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Investigation on the captive propagation of the endemic murrel *Channa stewartii* (Playfair, 1867)

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ABSTRACT

Channa stewartii occurring in the Eastern Himalayan region is an important ornamental fish commanding good value in domestic and international ornamental trade markets. Rampant exploitation from the wild has resulted in a declining population trend that necessitates immediate conservation measures for replenishing natural stocks. Since *ex-situ* propagation has shown reliability in restoring the population of many extant fishes, the present investigation was undertaken to propagate and standardize breeding protocols of the species. For captive propagation, five pairs of brooders were selected. The length/weight ranged from 19.5-20.4 cm/55.12-68.85 g in the cases of male specimens and in the case of females, it was 20.9-24.2 cm/69.61-83.92 g. The physicochemical parameters of water were maintained in the sustenance of brooders and breeds. Successful batches of fries were obtained from each pair via captive breeding following the pairing method. The highest fertilization rate (87-89%) was observed in the pair with standard length and weight of 19.8 cm and 58.13 g in males and 23.5cm and 83.92 g in females. Fertilized eggs were round, floating and 0.8-1.0 mm in diameter. Fries were measured as 0.4-0.6 cm and weighed in the range of 0.02-0.04 g. Parental care was distinct in both sexes. The breeding experiment carried out without the induction of synthetic hormones can be regarded as a novel approach toward the captive propagation of fish which will surely aid in formulating management and conservation approaches in near future.

INTRODUCTION

Channa stewartii **(Playfair, 1867)**, commonly known as "*Chenga*" in Assamese is an endemic murrel of the Brahmaputra basin of India and Bangladesh and reported from the swamps and lakes of Assam as well as from altitudes above 1,500 m from Meghalaya and Nepal **(Talwar and Jhingran, 1991; Kalita *et al*., 2018)**. The fish is often consumed locally as a cheap source of animal protein and commands good demand in the ornamental fish industry due to its attractive colouration pattern and energetic behaviour **(Raghavan *et al*., 2013; Kalita *et al*. 2018)**. Recently, it has been observed that the harvesting of the species from their natural habitats has increased manifold due to their burgeoning demand for both consumption and ornamental trades **(Goswami *et al*., 2006; Bhagabati *et al*., 2019)**. Even though the species is listed as Least Concerned **(Chaudhry, 2010)** in the IUCN Red List of Threatened Species **(**version 3.1**)**, the availability of this endemic ornamental snakehead has been greatly reduced over the past few years due to many anthropogenic factors. These factors, in turn, have created productivity pressure on the species to sustain themselves in their natural habitat, which necessitates immediate conservation measures **(Wagh and Ghate, 2003; Deka *et al*., 2005)**.

Mitigative strategies for species conservation are multifaceted and often involve varied approaches to achieve conservation goals. Among them, captive breeding interventions are a globally acclaimed approach undertaken to increase the productivity in fishes **(Bhattacharyya and Homechaudhuri, 2009; Nayak *et al.*, 2020)**. It is regarded as one of the viable methods for restoring wild populations of threatened, rare and endangered fish species (**Philippart, 1995; Sarkar *et al*., 2006**). The technique is also helpful in providing critical information of the fish’s life history, as well as restoring extirpated populations (**Rakes *et al*., 1999**). Beside conservation approaches, the technique has immensely helped both commercial food fish and aquarium industries to increase their output significantly (**Moorhead and Zeng, 2010**). Traditionally, this approach involves administration of synthetic hormones for inducing breeding in fishes (**Nandeesha *et al*., 1991; Sarkar *et al*., 2009**; **Raizada *et al*., 2013**) but propagation *sans* artificial inducing agents have also showed encouraging outcomes (**Hazarika *et al*., 2014**). Fishes bred without hormone induction through habitat manipulation also has certain advantages over fishes that are induced bred as it ensures minimal harm to the brooders and greater output due to conducible environmental conditions.

Recent studies suggests that with increased trade in the aquarium industry (**Raghavan *et al*., 2013; Harrington *et al*., 2022**) coupled with habitat degradation, the population of *C. stewartii* is declining in an alarming rate in their natural habitats. Keeping this in view, the present investigation aimed to develop captive breeding techniques for *C. stewartii* through habitat manipulation which in turn surely supplement to meet the ever-growing market demand.

MATERIALS AND METHODS

1. Collection of specimens

Suitable brooders were collected during the pre-monsoon season, from Motapung-Maguri beel ghat (27°34'27"N and 95°23'30" E) and Guijan Ghat (27°34'40"N, 95°19'30" E).

2. Disinfection

The specimens were exposed to 1ppm $KMnO_4$ for about 5-7 min. They were further treated with UV water overnight and then transferred into the breeding enclosures.

3. Breeding site

The breeding experiments were carried out at the Indigenous ornamental fish breeding and rearing facility center available at the "Hitendra Nath Boruah Science and Culture Park" premises of Dibrugarh University, Assam.

4. Acclimatization and conditioning the brooders

The brooders were acclimatized in a tank with sandy bottom containing aquatic plants like *Eichhornia crassipes*, *Pistia stratiotes*, *Hydrilla vertcillata* etc. Regular monitoring of water parameters was done during the entire rearing period. The brooders were conditioned both with live feed like mosquito larvae, earthworm, ant larvae etc. and protein rich commercial feed.

5. Pairing of the brooders and induction of spawning

a) Five experimental setups were taken for the breeding trials with a pair of brooders in each set up prior to the breeding season. The male and female initially showed hostility towards each other but later settled to live together and was seen to move together in the tank. Once pairing was established the aquatic plants in the tanks were minimized to have a clear view of the tank.

b) After 48 hours of settlement of brooders in the breeding enclosures, $3/4^{th}$ of spawning aquarium water was drained and replaced by colder water with a sprinkler which creates a rain like simulation from evening for about 4 hours.

6. Water quality parameters

The different parameters recorded were water temperature, pH, Dissolved O_2, alkalinity and hardness following standard protocols **APHA (2005)**.

7. Fertilization and hatching rate

Fertilization and Hatching rates were calculated as follows:

$$\textbf{Fertilization (\%)} = \frac{\text{No.of fertilized egg}}{\text{Total No.of eggs}} \times 100$$

$$\textbf{Hatching (\%)} = \frac{\text{No.of hatchling appeared}}{\text{No.of eggs in sub sample}} \times 100$$

8. Observation of embryonic developments of the fertilized eggs

The gradual developmental stages of the eggs right from fertilization to hatching were observed using LEICA DM 750 and LEICA DM 1000 microscopes. Scales were added with the help of ImageJ image processing program.

RESULTS

1. Sexual dimorphism of *C. stewartii*

Marked sexual dimorphism was observed in *C. stewartii*. The males are characterized by bright and prominent black spots distributed unevenly across the trunk region. On the other hand, the presence of somewhat prominent alternate bands and stripes along with faded spots indicates the female sex **(Fig. 1)**. Moreover, the genital papilla of the gravid male is prominent and oozes out milt when pressed gently. Similarly female genital pore oozes out eggs when gentle pressure is applied on the abdominal region.

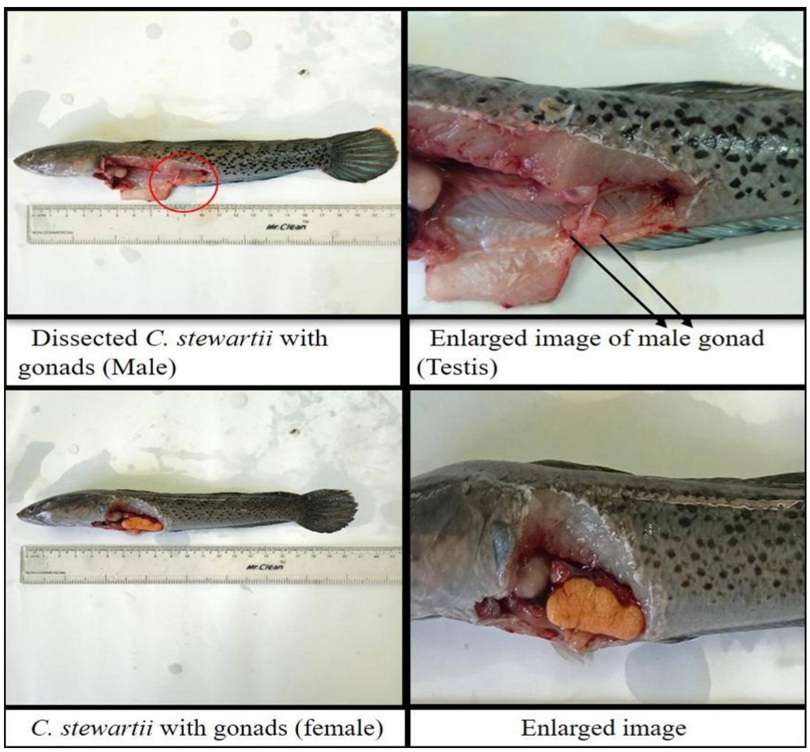

Fig. (1): Sexual dimorphism of *C. stewartii*

2. Breeding in captivity

Successful breeding was observed in 5 pairs of fishes with fertilization rate between 79-89% and hatching rate 79-81% respectively. Highest fertilization was observed in fish with standard length and weight of 19.8 cm and 58.13 g in male and 23.5cm and 83.92 g in female **(Table 2)**. The water quality parameters (water temperature, ºC; pH; dissolved oxygen, mg/L; alkalinity, mg/L and hardness, mg/L) during the breeding experiment were recorded in **Table 1.**

Prior to breeding, some breeding behavior like male chasing the female continuously was observed. After the release of eggs both the parents were continuously seen guarding the eggs. Both the parents did not feed for 5-7 days post breeding. The parents showed protective behaviour by taking the eggs in their mouth when experiencing threat. Parents move aggressively during seed development and could be seen to attack any moving object in the rearing enclosure. Fries were measured as 0.4-0.6 cm and weighed in the range of 0.02-0.04 g. Parental care was distinct in both sexes.

Table (1): Water quality parameters of the breeding experiment

Parameters	Feb	Mar	Apr	May
Water temperature (°C)	17.1-17.7	18.4-19.4	19.7-21.8	23.1-24.8
pH	7.1-7.3	7.3-7.4	7.3-7.4	7.2-7.4
D.O. (mg/L)	5.6-5.7	5.6-5.7	5.3-5.5	5.8-5.9
Alkalinity(mg/L)	76.55-80.12	74.13-77.41	78.33-80.19	76.84-81.46
Hardness(mg/L)	60.16-66.71	61.13-65.43	60.56-65.63	60.83-65.51

Table (2): Vital minutiae of the breeding experiment

Date of spawning	Batches of hatchlings	Length(cm)		Weight(g)		Fertilization (%)	Hatching rate (%)
		Male	Female	Male	Female		
27th March 2021	B1	19.8	23.5	58.13	83.92	87-89	79-81
10th April 2021	B2	20.1	23.2	56.2	80.1	82-84	76-78
18th April 2021	B3	19.5	24.2	58.92	83.40	84-87	74-79
26th April 2021	B4	19.6	20.9	55.12	69.61	79-83	75-78
5th May 2021	B5	20.4	23.4	68.85	76.62	81-84	77-80

3. Embryonic and larval developments of *C. stewartii*

The fertilized eggs are differed in terms of transparency, while the unfertilized eggs were opaque in appearance, the fertilized eggs were transparent. The gradual developmental stages of the fertilized eggs recorded during the experiment, were described in from sub-heading **3.1 - 3.10** along with **Fig. (2).**

3.1 Fertilized eggs: Fertilized eggs were non-adhesive, spherical, transparent, pale yellow in colour. Diameter of the eggs ranged from 0.8-1.0 mm and floating in nature. Blastodisc can be observed.

3.2 Formation of embryo: Division of blastodisc into 2 blastomeres is observed along with simultaneous cleavage. Gradually Morula stages are initiated.

3.3 Morula: Formation of multicellular blastodisc occurs and progression of blastulation formation occurs.

3.4 Blastula: Embryonic shield is formed; half of the egg gets invaded and differentiation of anterior and posterior end becomes evident.

3.5 Gastrula: Invasion of yolk becomes complete, with formation of two-layered structure i.e., outer epiblast and inner hypoblast.

3.6 Yolk plug stage: Yolk gradually getting absorbed, caudal fin starts separating, Heartbeat is distinct, development of somites.

3.7 Pre-hatching: Yolk sac lessened followed by invagination of buccal cavity, initial development of eye, streamlined body and tail is observed, embryo is bi-laterally organized, well developed notochord

3.8 1-day old larvae: Fully formed heart observed, clear demarcation of head, body and tail, little amount of yolk is left.

3.9 2-day old larvae: Mouth with fully developed lower jaw, rudimentary gill is observed, pectoral fin is distinct, yolk almost gone, vital organs completely developed, capable of locomotion.

3.10 4-5 day old (Hatchlings): Vital organs are well-developed, capable of feeding and locomotion on its own. The hatchlings are responses to stimulus.

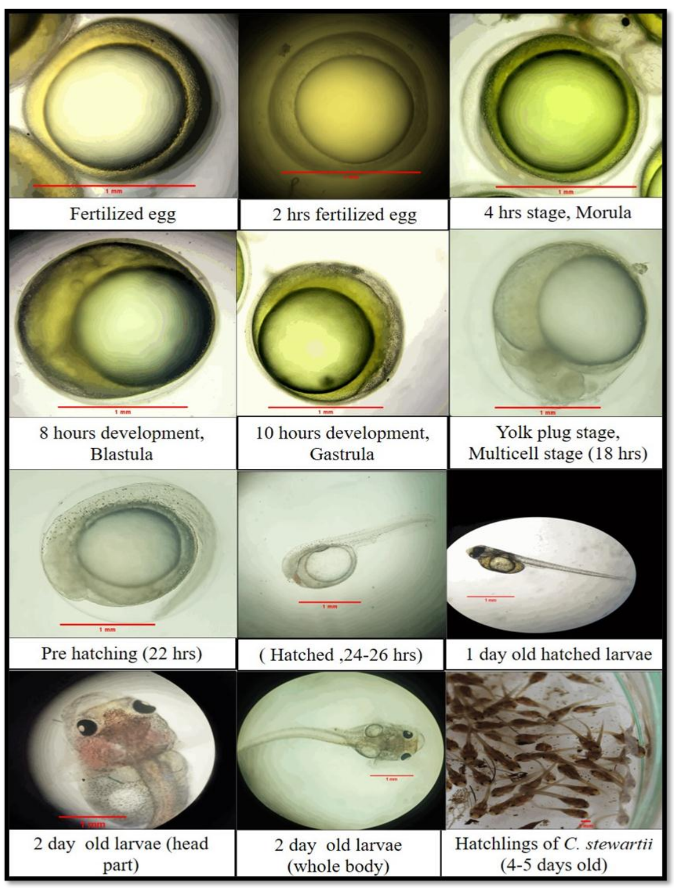

Fig. (2): Developmental stages of *C. stewartii*

DISCUSSION

The present investigation on captive breeding of *C. stewartii* indicated habitat manipulation as an effective technique for mass propagation of the species under confined conditions. During the investigation, marked sexual dimorphism of *C. stewartii* was established through morphological and as well as anatomical examination (**Fig. 1**). Sexual dimorphism is a widespread phenomenon which is often related to mate selection and competition between males. Information regarding the sexual dimorphism of a species is very much essential to comprehend the ecology, behaviour, and life history of a certain species (**Mercy *et al*., 2021)**.

Results of the current investigation indicated that breeding of *C. stewartii* in captivity could be conducted successfully *sans* hormone induction which produces healthy and viable fish hatchlings. In other Channids occurring in the region, **Hazarika *et al.* (2014)** captive bred *C. aurantimaculata* using this technique. Recently, **Nayak *et al*. (2022)** also captive bred *C. andrao* in aquarium conditions which highlights the efficacy of habitat manipulation for mass propagation of *Channa* species under captive conditions. Earlier observations and results of the present investigation thus revealed that *Channa* fishes show trends in reproduction from the advent of monsoon season. Similarly, **Dey *et al*. (2014)** captive bred and studied the larval development of ornamental hill stream fish *Devario aequipinnatus*, indicating the efficacy of habitat manipulation on other groups of fishes as well.

Comparative study with results of other studies and the present investigation further showed that different abiotic environmental factors play a crucial role in successful breeding through habitat manipulation. The current findings indicated *C. stewartii* is an early breeder with breeding season extending from late March to May (**Table 2**). The cold-water treatment in the form of artificial rain was verified to be the prime stimulus for the spawning. Similar observations were also made by **Breder and Rosen (1966)**. It was observed that the species bred in a variety of water conditions (**Table 1**), indicating its adaptability to changing water parameters. The maximum water temperature of the experimental setups was 24.8°C in May which seems to be suitable for the breeding of most tropical freshwater fishes including Channid fishes **(Kahn, 1924; Marimuthu *et al*., 2007; Yulintine *et al*., 2017).** Among different parameters, the role of temperature is paramount in captive breeding interventions as it is directly associated with mating, egg laying and hatching of the eggs. **Haung and Cheng (2006)** in this regard opined that a suitable water temperature can induce breeding in fishes even during a non-reproductive season. Similarly, a suitable range of pH is of utmost importance for successful fish culture. In the present study, water pH was maintained between 7.1-7.6. **Nayak *et al.* (2020)** earlier bred *C. bleheri*, an endemic fish from the region in similar pH range. The Dissolved Oxygen concentration was upheld at the range of 5.3mg/L to 5.8mg/L which have shown to be acceptable for other channid species in captive conditions **(Roy *et al*., 2016)**. Earlier studies on captive breeding in fishes have also revealed a positive correlation with water parameters such as pH, dissolved oxygen etc. with survival of different larval stages (**Purkayastha *et al*., 2012**). Furthermore, the level of alkalinity and hardness was also maintained at the permissible range during the experimental period (**Puspaningsih *et al*., 2018)** which plays an important role in overall fish health and growth. In summary, the findings of the present investigation highlight the possibilities of captive breeding in *C. stewartii* through habitat manipulation which in turn will have a positive impact on its management as well as formulating conservation strategies.

CONCLUSION

Due to the declining population trends of *C. stewartii* from its natural habitats owing to its exploitation for ornamental fish trade as well as its habitat degradation, captive propagation protocol developed for *C. stewartii* will create a positive impact on its wild population. Results highlighted that the species can be easily cultured if appropriate water conditions are maintained during its rearing. Furthermore, by analyzing the current food and ornamental fish market, the addition of cultured *C. stewartii* also will enhance the overall trade and socio-economic conditions of fish farmers and create new opportunities for the budding entrepreneurs. Most importantly, the knowledge void regarding the captive breeding and rearing of this endemic fish species from the region will also be fulfilled.

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