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Sanjib Sarkar
Ecology & Toxicology Laboratory, Department of Zoology, North Bengal University, West Bengal, India-734013

Benoy Kishore Rai
Ecology & Toxicology Laboratory, Department of Zoology, North Bengal University, West Bengal, India-734013

Dawa Bhutia
Ecology & Toxicology Laboratory, Department of Zoology, North Bengal University, West Bengal, India-734013

Swati Singh
Ecology & Toxicology Laboratory, Department of Zoology, North Bengal University, West Bengal, India-734013

Joydeb Pal
Ecology & Toxicology Laboratory, Department of Zoology, North Bengal University, West Bengal, India-734013

Correspondence
Joydeb Pal
Ecology & Toxicology Laboratory, Department of Zoology, North Bengal University, West Bengal - 734013,

Study on the breeding performance and developmental stages of climbing perch, *Anabas testudineus* (Bloch, 1792) in the laboratory (Siliguri, India)

Sanjib Sarkar, Benoy Kishore Rai, Dawa Bhutia, Swati Singh, Joydeb Pal

Abstract
Induced breeding of climbing perch, *Anabas testudineus* (Bloch, 1792) was done in aquaria in the laboratory using carp pituitary extract. An intramuscular injection was given with the single dose of 50 mg and 100 mgkg^{-1} body weight in males and females respectively. The female brood responded to lay eggs at 8-10 h latency period. The breeding performance and fertilization rate was higher when two males were used for every female. The present study observed fertilization rate of 96 ± 1.41and 84± 1.41 while male and female ratio was 2:1 and 1:1 respectively. The first cleavage began at a 20 min after fertilization. The morula, blastula and gastrula stage was completed at about 4 h, 5 h and 30 min and 7 h and 30 min respectively after fertilization. The details of fertilized eggs, cleavage and developmental stages are documented with photograph. Healthy larvae hatched after 21-22 h.

Keywords: *Anabas testudineus,* induced breeding, pituitary gland, fertilization, developmental stages

1. Introduction

The climbing perch, *Anabas testudineus* (Bloch, 1792) of the family Anabantidae also popularly known as 'Koi' in India is a small sized food fish, which inhabits both freshwater and brackish water. It can be found in most tropical or subtropical area including India, Pakistan, Bangladesh, Nepal, China, Myanmar, Thailand, Cambodia, Philippines, Indonesia, Singapore and Sri Lanka [1].

Among small indigenous fishes *A. testudineus* is an economically important fish of North Eastern parts of India. The fish is very popular for its delicious taste and flavour and its high restorage value and prolonged freshness out of water. At present due to high price and increasing market demand, koi fish culture has been expanding especially in certain part of India including North Eastern state [2]. Induced breeding of *A. testudineus* is thought to be difficult in laboratory conditions, however, reports of success of the same using pituitary extract [3, 4, 5] and synthetic hormones are available [6, 7, 8, 9]. To expand the culture of climbing perch the proper knowledge of induced breeding and early larval development is imperative. Most of the literatures on *A. testudineus* are on breeding, spawning and rearing. Only a handful studies have described the details of embryonic development of this fish [7, 10]. There is no literature on such studies of climbing perch from India.

The present study was designed to determine the efficiency of carp pituitary extract to induce breeding in *A. testudineus* and systematically documented the details of embryonic development.

2. Materials and methods

2.1. Collection and maintenance of brood fish

Healthy fish of both sexes weighing 40 g – 50 g were procured from local fish market, Shivmandir Bazer and Bagdogra Market, during the month of April and May, 2014. Male and female fish were kept separately in cemented tanks (2x1.5x1.5) m^3 for acclimatization during April to June, 2014. The water depth of 0.5-0.75 m was maintained in the tank. Fish were fed twice daily, i.e., 11 am and 6 pm, with small fishes that were finely chopped. Gonad maturation was monitored from time to time by observing the vent of both the sexes for the presence of eggs and sperms. Perfectly mature female fish have swollen, reddish vent and ooze eggs while the males ooze milky milt upon slight pressure in the abdomen.

2.2. Experimental design

Six fully mature male and four female fish weighing 40 g – 50 g were selected and transferred to glass aquarium measuring (30x25x25) cm^3 filled with pond water, with a water depth maintained at 20 cm. Fish were divided into replicates of two groups. One of the two groups comprised of one male and one female while the other group comprised of two males and a female fish. Experiments were done during the month of June and July, 2014.

2.3 Preparation of pituitary gland extract and administration of dose

Freshly collected carp pituitary glands or glands that were previously collected and preserved in absolute alcohol not more than 6 months old were used for the preparation of pituitary gland extract. The extract was prepared by grinding the pituitary glands in distilled water with the help of a Teflon pestle. An intra-muscular injection was administered to both sexes at the same time at a dose of 50 mg and 100 $mgkg^{-1}$ body weight in males and females respectively [3]. Injections were administered during late hours in the evening (8 pm). The timing of the injection was selected such that the brooders were subjected to minimum disturbance. After injection the brooders were kept together in aquarium and left undisturbed. When spawning was complete, the brood fish were removed from the aquarium. The fertilization rate was calculated by (number of fertilization eggs/number of eggs) x 100. In order to determine the common embryonic developments, 30 eggs were taken from each experiment and observed under the microscope (Olympus CK2). Whenever there was an evident difference during the embryonic development, photographs were taken using a digital camera (Olympus SP-350). The time required for the appearance of the first newly-hatched larvae, which would signal hatching out, was recorded.

3. Results

3.1. Induced breeding

Induced breeding using carp pituitary extract was successful in all the four experiments (Table. 1). Spawning of fish started after 8-10 h of post-injection. The fertilized eggs appeared clear, transparent and float on the surface of the water. Unfertilized eggs were opaque and remain suspended or sink to the bottom (fig. 1.a). The rate of fertilization was higher when two males were used (96 ± 1.41) as compared to experiments with a single pair of fish (84 ± 1.41) and the value was statistically significant at 0.05% level of significance ($p = 0.014$).

Table 1: Result of induced breeding and fertilization rate in *Anabas testudineus.* †= average weight of two fish.

Experiment No.	Weight (gm)		Male : Female (Ratio)	Fertilization rate*	Mean*±SD, n=4
	Male	Female			
1	40.5	47.5	1:1	85	84±1.41
2	48.5	50.0	1:1	83	
3	41.0†	42.0	2:1	97	96±1.41
4	40.7†	48.0	2:1	95	

3.2. Fertilized eggs

The development of eggs started with the formation of blastodisc (Fig. 1.b). The eggs were spherical in shape, clear, pearl-like in appearance, buoyant and non-adhesive. The size of eggs ranged between 850-900 µm.

3.3. Cleavage

The first cleavage began at approximately 20 min after fertilization (Fig. 1.c) and completed after 35 min giving rise to the first pair of blastomeres (Fig. 1.d). The second cleavage was observed at 1 h and 5 min (Fig. 1.e) and occurred perpendicular to the plane of first cleavage. The 8, 16, 32-cell stages of cleavage were completed after 2 h and 15 min (Fig. 1.f-h).

3.4. Morula, blastula and gastrula

The embryo continued further division and formed a ball of cells, morula at about 4 h after fertilization (Fig. 1.k). After 4 h post-fertilization, the blastocoel was formed by invasion of the yolk sac. At this stage the cell division becomes less synchronous and difficult to count the cells (Fig. 1.l). At about 5 h the beginning of gastrulation was noted by migration and arrangement of cells of the blastoderm (Fig. 1.m). At this stage, differentiation of embryonic tissue began and by the end of the process three distinct layers were observed (Fig. 1.n).

3.5. Embryonic body formation

The neurula stage became at about 7 h and 30 min – 9 h (Fig. 1.o). At about 9 h and 30 min after fertilization, organogenesis of the embryo was distinguishable (Fig. 1.p). The tail of the larvae was split from the yolk sac 13 h after fertilization (Fig. 1.q). The blood circulation could be observed. The movement of the tail was clearly visible at 15h. Larvae with distinct head, body and tail appeared between 17 h – 19 h (Fig. 1.r). The eyes and mouth though visible were not completely formed.

3.6. Newly hatched larvae

As a result of repeated tail movement against the egg membrane, the larvae emerged free from the egg (hatching). This was observed at about 19 h – 22 h after fertilization (Fig. 1.s). The larvae had round or oval shaped yolk sac. The hatchlings were transparent with some star or branch shaped melanophores distributed on both side of the body and back of the head and somites. The star shaped dark black brown melanophores were present on both side of yolk sac. The body of the larvae was straight, floating on the surface of the water.

Table 2. Time of different embryonic stages after fertilization in *A. testudineus*

Fig. no. 1	Stage of development	Time after fertilization
a	Fertilized egg	0 h
c	Start of cleavage	20 min
d	2-cell stage	35 min
e	4-cell stage	1 h and 5 min
f	8-cell stage	1 h and 25 min
g	16-cell stage	1 h and 50 min
h	32-cell stage	2 h and 15 min
k	Morula	2 h and 40 min – 4 h
l	Blastula	4 h – 5 h and 30 min
m-n	Gastrula	5 h and 30 min – 7 h and 30 min
o	Neurula	7 h and 30 min – 9 h
p-r	Segmentation	9 h and 30 min –19 h
s	Newly hatched larva	21 h – 22 h

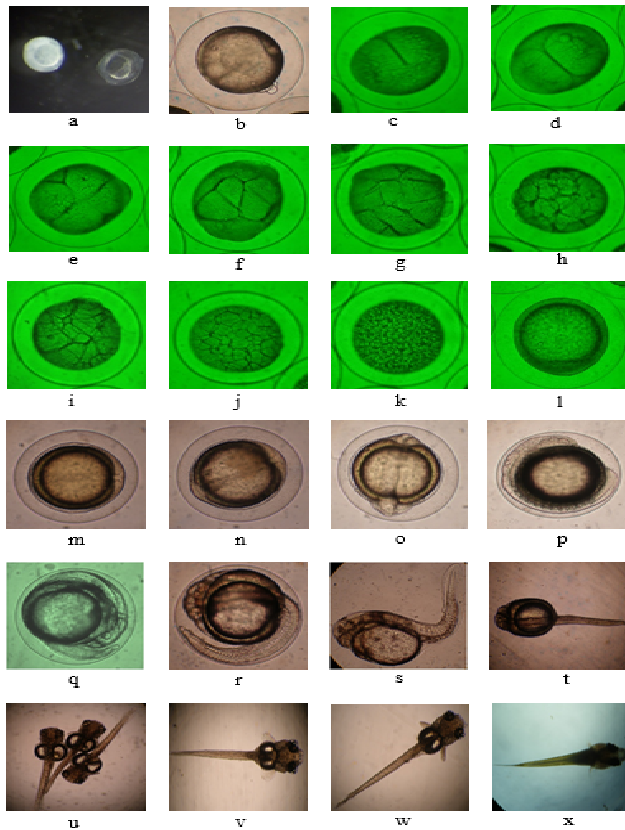

Fig 1: Developmental stages of *A. testudineus*. (a) fertilized and unfertilized egg, (b) blastodisc formation, (c) start of cleavage, (d) 2-cell stage, (e) 4-cell stage, (f) 8-cell stage, (g) 16-cell stage, (h) 32-cell stage, (i) 64-cell stage, (j) 128-cell stage, (k) morula, (l) blastula, (m) early gastrula, (n) late gastrula, (o) early neurula, (p) – (r) segmentation, (s) newly hatched larva, (t) 5 h larva, (u) 30 h old larvae, (v) 2 days old larva, (w) 3 days old larva (x) 10 days old larva.

4. Discussion

The start of cleavage and subsequent divisions vary considerably from the earlier studies on the development pattern of *A. testudineus* reported from Malaysia [7, 10]. Jalilah *et al.,* (2011) [7] reported a time of 30 min for first cleavage in *A. testudineus.* In the present study, the first cleavage was complete within 35 min. Both the results vary considerably from the result of Zalina *et al.,* (2012) [10] where the time taken was 1 h. Similarly, the appearance and completion of subsequent developmental stages including the time of hatching also differs between the earlier result [10] and that observed in the present study. In the present study, 4-cell, 8-cell, 16-cell, 32-cell stages were visualized at 1 h and 5 min, 1 h and 25 min, 1 h and 50 min, 2 h and 15 min respectively after fertilization. The same stages were reported at 2 h, 2 h and 30 min, 3 h, 3h and 30 min respectively after fertilization [10]. The time required for hatching of eggs, observed at 21 h - 22 h also differs with respect to earlier study where the reported time was 20 h [10].

Fertilization rates following induced breeding of climbing perch using synthetic hormones Wova-FH [2], LHRHa [10] and Ovatide [11] were 98.5%, 98.4 ± 1.2% and 90.2 ± 7.1% respectively. A comparative study on the rate of fertilization in climbing perch shows a slightly higher rates for carp pituitary extract (91.3 ± 5.1%) over synthetic hormone, Ovatide (90.2 ± 7.1) [11]. In the present study, the fertilization rate was 96 ± 1.4% when the ratio of male and female was 2:1. This result was higher than the result obtained by Singh *et al.* [11].

This trend of dissimilarity can be attributed to the differences in the environmental conditions, limnochemistry, topography and hydrodynamics [12]. The present study observed fertilization rate of 96 ± 1.41and 84 ± 1.41 while male and female ratio was 2:1 and 1:1 respectively which is as good as those obtained by use of synthetic hormones LHRH [10], Wova-FH [2], ovaprim [8] and ovatide [11].

5. Conclusion

Proper knowledge of induced breeding and early larval development is necessary for the captive culture of climbing perch. The present study gives encouraging result for use of carp pituitary in induced breeding of *A. testudineus*. In addition, the detailed information about the embryonic development of *A. testudineus* have been documented in this article will be helpful to the fish breeders as it will help in timely preparation of pond and feed for the hatchlings. The study also shows that use of pituitary extract for hypophysation can give excellent results.

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