Biological Forum – An International Journal **16(6): 185-188(2024)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Breeding and Larval Rearing of Koi, *Anabas testudineus* (Bloch, 1792) in an Improvised Technique

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(Received: 05 April 2024; Revised: 24 April 2024; Accepted: 19 May 2024; Published: 15 June 2024)
(Published by Research Trend)

ABSTRACT: The climbing perch, also known as Koi (*Anabas testudineus*), is in high demand in East and North Eastern India. It is one of the most hardy fish. They can flourish in specialized air-breathing organs that use atmospheric air for respiration. Koi, *A. testudineus*, were bred in a small improvised glass breeding chamber (6 ft ×2 ft ×1 ft), with the brooder within the brood cage. During breeding, floating eggs were automatically segregated from brood cages. It decreases egg losses during breeding, improving breeding effectiveness over open breeding in captivity. After multiple experiments, 0.4ml/kg for females and 0.2ml/kg for males diluted with sterile water 80-90% yields the best results. This minimizes wasted brooder costs and infection and usually releases 100% eggs. Fertilization was 83 ± 0.57 % and hatching was 85.5 ± 0.28 %. Feeding larvae egg yolk, infusoria, tubifex, and mosquito larvae led to 4.4 ± 0.44 g weight increase and 4.8 ± 0.1 cm length gain in 90 days, with a 22% survival rate.

Keywords: Koi, *Anabas testudineus*, breeding, glass chamber, larval rearing, captivity.

INTRODUCTION

Anabas testudineus, commonly known as the climbing perch, is a remarkable freshwater fish species native to South and Southeast Asia, particularly found in countries such as India, Bangladesh, Myanmar, Thailand, and the Philippines. *A. testudineus* can grow up to 25 cm in length and is characterized by its robust body, covered in ctenoid scales. The fish's coloration ranges from olive to brown, often with dark vertical bands along its sides (Froese and Pauly 2021). One of the most striking features of the climbing perch is its labyrinth organ, an accessory breathing structure that allows the fish to utilize atmospheric oxygen. This adaptation is crucial for survival in hypoxic waters, enabling the fish to inhabit areas where other species might perish (Liem, 1987). *A. Testudineus* is rich source of protein contains 18-20g of protein per 100g of its flesh, fat 1-3g /100g of fish, Omega-3 fatty acids and also contains vitamin A and D (Nasar and Roy 2018). Due to high demand and costs, climbing perch culture is expanding throughout India, especially in the Eastern and North Eastern states (Sarkar *et al.*, 2005). Despite possible risks such as habitat degradation and dryness, this species is a resilient, habitat-generalist fish that is categorized as 'Least Concern' in India. Despite its high potential for cultivation, the species has yet to be introduced into farming, owing mostly to a lack of seed, as artificial breeding and larval rearing techniques are not standardized in the country. Another issue with expanding *A. testudineus* culture is the scarcity of fry and fingerlings from natural sources, as it is unable to gather a viable quantity of fry and fingerlings from the wild. Larval-rearing techniques for the species have yet to be established.

Various hormone formulations have been used on *A. testudineus* to induce maturation and ovulation, such as Ovaprim (Perera *et al.*, 2013), Ovatide (Marimuthu *et al.*, 2009), Wova-FH (Sarkar *et al.*, 2005) has displayed successful attempts. In this present study, we have used the synthetic hormone 'Hatchme' for breeding which contains (SGnRH, Domperidone, and Propylene Glycol) by diluting with 80-90% sterile water.

Therefore, this study presents the breeding performance of Koi in a glass breeding chamber and to study the larval survivability at different stages of life under captivity. However, breeding and seed production of Koi is still a problem for fish farmers. So, hope this study will contribute to the development of a protocol for successful breeding and seed production.

MATERIALS AND METHODS

An overall total of 30 pairs of brooders with an average weight of 54.2 g, ranging from 50g-60g, and an average length of 17.1 cm, ranging from 15 cm to 20 cm were collected from Bandhab Aqua at Bunarhat, South 24

Pargana, West Bengal. Subsequently, the fish were securely moved to the Fish-breeding Training Centre located at Panchpota, Garia, Kolkata-700152. They were stocked and procured in four glass tanks with a 300-liter capacity and constant aeration.

Gonado Somatic Index: Before the hormonal injection dissected female fishes to collected the ovary to measure the GSI by using the formula

GSI = (Weight of gonads)/(Total weight of fish) × 100

Fecundity: Before receiving the hormone injection, fecundity was assessed. After dissecting mature female fish, the ovaries were carefully removed from the body. Subsamples from the front, posterior, and central regions of the right and left ovaries were then collected and fixed in 10% neutral buffered formalin (NBF). After that, the ovary was shaken to release the eggs, and each gram of ovary was carefully counted using only necked eye. Fecundity was calculated by using the formula

Fecundity = (Total ovary weight × No. of sample eggs) / Weight of total eggs

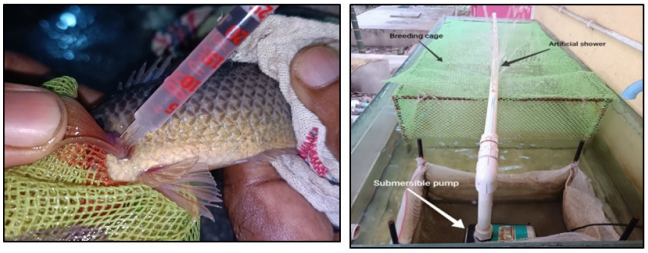
Experimental breeding setup: Koi, *Anabas testudineus*, were bred in a glass chamber (6 ft ×2 ft ×1 ft) with a brooder within the brood cage (5 ft ×1.8 ft ×1 ft dimension with 2 inches ground clearance). Used a submersible pump for an artificial shower. During the test, the fish were maintained in a natural photoperiod.

Breeding procedure: The breeding setup was done before hormonal injection. Initially, the breeding cage was placed inside the breeding tank. It not only helps to separate the brooders after breeding but also increases the egg efficiency. Used a submersible pump for an artificial shower by which the floating eggs automatically come outside from the breeding cage. After the breeding setup the fully mature male and female were segregated from the brood tank. Male and female were taken in a 2:1 ratio for hormonal injection. Used synthetic hormone 'Hatchme' for breeding which contains (SGnRH, Domperidone, and Propylene Glycol) by diluting with 80-90% sterile water. In 4 experimental trials the different doses were in T1(female-0.6ml/kg, male-0.3ml/kg), T2(female-0.4ml/kg, male-0.2ml/kg), T3(female-0.1ml/kg, male-0.05ml/kg), T4(female-1ml/kg, male-0.5ml/kg) and standardize the perfect minimal dose with best performance. After that, the breeding setup was left undisturbed and observations were made and recorded without disturbing the breeding pair. The water parameters like temperature and dissolved oxygen were checked with regular intervals. The spawning %, fertilization % and hatching% were also calculated.

Analysis of the growth performance and survivability rate of larvae: After hatching in a hatching tank (4 ft× 2 ft × 1ft), they were transferred to another tank size of (4 ft × 2 ft × 1 ft) for rearing hatchling to early fingerling for 90 days. After 18 hours of incubation period, when the yolk sac becomes absorbed, then start feeding boiled egg yolk in a 1/4 proportion and observe maximum feed consumption. Used to feed 6 times a day. After day 3 used to feed only green water and infusoria from days 4-8 for 3 times a day. After that used to feed infusoria and dry tubifex from days 9-15 for 2 times a day, next, we used only dry tubifex from days 16-20 for 2 times a day. After day 20 used to feed dry tubifex and mosquito larvae from days 21-45 for rearing fry to advance fry 2 times a day. After that, used to feed only artificial feed for rearing advanced fry to early fingerlings from days 46-90 for 2 times a day. Measured the growth performance of koi in different stages by using different types of feed and sampling the growth for 90 days to assess the final body weight and length and survival rate of the fish. The weight was taken in an electronic balance. The water quality parameters like temperature (°C), total Ammoniacal Nitrogen (TAN), dissolved oxygen (mg/l), pH, Nitrate (mg/l), Nitrate (mg/l), Total Hardness (mg/l) were measured by using 'Bionix Nitrate test kit'.

RESULT AND DISCUSSION

The gonado-somatic index (GSI) of *A. testudineus* was found out for 1 month. The gonad of the fish is slightly yellowish. All female specimens attaining a length of 14 ± 0.38 cm and weight of 52.5 ± 0.88 g were mature. The gravid females had a Gonado-Somatic Index (G.S.I.) ranged from 15.68 ± 0.31 with 15.68 on average. The pre-spawning absolute fecundity of *A. testudineus* was found to be 40528 as the mean and 2442.25 as the standard deviation and the range was 40528 ± 1155.12. The spawning success of Koi was found after four trials with different hormonal doses. Spawning success of 100% was observed in Trial 1, Trial 2, Trial 3, and Trial 4. The fertilization % of Koi after a successful spawning in Trial 1, Trial 2, Trial 3, and Trial 4 are 73.5 ± 0.64 %, 83 ± 0.57 %, 49 ± 0.57 %, and 57.5 ± 0.52 %. The highest fertilization % of 83 ± 0.57 % was observed in Trial 2 and observed brooder mortality in Trial 4. The hatching % of Koi found in Trial 1, Trial 2, Trial 3, and Trial 4 were 81 ± 0.57 %, 85.5 ± 0.28 %, 76.5 ± 0.64 %, and 73 ± 0.57 % respectively. The highest hatching % of 85.5 ± 0.28 % was observed in Trial 2.



(A) **(B)**

Fig. 1 (A) hormonal injection to *A. testudineus* **(B)** Breeding setup.

Table 1: Data on induced spawning of *A. testudineus*.

		T1	T2	T3	T4
Total body weight of brooders (g)	M	403	305	512	515
	F	210	160	260	252
No. of Brooders	M	8	6	10	10
	F	4	3	5	5
Dose of injection (ml/kg)	M	0.3	0.2	0.05	0.5
	F	0.6	0.4	0.1	1.0
Spawning %		100	100	100	100
Latency period (h)		6-7	6-8	9-10	6-7
Fertilization (%)		73.5 ± 0.64	83 ± 0.57	49 ± 0.57	57.5 ± 0.52
Incubation time (h)		14-15	12-15	20-22	12-14
Hatching (%)		81 ± 0.57	85.5 ± 0.28	76.5 ± 0.64	73 ± 0.57
Physico-chemical condition	AT(°C)	32	31	32	25
	WT(°C)	30	30	30	24
	DO(ppm)	8	7	8	8

AT- Air temperature; T- Trial, M- Male, h- Hour, F- Female, AT- Air temperature, WT- Water temperature; DO-Dissolved oxygen; Value are presented as Mean ± Standard Error.

Analysis of Larval Rearing of *A. testudineus* : Larval growth performance and survivability of *A. testudineus* (Bloch, 1792) for 90 days and used to feed different foods in different life stages. Observed the length gain of larvae from spawn to early fingerling. The length gain was achieved in nursery tank 0.55 ± 0.05 cm where we used to feed boiled egg yolk, green water, infusoria and dry tubifex; length gain in Rearing tank-1 0.85 ± 0.06 cm by using only dry tubifex and mosquito larvae; and in Rearing tank-2 4.8 ± 0.1cm where we used to feed only 0.8mm size artificial fish feed. Observed the weight gain of larvae from spawn to early fingerling. The weight gain in the nursery tank, rearing tank-1 and rearing tank-2 are 0.12 ± 0.01 g, 0.5 ± 0.08 g and 4.4 ± 0.44 g. The survival rate % achieved in HT, NT, RT-1 and RT-2 are 42.4%, 46.2%, 53.06% and 92.3% respectively.

Table 2: Larval rearing methodology of *A. testudineus.*

Parameters	HT	NT	RT-1	RT-2
Size of aquarium (ft)	4×2×1	4×2×1	4×2×1	4×2×1
Stage of fish	Hatchling to spawn	Spawn to fry	Fry to advance fry	Advance fry to early fingerling
Age (from fertilized eggs)	45-48 hr.	20 days	45 days	90 days
Days of Rearing	3 days	17 days	24 days	45 days
Nos. of fish stock	500	212	98	52
Stocking density (no. of fish/lt.)	2	1	1	1
No. of fish harvested	212	98	52	48
Survivability rate (%)	42.4	46.2	53.06	92.3
Length (cm)				
Initial	-	0.35 ± 0.05	0.9 ± 0.03	1.72 ± 0.08
Final	-	0.9 ± 0.03	1.72 ± 0.08	6.03 ± 0.31
Length gain	-	0.55 ± 0.05	0.85 ± 0.06	4.8 ± 0.1
Weight (g)				
Initial	-	0.03 ± 0.008	0.16 ± 0.02	0.5 ± 0.14
Final	-	0.16 ± 0.02	0.5 ± 0.14	4.96 ± 0.51
Weight gain	-	0.12 ± 0.01	0.5 ± 0.08	4.4 ± 0.44

Abbreviations: HT= Hatching tank, NT= Nursery tank, RT-1= Rearing tank-1, RT-2= Rearing tank-2. Value are presented as Mean ± Standard Error.

Fig. 2. Length measuring of Koi fingerlings.

Fig. 3. Weighing of Koi fingerlings.

Table 3: Details of feeding in different stages of larval rearing of *A. testudineus*.

Sr. No.	Stage	Age	Feed types	Remarks
1.	Fertilized eggs	0 hr	-	No feed
2.	Hatchling	21-22 hr	-	No feed
3.	Spawns	42-44 hr	Boiled egg yolk	6 times
4.	Spawn to fry	3 days	Boiled egg yolk, Green Water.	6 times
		4-8 days	Green water, Infusoria.	3 times
		9-15 days	Infusoria, dry tubifex	2 times
		16 – 20 days	Dry tubifex	2 times
5.	Fry to advanced fry	21- 45 days	Dry tubifex, Mosquito larvae.	2 times
6.	Advanced fry to early fingerlings	46- 90 days	Artificial fish feed	2 times

Koi, Anabas testudineus is one of the most popular fish in eastern and north eastern India. According to IUCN it is a Least Concern species because of less culture and lac of quality seed production. This research employed a glass breeding setup to decrease egg losses during breeding, improving breeding effectiveness over open breeding under captivity. Glass breeding chamber (6 ft ×2 ft ×1 ft) with brooder put in brood cage after hormonal injection and artificial shower. After several hormonal doses of (female-0.6ml/kg, male-0.3ml/kg), (female-0.4ml/kg, male-0.2ml/kg), (female-0.1ml/kg, male-0.05ml/kg), (female-1ml/kg, male-0.5ml/kg) observed the best performance of breeding by using the minimal dose of 0.4 ml/kg for female and 0.2 ml/kg for male. Where get 100% spawning rate, fertilization % 83 ± 0.57 and Hatching% 85.5 ± 0.28 with a latency period of 6-8 hours and incubation time of 16-20 hours at 30°C temperature.

Perera *et al*. (2013) according to them *A. testudineus* could be successfully stimulated to generate sperm and eggs by injecting ovaprim (SGnRHa) intramuscularly. A dose of 0.5 ml/kg of ovaprim was necessary for a successful ovulation. Rajbongshi *et al*. (2020) used the synthetic hormone ovatide to induce spawning of the *A. testudineus* and got the highest performance by using the dose of 0.3 ml/kg body weight, where the fertilization, hatching and survival rate was 98.3±0.3, 87.98±0.82, 80.92±0.94 percentage at the temperature of 26-28°C.

CONCLUSIONS

The study offers a comprehensive examination of the breeding and larval rearing practices for the climbing perch (*Anabas testudineus*) using a specialized glass breeding chamber. Within this setup, the strategic placement of a breeding cage proves advantageous in breeding. It streamlines the process of separating eggs from the brooder post-breeding, significantly enhancing egg efficiency compared to conventional open breeding systems. This innovation not only optimizes production levels but also simplifies the rearing of larvae by low-cost feed within the controlled environment of the chamber. Moreover, the utilization of this system provides a unique advantage point to closely observing the intricate breeding and spawning behaviours of the climbing perch. By facilitating easier egg and spawn collection. What is particularly noteworthy is the cost-effectiveness of this setup, coupled with its compact design, rendering it accessible even in limited-space environments. Beyond its practical applications, the study also described morphological development stages during the early life of the climbing perch.

FUTURE SCOPE

The research offers invaluable guidance for comparative studies exploring various hormonal interventions by providing detailed insights into these crucial developmental milestones. Furthermore, it furnishes essential information about the embryonic, larval, and juvenile phases of climbing perch development, serving as a foundational resource for aqua-culturists aiming to optimize rearing practices and maximize yield.

Acknowledgement. I sincerely thank Dr. Shriparna Saxena, Head of the dept. Aquaculture, Sanjeev Agrawal Global Education University, Bhopal. Thanks to Dr. B.K. Mahapatra, former Principal Scientist & Scientist In-Charge, ICAR-CIFE, Mumbai and thanks to Dr. Deepak Kher, DEAN, School of Agriculture, Sanjeev Agrawal Global Education University, Bhopal.

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How to cite this article: Chirantan Jana, Shriparna Saxena, B.K. Mahapatra and Deepak Kher (2024). Breeding and Larval Rearing of Koi, *Anabas testudineus* (Bloch, 1792) in an Improvised Technique. *Biological Forum – An International Journal, 16*(6): 185-188.