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Induced breeding of climbing perch *Anabas testudineus* by SGnRHa and study of its embryonic development under controlled conditions at Mawpun Ri-Bhoi, Meghalaya

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Abstract
The climbing perch (*Anabas testudineus*) is a highly sought-after fish in northeastern India, but the lack of a standardized breeding method presents a significant challenge for the development of its aquaculture. This study aimed to induce the breeding of *Anabas testudineus* in an aquarium using SGnRHa. The broodstock was injected intramuscularly, typically in the muscle just below the tip of the dorsal fin, at a 45° angle. The fish were left to spawn in the aquarium at a male-to-female ratio of 2:1. Males received an injection of 0.1 ml/kg, while females were given 0.3 ml/kg. Spawning was observed 8-10 hours after the SGnRHa injection, followed by fertilization. The first cleavage occurred within 20 minutes, with the 4-cell, 8-cell, 16-cell, and 32-cell stages observed at approximately 1 hour and 5 minutes, 1 hour and 25 minutes, 1 hour and 55 minutes, and 2 hours and 15 minutes, respectively. The morula, blastula, and gastrula stages were completed at around 3-4 hours, 7 hours, and 8-9 hours post-fertilization. Detailed photographs were taken to document the fertilized eggs, cleavage, and various developmental stages. Healthy larvae hatched 32-34 hours after fertilization.

Keywords: Induced Breeding, *Anabas testudineus*, development stages, fertilization

1. Introduction
The climbing perch, *Anabas testudineus* (Bloch, 1792), known as 'Koi' in India, belongs to the Anabantidae family. This small-sized fish is commonly found in both freshwater and brackish water habitats. It is widely distributed across tropical and subtropical regions, including India, Pakistan, Bangladesh, Nepal, China, Myanmar, Thailand, Cambodia, the Philippines, Indonesia, Singapore, and Sri Lanka (Misra *et al.*, 1959) [5].

In northeastern India, *A. testudineus* is an economically valuable species among small indigenous fishes. It is highly regarded for its tasty flavor, high nutritional value, and ability to maintain freshness for extended periods outside water. Due to its increasing market demand and high prices, koi fish farming has been growing, particularly in northeastern Indian states (Sarkar *et al.*, 2005) [11]. While inducing the breeding of *A. testudineus* in laboratory settings is considered challenging, there are reports of successful breeding using pituitary extracts and synthetic hormones (Patowary *et al.*, 2012). To promote the culture of this species, a deeper understanding of induced breeding and early larval development is essential (Zalina *et al.*, 2012) [14]. Most research on *A. testudineus* has focused on breeding, spawning, and rearing, with only a few studies detailing its embryonic development.

The species occupies various aquatic habitats across its native range, including swamps, marshes, lakes, canals, pools, rice paddies, oxbows, tributaries, and main river channels. Although it primarily inhabits lowland freshwater environments, *A. testudineus* can also be found in brackish coastal areas. During the wet season, it often enters temporary flooded habitats, where it may become stranded as the water levels recede. If necessary, the fish can travel over land for distances of several hundred meters, provided it stays moist. These movements are not frequent but are likely triggered by overcrowding, contamination, or water scarcity, compelling the fish to search for more suitable habitats.

In extreme conditions, it can even enter a state of aestivation by burrowing into damp ground for several weeks (Biswas *et al.*, 2009) [2].

Induced breeding is a method used to breed economically significant fish species, which typically do not reproduce in captive environments, through artificial stimulation. In this process, mature fish are induced to release eggs and sperm through the introduction of pituitary or synthetic hormones, promoting breeding under controlled conditions (Paul *et al*., 2014) [9].

Materials and Methods

The current research was carried out between July and December 2023 during the monsoon season at the Meghalaya State Fisheries Research & Training Institute in Mawpun, Meghalaya. Breeding experiments on *Anabas testudineus* were conducted throughout this period.

Tank Preparation

The tank used for broodstock management is thoroughly washed, then cleaned with a KMnO4 solution, and subsequently rinsed with water to eliminate any germs or bacteria that could infect the brooders.

Collection of brooders

Brooders are being collected from Meghalaya State Fish Brood Bank (Umktieh, Ri Bhoi District). From there they are being transported in a 10-litre bucket. Since it is an airbreathing fish we do not use oxygen as it can survive up to 16 hours without water. Changing of water was done only one time on the way. Also, it was again treated by KMnO4 for 2 minutes per each brooder. So, as to keep them and control from stress or any kind of infection. After that, it was released into the tank for 45 days (July – August).

Broodstock management

In the experiment of the breeding period, broodstock management was done for 45 days (July- August 2023)

*Water quality***:** Throughout the experimental period, various physical and chemical parameters of the broodstock tank water and hatchery water, including flow rate, temperature, pH, dissolved oxygen, free carbon dioxide, hardness, alkalinity, nitrate, nitrite, total ammonium, phosphate, iron, and calcium, were measured following standard methods. Water quality is a critical factor in fish breeding, making it an essential parameter for the success of the process.

Feeding methods

The brood fishes were fed twice a day once in the morning and in the afternoon. The feeds used are earthworms, white portion of boiled eggs, and larvae of insects, etc. The brooders were also treated with cow dung and soil manure respectively.

Length and weight measurement

During July and August, the fish weight was attained 100-120 grams for females and 60-70 grams for males, and the length was measured with a scale of approximately 23-26 cm.

Injection of brooders

Brooders were injected in the intra-muscular part which is generally given in the muscle below the tip of the dorsal fin, at a 45° angle. The brooders were injected in the evening (2:30. 24th -aug-2023) with a dosage of 0.3 ml for females and 0.1 ml for males at the ratio of 2:1. Then they started showing courtship behavior after 3-4 hours of injection and their spawning time is 7-10 hours.

Study of Embryonic development

Some of the fertilized eggs are being taken in a petri dish for further study of embryonic development. Then they were taken in a slide with the help of a dropper and observed under a trinocular compound microscope, then the developmental stage was documented with a photograph.

Results

Water Parameters

During the present experiment, it was observed that water temperature ranged between 24-29 ºC, water pH from 6-7, total alkalinity ranged from 138-156 mg/L, total hardness ranged from 118128 mg/L and DO range was 4-5ppm.

Table 1: Water quality parameters of broodstock management and breeding of *A. testudineus*

Parameters	Values
Temperature (°C)	24-29 °C
Hardness (ppm)	118-128
pH	7-Jun
Alkalinity (ppm)	138-156
DO (ppm)	5-Apr

Induced breeding

Induced breeding using carp pituitary extract proved successful, with spawning occurring 7- 10 hours after the injection. The fertilized eggs were clear, transparent, and floated on the water's surface, while the unfertilized eggs appeared opaque and either remained suspended or sank to the bottom. The fertilization rate was higher when two males were used compared to experiments with a single pair of fish.

The brooders were injected intramuscularly, typically in the muscle below the tip of the dorsal fin at a 45° angle. In this experiment, a 1ml syringe was used, and the brooders were injected in the evening (2:30 PM, August 24, 2023) with a dosage of 0.3 ml for females and 0.1 ml for males, following a 2:1 ratio. After the injection, the brooders were kept together in the aquarium and left undisturbed. Once spawning was completed, the brooders were removed from the aquarium.

Fertilization rate and survival rate

From the experiment, the data has found that

Table 2: mean of fertilization rate (%) and survival rate (%).

Species	Fertilization Rate	Hatching Rate (%)	Survival Rate (%)
Anabas testudineus	80%	60%	30%

Where fertilization rate and survival rate were calculated by using the following formula:

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

= 80%

$$Survival\ rate\ (\%) = \frac{\text{No. of survival fry}}{\text{Total no of hatching}} \times 100$$

= 30%

Here below are the results of the embryonic development of *A. testudineus*

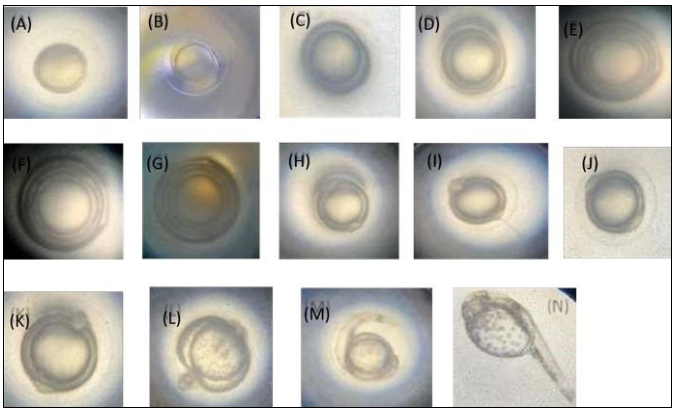

Fig 1: Developmental stages of *A. testudineus*. (A) fertilized eggs zygote, (B) 1-cell stage (C) 2-cell stage (D) 4-cell stage, (E) 8-cell stage, (F) 16-cell stage, (G) 32-cell stage, (H)morula (I)blastula (J) early gastrula (K) gastrula (L) Organogenesis stage, (M) segmentation, (N) newly hatched larva

The stages of embryonic development were monitored using a binocular microscope. The eggs appeared bright golden, with a clear, pearl-like appearance, and were free-floating. Newly fertilized eggs had a diameter of 0.72±0.05 mm, were non-adhesive, and floated to the water surface.

The onset of cleavage and the subsequent cell divisions differed significantly from earlier studies on the development of *A. testudineus*. In this study, the first cleavage occurred rapidly, with the 4-cell, 8-cell, 16-cell, and 32-cell stages observed at 1 hour and 5 minutes, 1 hour and 25 minutes, 1 hour and 55 minutes, and 2 hours and 15 minutes, respectively, after fertilization. The detailed description of all the stages is given below.

Table 3: Time of different embryonic stages after fertilization in *A. testudineus*

Stages of Development	Time after Fertilization
Fertilized egg	0 hrs
Start of cleavage	20 min
2-cell stage	35 min
4-cell stage	1 hour and 5 min
8-cell stage	1 hour and 25 min
16-cell stage	1 hour and 55 min
32-cell stage	2 hours and 5 min
Morula	2 hours – 4 hours
Blastula	6 hours – 7 hours 30 min
Gastrula	8 hours – 9 hours 20 min
Neurula	9 hours 30 min – 11 hours
Segmentation	15 hours – 24 hours
Newly hatch larva	32 hours – 34 hours

Discussion

In the current experiment, the water temperature ranged from 24 °C to 29 °C, the pH was between 6 and 7, total alkalinity varied from 138 to 156 mg/L, total hardness ranged from 118 to 128 mg/L, and dissolved oxygen levels were between 4 and 5 ppm. According to Moitra *et al.* (1979) [6], the optimal water temperature for breeding *A. testudineus* in laboratory conditions is 28±1 °C. In this study, the water temperature was 27±2.5 °C, which was favorable for breeding, potentially contributing to the high hatching rate.

The goal of this study was achieved, with SGnRHa administered at 0.3 ml/kg body weight resulting in the highest spawning, egg production, and hatching rates for *A. testudineus*. The positive response of both males and females to a single dose of SGnRHa is significant for commercial seed production. This breeding protocol does not require significant investment, making it suitable for small-scale farmers for seed production and for species restoration and conservation (Himadri *et al.*, 2011).

In this study, the fertilization rate was 80%, the hatching rate was 60%, and the survival rate was 30%. These results were slightly lower compared to the findings of S. Patra and P. K. Pathak (2019).

The eggs were bright golden, clear, pearl-like in appearance, and free-floating. Freshly fertilized eggs measured 0.72 ± 0.05 mm in diameter, were non-adhesive and floated on the water's surface. This characteristic was also observed by Zworykin (2012) [15] in Vietnam. The fish produced between 490 and 810 eggs per gram of body weight, with a fertilization rate of approximately 92±3%, similar to findings reported by Zalina *et al*. (2012) [14] and Perera *et al*. (2013) [10]. The fertilized eggs began hatching within 32 hours of fertilization.

In this study, the first cleavage was completed within 20 minutes, which differs significantly from the 1-hour duration reported by Zalina *et al.* (2012) [14]. Similarly, the timing and appearance of subsequent developmental stages, as well as the hatching time, also varied. In this study, the 4-cell, 8-cell, 16-cell, and 32-cell stages were observed at 1 hour and 5 minutes, 1 hour and 25 minutes, 1 hour and 55 minutes, and 2 hours and 5 minutes after fertilization. In contrast, Zalina *et al*. (2012) [14] reported these stages at 2 hours, 2 hours and 30 minutes, 3 hours, and 3 hours and 30 minutes, respectively, after fertilization. The time for egg hatching, observed between 32 and 34 hours, also differed from previous studies, likely due to temperature fluctuations in the hilly regions of Northeast India.

Conclusion

Based on the study, it can be concluded that careful monitoring of water quality is essential to achieve optimal results. For the successful induced breeding of *Anabas testudineus*, maintaining good water quality and effective broodstock management is crucial. A solid understanding of induced breeding techniques and early larval development is also vital for the captive cultivation of climbing perch. The study demonstrates promising outcomes with the use of SGnRHa for the induced breeding of *A. testudineus*.

Induced breeding is a straightforward technique that can be easily learned with minimal training. It ensures a consistent and timely supply of spawn for fish culture, meeting demand at any time. However, spawn availability from natural sources is unreliable, as it is dependent on the monsoon season.

Further improvements can be made by addressing certain limiting factors such as temperature, hardness, pH, water circulation, and hormonal dosages. Air-breathing fish hold commercial value as they are sold alive. They are highly sought after for their rich protein and iron content, low fat, and easy digestibility.

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