigma, Germany). Due to limited availability and conservation importance of *C. stewartii,* destructive sampling was minimized to reduce the impact on local population, following ethical considerations for rare species. GSI was calculated using the following formula: Gonadosomatic index (GSI) = (Gonad weight/total body weight) x 100. Oocyte diameter was estimated to determine the female gonad status. Female brooders of *C. stewartii* generally exhibit a broader and softer abdomen during breeding season and genital vent in females appear round, swollen and slightly reddish or pinkish in colour. In contrast, the mature male fish possess a comparatively slender body with brighter body colouration and numerous black spots distributed over the body surface, while the genital papilla is elongated, pointed and reddish in colour (Fig. 2a,b,c).

Hormonal administration: Fish were administered different doses of commercial fish hormones available in the market. The inducing agents such as Salmon Gonadotropin-Releasing Hormone (sGnRH) and Human Chorionic Gonadotropin (hCG) were tried at different dosages as per earlier studies on *Channa striata* (Kumar *et al.,* 2021b). Hormone injected fish were released in concrete tanks containing 1000 l of water with a male: female ratio of 1:1.Tanks were covered with net to avoid jumping during the mating and brooders were kept for three days for observation of spawning.

Natural spawning: Three distinct breeding sets other than hormonal administration, each in duplicate, were organized in RCC tanks (2 x 1.75 x 0.75 m^3) for natural spawning with 1000 l of water in each tank. In the first treatment group (T_1), tanks were prepared with a 5-6-inch soil base, and PVC pipe hideouts were provided. In the second treatment group (T_2), tanks had a 5-6-inch soil base, PVC pipe hideouts, and floating aquatic macrophytes (*Pistia stratiotes*) covering 10-15% of water surface area. In the third treatment group (T_3), no hideouts or floating aquatic macrophytes were provided. Male and female brooders were individually selected based on physical examination and paired at a 1:1 ratio in each tank. The breeding trial was conducted during 2020-2021.

Fry rearing under different feeding treatments

First phase of the experiment: Three-week old *C. stewartii* fry (Average length- 27.19 ± 0.33 mm and average weight 0.21 ±0.01 g) were reared under different feeding regime, namely plankton, tubifex and powdered feed in triplicate for 30 days. Fish were stocked in ferrocement tanks measuring 1.5 m x 0.8 m x 0.8 m at a density of 30 nos m^3. Feeding as per the experiment was done thrice daily in the morning (9.00 am), afternoon (1.00 pm), and evening (5.00 pm). Plankton was collected from the earthen pond which was fertilized with groundnut oil cake, cow dung and single super phosphate (SSP) in a phased manuring protocol (Jena *et al.,* 2005). Tubifex was grown in a running water system with a muddy soil base enriched with groundnut oil cake in shape of dough balls. Tubifex worms get congregated and multiply near the balls. This helps in collection of tubifex in lumps and washed in clean water 3-4 times before feeding. The powdered feed of size 300-500 µm (Prince Wean, Aqua Marine, Chennai, India) was used. Water quality parameters such as temperature, pH, alkalinity, hardness, and total ammonia were measured routinely as per standard procedures (APHA, 2018). The bottom of the tanks was siphoned carefully every alternate day in the morning hours and a water exchange of 10-20 % was carried out. Tanks were aerated with the help of an air blower with a common supply to all the tanks.

Second phase of the experiment: After 30 days, the fish were graded as per their size and similar sized individuals (1.51± 0.33 g) were taken for the second phase of the experiment in triplicate for another 60 days. All the tanks were cleaned properly and filled with clean water for the conducting the next experiment. Each tank was stocked with 20 numbers of early fingerlings for the experiment. The treatments included plankton and floating feed (1:1); tubifex and floating feed (1:1); and floating feed (solely) fed thrice a day, similar to first phase of the experiment, for a period of 60 days. The floating pellet used measured 1.2 mm with crude 42% protein and 8% lipid (Growel Pvt. Ltd., India) Feeding was done @ 4-6% of fish body weight, fish were sampled randomly for growth parameters on 0, 15, 30, 45 and 60 days. Growth and survival were calculated by the following formula:

Length gain (mm day^{-1}) = (Final body length (mm) – Initial body length (mm)/ number of days; Weight gain (mg day^{-1}) = (Final body weight (g) - Initial body weight (g))/ number of days

$$\text{Specific growth rate (\%)} = \frac{\ln\text{(Final body weight)} - \ln\text{(initial body weight)}}{\text{Number of days}} \times 100$$

Survival rate (%) = (Number of fishes survived/ Number of fishes stocked) x 100; Cannibalism (%) = [(Total number of fish mortality- Number of fishes died showing no signs of cannibalism)/ number of fishes stocked] x 100

Statistical analyses: In this study, all data are presented as mean ± standard error (SEM). All data variables were examined for homogeneity of variance using the Levene's test and for normality using the Kolmogorov-Smirnov test. Data that did not follow a normal distribution were subjected to arcsine transformation prior to statistical analysis. One-way analysis of variance (ANOVA), followed by Tukey's test was used to

Fig. 1: Broodstock of *Channa stewartii*.

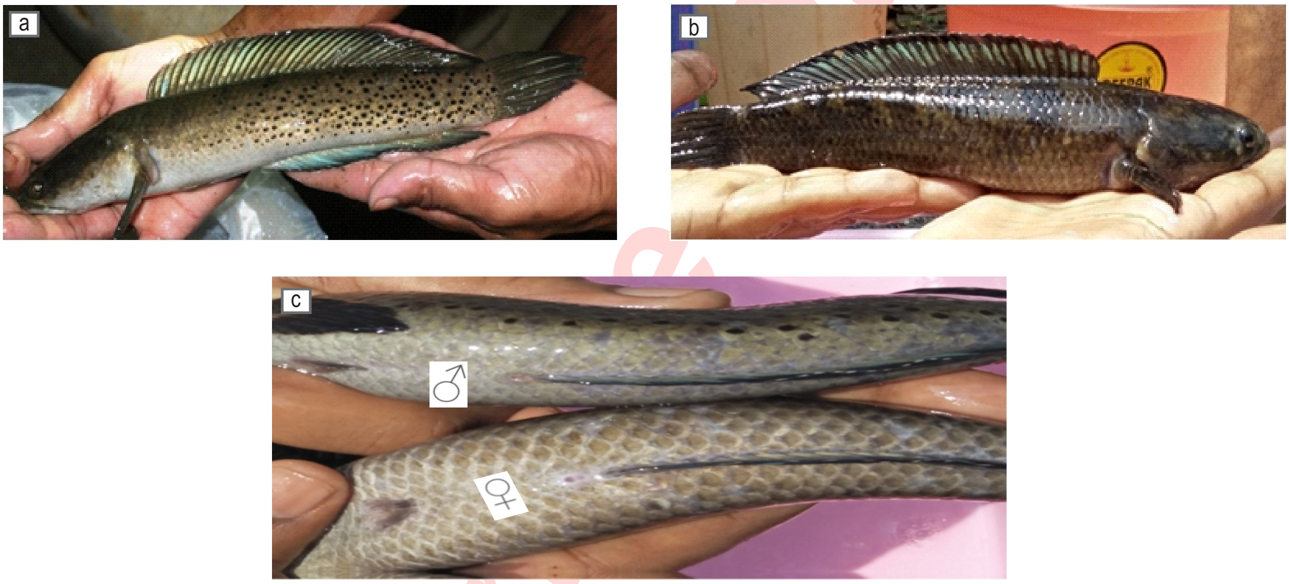

Fig. 2: (a) Male brooder of *C. stewartii*; (b) Female brooder of *C. stewartii* and (c) Female and male brooders of *C. stewartii* showing their genital vents.

determine significant differences among the means using IBM SPSS Statistics version 20. The level of significance among the treatment groups was set at $p < 0.05$.

Results and Discussion

The water quality parameters of samples collected from the broodstock collection site at Dibrugarh, Assam were as follows: pH (6.4-7.6), hardness (40-61 mg l^{-1} $CaCO_3$), conductivity (70-116 µmhos cm^{-1}) and total alkalinity (40-70 mg l^{-1} $CaCO_3$). The water quality parameters of samples collected from the broodstock collection site at Tinsukia, Assam were as follows: pH (7.0-7.5), hardness (60-110 mg l^{-1} $CaCO_3$), conductivity (85-118 µmhos cm^{-1}) and total alkalinity (65-130 mg l^{-1} $CaCO_3$). The water quality parameters of the broodstock rearing unit at ICAR-CIFA, Bhubaneswar was as follows pH (7.8- 8.2), alkalinity (120-150 mg l^{-1} $CaCO_3$), and hardness (100-120 mg l^{-1} as $CaCO_3$). The total alkalinity of the bheel water was comparatively low, as the site is located near a river, and it ranged between 40-130 mg l^{-1} as $CaCO_3$. This indicates that *C. stewartii* possesses a relatively wide tolerance to varying alkalinity conditions in its natural habitat, which is influenced by the dynamic hydrology of floodplain wetlands (beels). Such wetlands are characterized by fluctuating physico-chemical parameters, often remaining alkaline and supporting diverse fish communities, while *C. stewartii* inhabits these variable environments across the Brahmaputra basin, reflecting its ecological adaptability (Bordoloi *et al.,* 2024). At the experimental site, bore-well water with an

Table 1: Growth and survival of *C. stewartii* brooders during broodstock development in RCC tanks

Parameters	Male	Female
Initial Mean Length (mm)	230.8 ± 26.61	286.8 ± 2.49
Initial Mean weight (g)	127 ± 4.47	222.6 ± 5.59
Final Mean Length (mm)	248 ± 11.37	290.2 ± 3.70
Final Mean Weight (g)	176 ± 25.09	289.8 ± 11.19
Weight gain (g/day)	0.54 ± 0.25	0.69 ± 0.13
SGR	0.35 ± 0.144	0.27 ± 0.05
Survival (%)	66 ± 4.95	70.2 ± 6.38

Table 2: The gonado-somatic index (GSI) and oocyte diameter of *C. stewartii* during the pre-spawning and spawning period

Parameters	Pre-spawning period (December)	Spawning period (April)
GSI (Female)	0.54 ± 0.01	3.06 ± 0.11
GSI (Male)	0.06 ± 0.01	0.16 ± 0.01
Oocyte diameter (mm)	0.35 ± 0.02	1.11 ± 0.03

alkalinity range of 120-150 mg l^{-1} as $CaCO_3$ was used, which falls within the natural range observed in their native habitats. Consequently, *C. stewartii* acclimatized successfully and exhibited breeding under controlled conditions.

Initially, the adult fish showed leaping and jumping behaviour during rearing in the concrete tanks that led to loss of few fish, therefore, a free board of 1.5-2 feet was provided and tanks were covered with 30 mm nylon mesh net which prevented the escape of fish. Further, the addition of aquatic macrophytes in 10-15 % of water spread area slow down the jumping behaviour. *C. stewartii* also showed ambush predation, strong territoriality and seasonal breeding with pronounced parental care. Sex-wise growth and survival parameters of the broodstock reared in concrete tanks for 90 days are summarized in Table 1. Weight gain was higher in female broodstock (0.69 g day^{-1}) than males (0.54 g day^{-1}) whereas specific growth rate (SGR) was higher in males (0.35%) compared to females (0.27%), it was due to the differences in initial body weight. The female broodstock showed more survival during captive rearing in comparison to male broodstock of *C. stewartii* (Table 1).

Female showed a little swollen abdomen with round vent and few blurred dark spots near the caudal peduncle. Male showed slender body with oval shaped vent and dark black spots throughout the body. The ovaries were paired, elongated, and fleshy with a cylindrical shaped and smooth outer surface. They were pale pink to yellowish in colour with visible vascularization, indicating mature ovarian condition (Fig. 3a). The ventral and dorsal fins of males were bluish in colour. The female gonadosomatic index (GSI) was found to be 3.06 ± 0.1% during the month of April (spawning period) and 0.54 ± 0.01 % during December (pre-spawning period). The male gonadosomatic index (GSI) was found to be 0.16 ± 0.01 % during April (spawning period) and 0.06±0.01 % during December (pre-spawning period). The ova diameter was found to be 1.11 ± 0.03 mm during the April month (spawning period) and 0.35 ± 0.02 mm during the December month (pre-spawning period) (Table 2). The higher gonadosomatic index (GSI) in April indicates peak reproductive activity, while the lower GSI in December reflects a resting or preparatory phase. A similar pattern was observed in ova diameter, with larger oocytes in April suggesting advanced vitellogenesis and maturation (Fig. 3b), whereas smaller ova in December indicate early developmental stages (Bromage and Roberts, 1995). The testes were paired, compact, pinkish to creamy colour and fleshy with an oval to pyriform (pear-shaped) appearance and slight anterior lobulation. Increased redness and enlargement occured due to gonadal development and enhanced blood supply during the spawning season (Fig. 3c). Different attempts of breeding trials with hormones are presented in Table 3. Three breeding trial attempts with Salmon GnRH and different sex ratio failed. However, breeding trial with HCG yielded spawning but eggs were unfertilized. Breeding attempts of murrels are mostly limited to food fish mainly, *Channa striata, C. marulius* and *C. punctatus.* Understanding the reproductive behaviour and breeding biology of a species is important before attempting captive breeding of non-domesticated fish species.

Breeding trials from the same genera, has been attempted in *C. striata* (Parameswaran and Murugsan, 1976; Haniffa *et al.,* 2000; Pati *et al.,* 2004; Marimuthu *et al.,* 2007; Hossain *et al.,* 2008; Roy *et al.,* 2016; Kumar and Mohanty, 2018; Rawat *et al.,* 2020; Kumar *et al.,* 2021a, Kumar *et al.,* 2021b; Damle *et al.,* 2023). *C. stewartii* has parental care as found in most *Channa* spp. The failure of induced breeding with GnRHa may be due to its delayed action on final maturation and ovulation, similar result was reported by Kumar *et al.* (2018) in *C. striata.* Further, administration of hCG successfully induced the spawning but eggs were unfertilized, this indicated that possibly both male and female were not synchronised in their maturity.

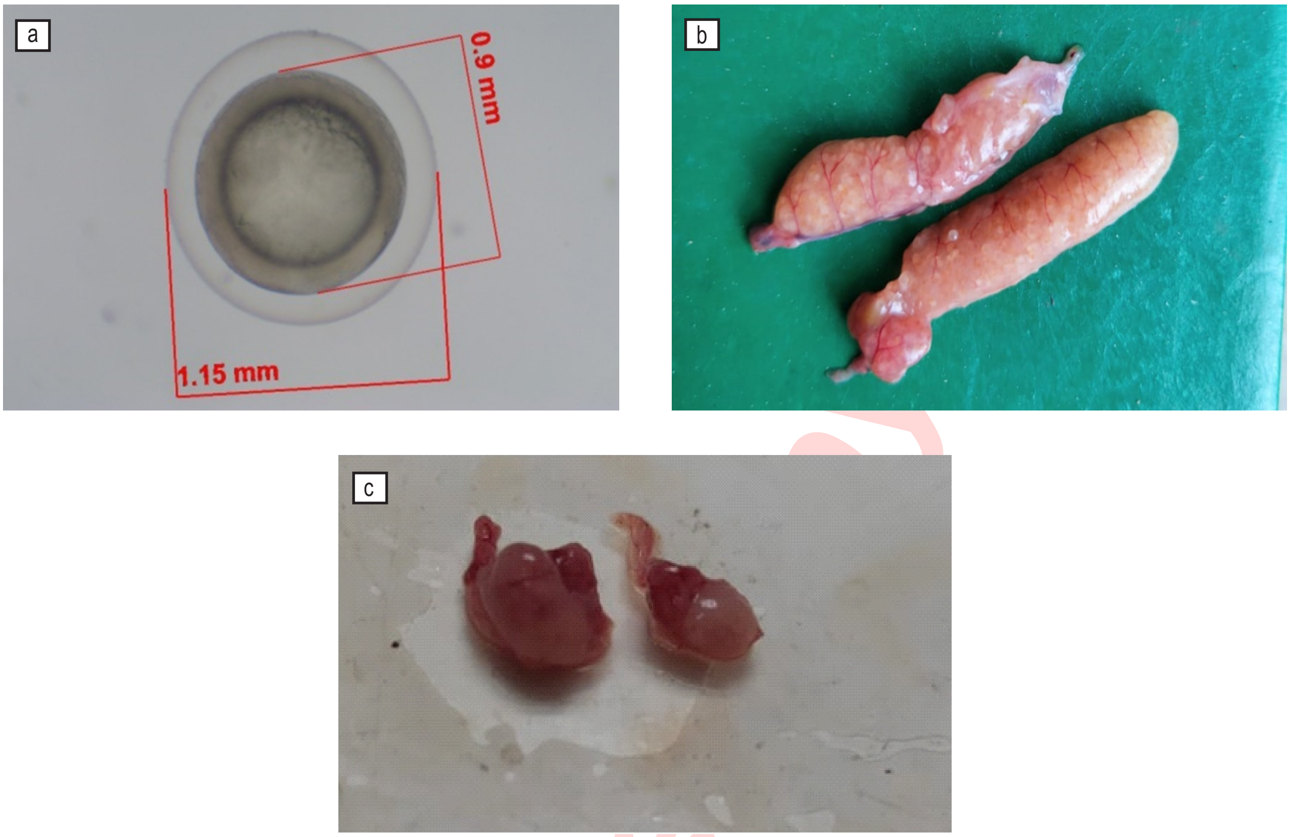

Fig. 3: (a) Mature ova of *C. stewartii*; (b) Gonad of *C. stewartii* and (c) Mature testes of *C. stewartii.*

Spawning by administration of hCG might be due to its direct action on the gonad. This result gave further hope that may be with permutation and combination of different hCG dosages may help in successful induced breeding of *C. stewartii.* Kumar *et al.* (2021b) also reported that hCG gives better spawning success in *C. striata* in comparison to GnRHa. As the initial trials using hormonal inducement were unsuccessful, environmental simulation for natural spawning was attempted.

For spontaneous breeding under captive conditions, selected males and females were stocked in 1:1 ratio in each tank and fed fresh squid pieces (~1 cm) twice a day. The best female and male brooders were selected based on their secondary sexual characters. Female showed a little swollen abdomen with round vent and few blurred dark spots near the caudal peduncle. Males showed slender body with oval shaped vent and dark black spots throughout the body. Relatively similar size of female and male 1:1 ratio was stocked in each tank and fed with squid cuts. The tanks were checked for any breeding activity during feeding time. Before spawning, the fish were seen chasing each other and hiding near aquatic macrophytes, and at times both male and female were found inside the PVC pipes. The tanks were observed daily for spawning and after a few days the breeding response was seen in the tanks. In the T_1 group one replicate responded on 9^{th} day of stocking and in the T_2 groups both the replicates responded (one on the 5^{th} and another on the 7^{th} day of stocking). The fish in T_3 group did not respond even after three weeks. To minimize the workload and also to maximize hatchling output, the young ones were co-reared with their parents in the same tanks and fed ad-libitum with mixed zooplankton.

The zooplanktons were collected from the fertilized pond and sieved through a 100µ filter and fed for 5 days, and later filtered through a 200µ filter. Hatchlings were allowed to co-rear with the parents for three weeks and then collected. Parental care was observed in all the bred groups. Co-rearing of hatchlings with parents under captive conditions likely reduced handling stress and enhanced survival, as parental care provides protection, improved environmental stability, and favourable developmental conditions for offspring (Wootton, 2012; Goldberg *et al.,* 2020; Thünken *et al.,* 2024). The consistent parental care observed across the groups may have enhanced early larval performance. Ad libitum feeding with appropriately sized live zooplankton supports growth and survival, as larvae rely on small, motile prey. The total number of hatchlings collected in different groups is presented in Table 4. Spawning was first observed in one replicate of T_2 on the 5^{th} day after stocking, while the other replicate responded on the 7^{th} day. This indicated that soil base,

Table 3: Hormonal doses applied for induced breeding attempt in *C. stewartii*

Weight (g)		Male Female Ratio	No. of pairs/ tank	Hormones	Hormonal dose (ml kg^{-1})		Results
Male	Female				Male	Female	
121	160	2:1	1	SGnRH (Ovatide)	0.3	0.2	Negative
205	298	1:1	1	SGnRH (Ovatide)	1	1	Negative
155	215	1:1	2	SGnRH (Ovatide)	0.5	0.5	Negative
210	288	1:1	1	HCG	300 I.U.	200 I.U.	Spawning successful (Unfertilized)

Table 4: Collection of *C. stewartii* hatchlings from the different treatment groups

Treatments	Number of hatchlings	Length of fry (mm)	Weight of fry (g)
T_1	180	27.31 ± 1.31	0.23 ± 0.03
T_2	253 ± 45	25.75 ± 1.31	0.22 ± 0.02
T_3	-	-	-

hideouts and floating macrophytes might have provided natural environment to the fish and triggered spawning. Since *C. stewartii* exhibit parental care, it cannot breed unless it feels that their offspring's will be safe in the given environment. Therefore, it is indicated that the T_2 provided best natural environment and both the replicate responded positively. In T_1 only one of the replicate successfully responded on the 9^{th} day, which indicated that floating macrophytes is playing important role in simulating natural environment for successful spawning. The T_3 group did not spawned even after three weeks, which showed that hideout and floating aquatic macrophytes is required for the successful spawning. Damle *et al.* (2023) recommended habitat enrichment in captive systems as an effective strategy to promote early gonadal maturation and reproductive performance in *C. striata* broodstock.

Hatchlings were allowed to co-rear with the parents for three weeks and then collected. During the co-rearing period, offspring were fed with mixed zooplanktons collected from pond and parents were continued to be fed with squid/fish cuts. The number of hatchlings collected from T_2 (253 ± 45 numbers) was more than T_1 (180 numbers) group (Table 4). In the present study, the better breeding response form T_2 might be due to floating aquatic macrophytes in combination with soil base providing better environment for the fish to positively respond for spawning. Fish exhibited parental care and upon danger the parents carry their spawn in their mouth for some time and once they feel safe then again releases back. Weijola (2022) emphasized that complex parental care behaviours, including trophic egg provisioning and offspring guarding in snakeheads, play an important role in enhancing early survival and adaptive fitness of progeny. Fish exhibiting parental care often protect their offspring by temporarily taking eggs or fry into their buccal cavity during disturbance or predation threat, and releasing them again once the conditions are favourable, such behaviour commonly observed in mouth brooding fish including cichlids and snakeheads (Abecia *et al.,* 2022). It may also be correlated that floating macrophyte helps in hiding of their offspring and, hence, responded better in T_2 treatment in comparison to other treatment groups. It may also be correlated that the presence of floating macrophytes provided shelter and concealment for the offspring, thereby reducing stress and enhancing protection from predators and external disturbances. The increased habitat complexity created by aquatic vegetation likely offered a favourable microenvironment for parental care and larval retention, which may have contributed to the comparatively better survival observed in T_2 treatment than in other treatment groups. Aquatic macrophytes are known to function as important refuge habitats for juvenile fish and enhance early survival by reducing predation risk and improving habitat suitability (Peterson *et al.*, 2025).

During fry rearing, water quality remained within suitable ranges (temperature 25-29 °C, pH 7.4-8.2, alkalinity 90-130 mg l^{-1} $CaCO_3$, hardness 94-120 mg l^{-1} $CaCO_3$, dissolved oxygen 4.8–5.4 mg l^{-1}, and ammonia <0.01 mg l^{-1}). In the first 30 days of rearing, significantly higher ($p<0.05$) survival was recorded in the plankton-fed group (66.67 ± 2.8%) compared to the tubifex (62.5 ± 3.53%) and powdered feed groups (56.67 ± 2.8%) (Fig. 4). The superior survival in the plankton-fed groups may be attributed to higher acceptability, continuous motility, and better digestibility of live plankton during larval stages. Live feeds stimulate feeding responses through prey movement and provide essential nutrients and digestive enzymes that improve larval growth and survival. In contrast, powdered feeds are often poorly ingested

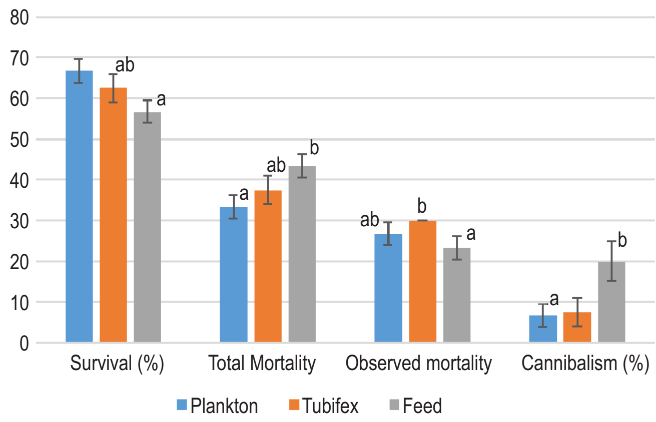

Fig. 4: Survival (%), mortality and cannibalism (%) during first month of the experiment in different feeding groups.

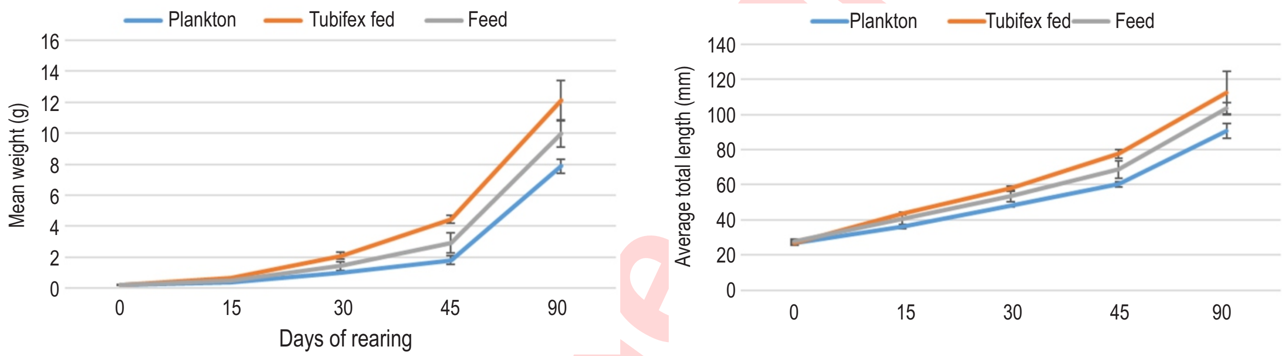

Fig. 5: Average weight and average total length of different experimental groups during different rearing months. Values are mean ± S.D.

and digested during early larval stages, leading to nutritional stress, size heterogeneity, and increased mortality. Similar findings have been reported in larval and juvenile rearing of several carnivorous fishes, including striped murrel, where live feed significantly improved survival and growth performance compared to formulated diets (Qin and Fast, 1997; Mamun *et al.,* 2020; Sahoo *et al.,* 2025). At this stage, the fish larvae and fry preferentially consume live feed due to their underdeveloped stomach and digestive systems, which limits their ability to efficiently utilize artificial diets (Govoni *et al.,* 1986; Holt, 1993; Roy *et al.,* 2009).

The non-significant difference in final length between the tubifex- and formulated feed-fed groups indicates comparable linear growth; however, the significantly higher final weight in the tubifex-fed group suggests better nutrient utilization and biomass accumulation (Table 5). Tubifex worms are highly palatable and rich in essential nutrients, which enhance feed intake and digestion during larval stages. Similar findings have been reported in larval fish rearing studies where live feeds promoted superior weight gain compared to artificial diets (Mamun *et al.,* 2020). Similar preferences for live feeds have been reported in several studies on *C. striata* reared under captive conditions, where improved growth and survival were observed in fish fed natural diets (Kumar *et al.,* 2008; Qin and Fast, 1996). Comparable findings have also been documented in other cultured fish species, including cod (Folkvord, 1991), Asian seabass (Curnow *et al.,* 2006), and pikeperch (Bódis *et al.,* 2007; Szczepkowski *et al.,* 2011), emphasizing the universal importance of live feed during early developmental stages.

In addition to promoting growth and survival, live feeds also contribute to better water quality management in larval rearing systems. Unlike powdered or pelleted feeds, live feeds remain active in the water column and are consumed gradually, thereby minimizing feed wastage and organic matter accumulation. In contrast, uneaten artificial feeds tend to settle at the tank bottom, causing deterioration of water quality and negatively affecting fish health and growth if not properly managed. Therefore, the present findings further emphasize the advantages of live feeds, particularly tubifex and plankton, in improving growth performance while maintaining favourable rearing conditions during early developmental stages. Furthermore, cannibalism did not differ significantly between plankton and tubifexfed groups, suggesting that both live feeds

Table 5: Growth parameters during the first month (30 days) in different experimental groups (Mean ± SD)

Group	Initial total length (mm)	Initial weight (g)	Final total length (mm)	Final weight (g)	Length gain (mm day^{-1})	Weight gain (g day^{-1})	Specific growth rate	Survival (%)	Cannibalism (%)
Plankton	27.0± 1.00	0.20± 0.01	47.93± 0.31^{a}	0.98± 0.01^{a}	0.69± 0.03^{a}	0.026± 0.001^{a}	5.16± 0.19^{a}	66.67 ±2.8^{b}	6.67 ±2.8^{a}
Tubifex	26.75± 1.06	0.21± 0.03	58.1± 1.27^{b}	2.11± 0.24^{b}	1.05± 0.01^{c}	0.063± 0.01^{b}	7.69± 0.07^{b}	62.5± 3.53^{ab}	7.5± 3.54^{a}
Feed	27.83± 1.04	0.23± 0.02	53.4± 2.96^{b}	1.43± 0.29^{a}	0.85± 0.08^{b}	0.39± 0.01^{a}	5.98± 0.57^{a}	56.67± 2.8^{a}	20.0± 5.0^{b}

*Values are mean ± S.D.; Different superscripts in the same column show significant differences ($p<0.05$)

Table 6: Growth parameters during 2nd-3rd month (60 days) in different experimental groups (Mean ± SD)

Group	Final length (mm)	Final weight (g)	Length gain (mm day^{-1})	Weight gain (g day^{-1})	SGR	Yield (g l^{-1})	Survival (%)
Plankton+ feed	93.22±4.22^{a}	7.89±0.78^{a}	0.75±0.07	0.11±0.01	3.47±0.17^{b}	0.37±0.03^{a}	95±5.00
Tubifex + feed	112.17±8.84^{b}	12.13±2.23^{b}	0.90±0.15	0.17±0.04	2.89±0.31^{a}	0.58±0.09^{b}	96.67±2.89
Feed	103.42±3.17^{ab}	9.97±1.46^{ab}	0.83±0.05	0.14±0.02	3.22±0.25^{ab}	0.48±0.08^{ab}	96.67±2.89

Values are mean ± S.D.

adequately satisfied the predatory feeding behaviour of the larvae. However, the highest cannibalism observed in powdered feed groups could be associated with inadequate feed acceptance and increased size variation among individuals, which are known to trigger aggressive and cannibalistic behaviour in carnivorous fish larvae (Fig. 4). Similar observations have been documented in snakehead and catfish larvae, where poor-quality or insufficient feed enhanced intra-cohort cannibalism and reduced survival (Sahoo *et al.,* 2025). Although this finding contradicts earlier reports by Roy *et al.* (2020), the present study indicated that few fishes readily consumed the artificial feed, while others showed low appetite or poor acceptance. In contrast, live feeds such as zooplankton and tubifex were more readily accepted and available upto satiation level, thereby reducing feeding competition and cannibalism.

Survival and weight gain varied significantly among the feeding regimes during the second phase of rearing (Table 6). The significantly higher final weight (12.13 ± 2.23 g), length (112.17 ± 8.84 mm), and yield (0.58 ± 0.09 g l^{-1}) observed in tubifex and feed-fed groups, and lowest in plankton and feed fed groups (7.89 ± 0.78 g, 93.22 ± 4.22 mm and 0.37 ± 0.03 g l^{-1}, respectively), indicate that the combination of live and formulated feed provided balanced nutrition and improved feed utilization (Fig. 5). Tubifex worms are highly palatable and rich in protein, which enhances nutrient assimilation and biomass accumulation in carnivorous fish juveniles. In contrast, the comparatively lower growth and yield in the plankton- and feed-fed groups may be due to relative lower nutritional density and smaller prey size of plankton during advanced larval stages. However, the higher SGR in plankton-fed fish suggests better digestibility and rapid early growth associated with small live prey.

This study successfully demonstrated the captive breeding and seed rearing of *C. stewartii,* an indigenous ornamental fish species facing population decline due to habitat degradation and overexploitation. The study, further it highlighted the importance of appropriate live feed management in reducing cannibalism and maintaining favourable rearing conditions. Collectively, these results provide a practical framework for the captive propagation, conservation, and sustainable ornamental aquaculture of *C. stewartii,* thereby reducing pressure on wild populations and supporting biodiversity conservation. Future research should focus on standardizing induced breeding and larval weaning protocols, optimizing broodstock nutrition, and developing cost-effective formulated feeds for large-scale seed production. Studies on reproductive biology, genetic diversity, stress management, and disease control under captive conditions would further support domestication and conservation programmes. Additionally, assessment of market potential and farmer-level adoption could promote the sustainable ornamental culture of *C. stewartii.*

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