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Captive Breeding of *Anabas testudineus* (Climbing Perch) under Semi-artificial Conditions for the Mass Production of Fish Seed for Conservation and Aquaculture

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ABSTRACT
Background: *Anabas testudineus* is a freshwater perch in tropical and subtropical Asia. Market demand for this fish is high due to favourable qualities in their muscle meat. Nevertheless, availability of this fish in the Sri Lankan market is at a low level due to introduction of exotic species and unavailability of research and development of its aquaculture. Hence, method of mass production of seedlings of this fish has become mandatory for the conservation purposes and to use this fish as a cultured fish in Sri Lankan reservoirs. **Aim of study:** The objective of this study was to develop a semi-artificial breeding technique for *A. testudineus* under local conditions. **Methods:** Matured fish captured from the wild were acclimatized for two weeks. Randomly selected male and female fish were stocked at 1:1 ratio in three different water systems in order to study the possibilities of breeding. Further investigations were carried out by induce their breeding with Salmon GnRHa (Ovaprim®). Seven breeding trials were conducted each with five replicates using seven Ovaprim doses (viz., 2, 2.5, 3, 3.5, 4, 4.5 and 5 mL kg^{-1} b.wt. of fish) and administered as a single intramuscular injection for females. Male fish received half of the dose. Hormone treated fish were kept in aerated glass aquaria. **Results:** It was observed that *A. testudineus* did not breed naturally under captive conditions. Successful ovulation was only obtained with Ovaprim in the group treated with 0.5 mL kg^{-1} b.wt.

Key words: GnRHa, intramuscular injection, ovaprim, ovulation

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INTRODUCTION
Anabas testudineus (Bloch, 92; Climbing perch) is an anabantid which occurs mainly in lakes, swamps and estuaries[1,2], sluggish flowing canals, medium to large rivers, flooded fields and stagnant waters[3] in most tropical and subtropical Asia[4,5,6,7,8]. It is one of the hardiest fish in Sri Lanka's inland waters[9] and locally known as "Kavaiya". They can thrive in oxygen depleted water bodies using their special accessory air breathing organ which facilitates the utilization of atmospheric air for their respiration[10]. Therefore, well known for their ability to migrate overland from one water body to another[9,11,12] and these migrations can be observed frequently during the drought in the Dry Zone of Sri Lanka. *Anabas testudineus* is described as insectivorous[13] or omnivorous[8,9,14] by some authors. They are well known due to taste, high nutritive value and recuperative qualities in their muscle meat. It contains high iron and copper which is essential for haemoglobin synthesis[15]. Therefore it is mainly given to nursing mothers, children and convalescent people in the rural villages of Sri Lanka.

In Sri Lanka, the exotic species, such as tilapias (*Catla catla* and *Labeo rohita*), the Chinese carps (*Oreochromis mossambicus* and *O. niloticus*) and the Indian carps (*Ctenopharyngodon idella*, *Hypophthalmichthys molitrix* and *Cyprinus carpio*) have so far been the major target species for aquaculture. Such introductions of exotics, water pollution and increasing land use for cropping have already instigated the vulnerability of *A. testudineus*. Presently, they can only be collected from the lotic and lentic waters where they breed naturally. High price and market demand for this fish persuade fishers to capture them especially from man-made lakes in the dry zone of the country. However, the major constraint in marketing of this species is the non-availability of this fish in daily fish catches. Therefore, the objective of the study is to introduce an enduring system to produce seeds of *A. testudineus* through

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induced breeding as a solution to supply fish fry to inland reservoirs and fish farmers which will ensure conservation of this species as well.

Several successful studies have been carried out to stimulate maturation and ovulation of *A. testudineus* using various hormone preparations such as Ovaprim[16], Wova-FH[17], LHRHa combination with a dopamine inhibitor (Motillium[18]) and pituitary gland extract[19]. Therefore, Salmon GnRHa (Ovaprim) was used for the present study in order to stimulate breeding of wild-captured *A. testudineus*. It is expected that the information of the present study will also be helpful to develop similar techniques in captive breeding, seed production and propagation of *A. testudineus*.

MATERIALS AND METHODS

Collection of fish: This study was carried out at the Department of Animal Science (Faculty of Agriculture) of the University of Peradeniya, Sri Lanka. Fish were collected from the river "Malwatu Oya" near Anuradhapura city in the North Central Province one month before the onset of Northeast Monsoon rain (December-February) and kept them in cement tanks alfresco for acclimatization.

Induce breeding under natural water conditions: After one month, three different water systems were arranged using 36 cement tanks (area-1×1 m; water depth -30 cm) in order to stimulate the spawning of fish without hormonal manipulation. The fist twelve tanks were provided with water flowing system with some aquatic plants. The second set of twelve tanks was provided with water flowing system without aquatic plants. The third set of twelve tanks was provided with stagnant water with some aquatic plants. Bottom of each tank was covered with fine sand and mud up to 5 cm height. Water was taken by a natural pond located at the Department premises. These tanks were kept for three days to settle silt and mud particles prior to the introduction of fish. Thirty six pairs of matured male and female fish at 1:1 ratio[20] were randomly selected and transferred to these spawning tanks. Hence, each tank occupied one pair of brooder fish. Body weight of each fish was recorded at the time of transfer using an electronic top-loading balance. Total Dissolved Solids and Conductivity were measured with a multi-parameter (HANNA multiparameter meter, HANNA Instruments, USA, HI 991404-01) and Dissolved Oxygen and temperature were measured with HACH Sension Waterproof Dissolved Oxygen Meter (HACH-USA, DO6 5020). All the brooders were fed with live feed twice a day and kept undisturbed. They were observed for spawning for two weeks. The water quality parameters in different systems were analysed using one-way ANOVA and the means were compared at 95% significant level.

Mode of reproduction: *Anabas testudineus* is bisexual and mature in six months[20]. Sexual dimorphism is generally observed with the approach of the breeding season. It is also a seasonal breeder with a long period of breeding. Spawning occurs once a year. Spawning season in different localities of India and Bangladesh commence in April and extends to October[20]. However, it has been observed that in Malwatu Oya, female *A. testudineus* possessed mature eggs from October to April of the year.

Identification of male and female fish: Sexual dimorphism in *A. testudineus* does not distinctively appear[21]. However it is practically possible during the breeding season. Therefore, identification of male and female brooders was done on the basis of some external features (Table 1).

Hormone administration: The use of GnRH analogues in aquaculture serves several purposes; advancing the spawning season, initiating the process of final oocyte maturation and synchronizing the spawning of brood-stock[22]. Under captive conditions, fish often develop mature gonads and gametes but fail to undergo the process of final oocyte maturation and spawning, due to an insufficient surge of GtH from the pituitary. Thus, females may develop vitellogenic oocytes; yet remain incapable of ovulating. Captive stocks which may have not fully prepared for spawning can be induced to ovulate artificially by hormonal injections[20]. Ovaprim (Syndel International Inc., Canada) is a combination of SGnRHa and dopamine antagonist[23,24]. It is one of the most widely acceptable and readily available synthetic hormones because it has been found to be very effective[25,26]. In order to measure the hormone accurately, a stock solution was prepared using the original Salmon GnRHa (Ovaprim).

Table 1: External features used to identify male and female brood fish of *A. testudineus*

Female	Male
Swollen abdomen/girth is distended with the develop ovary[#]	Slender in appearance
Distance between the base of the pectoral fins is greater than the length of the isthmus[##]	Lesser
Eject yellowish eggs following gentle pressure on the abdomen	Eject milt when light pressure is applied on the abdomen
Body coloured and slightly brighter	Body coloured and darker
In the breeding season female exhibits a prominent bulge at the vent resembling genital papilla[###]	This structure is absent

[#]Morioka *et al.*[18], [##]Pehiyagoda[9], [###]Patowary and Dutta[29]

One part of hormone was dissolved in nine parts of saline NaCl (0.7%) and frozen at -20°C until use. One millilitre of stock solution contained 0.10 mL of original Salmon GnRHa. Seven different doses (Table 2) of Salmon GnRHa (Ovaprim) were administered intramuscularly according to their body weight into the dorsolateral region of each fish in a single dose as described by Haniffa *et al.*[27]. The females were treated with full doses of GnRHa (Ovaprim) and male fish were treated with half of the dose at the same time. Thirty-five pairs of fish were carefully transferred to $0.75 \times 0.30 \times 0.30$ m size indoor glass aquaria with sufficient aeration immediately after the hormonal administration. Hence, each tank was occupied by a pair of brood fish. Each dose was tested using five pairs of brooders. Response of each pair of brooder to each dose of GnRHa (Ovaprim) was observed (Table 2).

Fertilization and hatching: Eggs released by female fish were allowed to fertilize naturally by the milt of the male in the same spawning tank. Once complete ovulation was observed, the time taken for ovulation was noted, the male and female fish were removed and excreta if any, was removed by careful siphoning. Fertilized eggs were incubated at room temperature with continuous aeration. The latency period (h), incubation period (h), fertilization rate (%), hatching rate (%), water temperature (°C) and pH were determined (Table 4).

Table 2: Different doses and volumes of Ovaprim administered to brood fish of *A. testudineus*

	Ovaprim dosage			
Experimental group No.	Female fish (mL kg^{-1})	Stock solution (μL)	Male fish (mL kg^{-1})	Stock solution (μL)
1	0.20	200	0.100	100
2	0.25	250	0.125	125
3	0.30	300	0.150	150
4	0.35	350	0.175	175
5	0.40	400	0.200	200
6	0.45	450	0.225	225
7	0.50	500	0.250	250

RESULTS

Breeding under natural conditions: Though three different natural water systems were provided *A. testudineus* did not ovulate under captive conditions. However, each female was found to pair with a male. Nest building characteristic was not observed. Males were more active and aggressive (Table 3).

Ovaries: The ovary of *A. testudineus* is a bilobed structure (Fig. 1) lying just ventral to the air bladder and is attached to the body cavity by a thin membrane. The left ovary is slightly larger in length than that of the right. The well matured ovary is granular in appearance and yellow in colour.

Semi-artificial breeding of *A. testudineus*: All the fish in thirty five experimental units survived after the hormone administration. Induced females were found to ovulate 12-15 h after hormonal administration. It was observed that the male Climbing perch rubbed its body with that of the female and released mint and the eggs

Table 3: Water quality parameters in the three systems

Parameter	Flowing water with aquatic plants	Flowing water without aquatic plants	Stagnant water with aquatic plants
Total dissolved solids (mg L^{-1})	121.00±16.2^{a}	86.00±8.9^{b}	77.00±5.6^{b}
Dissolved oxygen (mg L^{-1})	8.60±1.2^{a}	7.90±1.5^{a}	7.10±2.2^{a}
Conductivity (μs cm^{-1})	242.00±29.3^{a}	120.60±5.1^{b}	107.40±8.7^{b}
pH	8.23±1.5^{a}	8.31±1.3^{a}	8.09±1.1^{a}
Temperature (°C)	24.50±0.6^{a}	24.70±1.0^{a}	25.60±1.2^{a}

Values within rows having differet superscripts are significantly different ($p < 0.05$), Values are expressed as Mean±SD

Table 4: Results of semi-artificial breeding experiment of *Anabas testudineus* treated with different doses of Ovaprim (GnRHa) hormone

	Experiment No.						
Parameters	1	2	3	4	5	6	7
Ovaprim dosage (mL kg^{-1}) b.wt.	0.2	0.25	0.3	0.35	0.4	0.45	0.5
Weight of fish (g)							
Female	55.9±3.3	50.9±3.2	50.5±2.5	49.5±3.3	53.6±1.6	50.9±1.0	51.3±2.5
Male	56.2±1.9	51.7±6.4	53.6±4.0	56.1±2.0	52.0±5.9	53.4±3.6	56.2±1.9
Length of fish (cm)							
Female	15.0±0.5	14.6±0.3	15.1±0.3	15.1±0.4	14.9±0.3	15.2±0.3	15.0±0.4
Male	15.4±0.1	15.0±0.4	15.5±0.3	15.0±0.5	15.4±0.4	14.9±0.4	15.2±0.4
Latency period (h)	Nil	Nil	Nil	Nil	Nil	Nil	14.6±0.15
Fecundity	Nil	Nil	Nil	Nil	Nil	Nil	40220±7676
Fertilization (%)	Nil	Nil	Nil	Nil	Nil	Nil	93.0±10.32
Hatching (%)	Nil	Nil	Nil	Nil	Nil	Nil	87.0±3.9
pH	8.13±0.1	8.30±0.2	8.33±0.3	8.13±0.2	7.90±0.2	8.18±0.2	8.3±0.2
Temperature (°C)	24.5±0.4	24.9±0.2	23.7±0.7	24.6±1.4	24.6±0.8	24.5±1.1	24.7±1.2
Remarks	Not spawned	Not spawned	Not spawned	Not spawned	Not spawned	Not spawned	Spawned

Values are expressed as Mean±SD

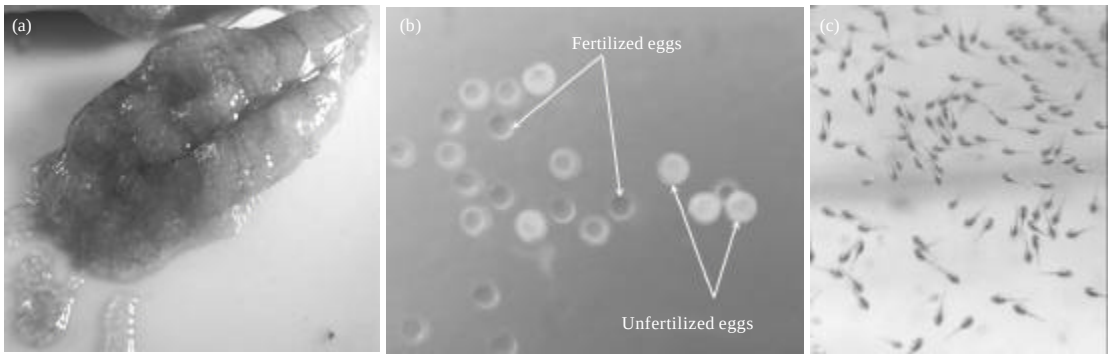

Fig. 1(a-c): (a) Matured ovary, (b) Released eggs after fertilization and (c) Newly hatched larvae of *A. testudineus*

were fertilized externally. Table 4 indicates the average body weight, dose of Ovaprim, latency period, number of eggs spawned, fertilization and hatching rates.

Ova: Eggs were visible to the naked eyes. Fertilized floating eggs were translucent and non-adhesive whereas unfertilized eggs gave an opaque appearance (Fig. 1).

DISCUSSION

Fish breed naturally in their inhabitant waters once they receive the environmental cues above the respective threshold value. Until all the environmental cues are satisfied, they are physiologically not ready for spawning. Pethiyagoda[9], states that *A. testudineus* breed prolifically with the onset of monsoon rains in their natural environments. Failure to spawn in the three different systems (flowing water with aquatic plants, flowing water without aquatic plants and stagnant water with aquatic plants) has left uncertainty of their breeding habits. However, this indicates that flowing water, aquatic plants, mud bottom and aeration of water (as given in this experiment) are not sufficient to induce breeding of *A. testudineus*. The absence of natural breeding infers that there are some other unidentified conditions that affect natural breeding of this fish. The other factors such as flow rate and water quality may also have contributed to the induction of spawning behaviour. Successful spawning in majority of fishes has been induced on cloudy and rainy days, especially after heavy showers[28]. However, during the present experiment, these environmental variables were not considered.

The hormonal signals that initiate ovulation are naturally induced by specific environmental cues. Mature females of *A. testudineus* having stage IV ova were always used to ovulate by using hormones. Fish which were not successful in natural breeding under stimulated water conditions were subjected to different doses of Ovaprim in glass aquaria inside the laboratory. Similar environmental conditions were supplied for all the fish (Table 4). Patowary and Dutta[29] have observed low temperatures of about 28°C and darkness are important factors for spawning of *A. testudineus*. However, complete darkness was not provided in this experiment. Nevertheless, an undisturbed, quiet environment was guaranteed. Moitra *et al.*[19] recommended an optimum ambient temperature of 28.6°C for breeding under laboratory conditions. Morioka *et al.*[18] states that in the region where the climbing perch occurs, the reproductive season is considered as the pre-rainy, the rainy and the post-rainy seasons when the water temperature is relatively higher (>25°C). Nevertheless, Morioka has also observed the presence of sexually well mature females when the water temperature was around 18-21°C and using these broodstock, fertilized eggs were successfully obtained by semi-artificial propagation. However, present study has demonstrated 23.7±0.7 to 24.7±1.21 mean temperatures in experimental tanks. The other factors such as suitable water pH and Dissolved Oxygen levels may also have contributed to the induction of ovulation of *A. testudineus*.

The present study also demonstrated that *A. testudineus* can be successfully induced to produce sperms and ova using an intramuscular injection of Ovaprim (sGnRHa). Stripping of the male fish was not carried out and eggs were allowed to fertilize naturally. Therefore, one can practice this method even at the farm level with limited facilities and knowledge. However, the dosage of Ovaprim required to successfully ovulate was 0.5 mL kg^{-1}. Patowary and Dutta[29] have observed spawners of *A. testudineus* bred 10 to 12 h after the Ovaprim injection at 2 mL kg^{-1} b.wt. Nevertheless, for complete spawning it has taken 14.63±0.15 h in the present study. Jacob[20] states that, Ovaprim diluted in distilled water or 0.6% saline at the recommended dose

of 0.5 mL kg^{-1} b.wt. was ineffective in inducing successful ovulation of *A. testudineus*. However, it was revealed by the present study that *Anabas* did not ovulate at Ovaprim levels below 0.5 mL kg^{-1}. Therefore, the minimum dose of Ovaprim required to induce ovulation of *A. testudineus* is 0.5 mL kg^{-1}.

In the present study, mean number of eggs (40 220±7676) released by a female having a mean length of 15.0±0.4 cm and mean weight of 51.3±2.5 g indicates high fecundity. Marimuthu *et al.*[30] recorded fecundity ranging from 3 120 to 84 690 with a size range of 12.4-19.2 cm (16.13±0.249 cm) in total length and with 33.2-137.1 g in total body weight. Khan and Mukhopadhyay[31] has observed fecundity ranging from 10 002 to 36 477 in size range of 99-166 mm. However, Banerjee and Prasad[32] reported a fecundity of 4 588 to 34 993 in size range of 73-182 mm. Zalina *et al.*[8] has observed mean fecundity ranging from 2 785±411 to 30 499±7935 in size range of 56.1±4.4 g after treat with 2, 20 and 200 µg kg^{-1} b.wt. of LHRHa hormone. Jacob[20] observed *A. testudineus* induced with Ovaprim doses, 1-3 mL and 3-6 mL kg^{-1} b.wt., has resulted 9 804 and 8 792 eggs, respectively. Some of the identified fecundities are more or less deviate from the values observed in the present study. Nevertheless, in general, it indicates a comparatively higher fecundity in *A. testudineus*.

Fertilization was higher than 90% and the fertilized eggs had hatched within 15 h after spawning. Jacob[20] has observed 95.0±5.0 and 93.31±5.0 mean fertilization rates after injecting Ovaprim doses, 1-3 mL and 3-6 mL kg^{-1} b.wt. respectively. Zalina *et al.*[8] has observed 93.9±3.0-98.4±1.2% fertilization rates when treated with 2, 20 and 200 µg kg^{-1} b.wt. of LHRHa hormone. Nevertheless, the fertilization rates observed in the present study (Table 4) is slightly less than the vales observed in the above studies. However, reduced fertilization rates were not attributed to dose or treatment, but rather time to ovulation after initial injection[20]. It was concluded that eggs induced to spawn early in the season had insufficient time to resume meiotic maturation before being ovulated and therefore were incapable of being fertilized.

Jacob[20] has observed hatching percentage of 95.4-98.2%. When treated with 2, 20 and 200 µg kg^{-1} b.wt. of LHRHa hormone, Zalina *et al.*[8] has observed 65.33±2.69, 59.61±2.18 and 56.52±35% hatching rates respectively. Sarkar *et al.*[17] reported a hatching rate of 90.5±3.65[33], 68.57 to 73.11[18] and 100% hatching. The mean hatching rate (86.97±3.88%) observed in the present study is not much deviated from[17,20]. Nevertheless, the hatching rates are better than that of Zalina *et al.*[8]. Very high hatching percentages recorded by Morioka *et al.*[18] might be related to comparatively higher temperature range (27-30°C) observed during the period of the experiment. The temperature observed in the present study was considerably lower (Table 4) during the experiment. As temperature is considered one of the main environmental factors[34] influencing hatching percentage and survival of embryo and larvae[35]. Therefore, it can be the reason for not having very high hatching rate in the present study as observed by[18,20]. However, the success of induced spawning depends on a number of factors, which in most of the fishes are not clearly understood.

CONCLUSION

The present study shows that *A. testudineus* does not breed naturally under captive conditions provided. Their requirements for natural breeding are more complicated and yet to be identified. The attempt on induced breeding however was found effective when Ovaprim (sGnRHa) was injected as a single dose for females (0.5mL kg^{-1} b.wt.) and half dose for males. Ovaprim administration produced high rates of ovulation, fertilization and hatching of normal larvae of *A. testudineus* and breeding was semi-artificial. Stripping of the male fish is not necessary.

In Sri Lanka, most of the native fish species are currently not well utilized for aquaculture. Nevertheless, seeds of *A. testudineus* can be produced semi-artificially at the farm level. The inclusion of this species in Sri Lankan aquaculture can change the current aquaculture systems which depend excessively on exotic fish species.

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