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# Comparative study of analogue hormones and the embryonic, larval and juvenile development on the induced breeding of climbing perch (*Anabas testudineus*, Bloch, 1792)

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**Abstract**
This study showed that average number of eggs produced by the *Anabas testudineus* female using LHRHa hormone (2773 eggs in LHRHa; 393 eggs in SGnRHa) were significantly ($P < 0.05$) higher than those in the SGnRHa hormone group. However, the diameters of eggs were smaller using LHRHa hormone (0.87mm in LHRHa; 1.04 mm in SGnRHa). No significant difference was found in egg fertilization rates irrespective of the treatment. The latency period of the *A. testudineus* was 1 h shorter when using LHRHa. First cleavage occurred at 2.30 h after the egg fertilization. The eggs completed the 128-cell division within 4 h after fertilization and entered the early gastrula stage at 6 h. The embryonic body was formed at 12 h and the fish larvae hatched at 17 – 18 h. The larvae entered the juvenile stage in 16 days and reached maturation in 2 months. This study suggests that LHRHa was more effective than SGnRHa in climbing perch.

**Keywords:** Climbing perch, larval development, LHRHa hormone, induced breeding, SGnRHa hormone

## 1. Introduction
The climbing perch, *Anabas testudineus*, is an omnivorous freshwater species. They feed on a variety of food items such as detritus, aquatic plants, shrimps, worms, mollusks, insects and other fish fries [26]. Owing to the advantages of its robust biological characteristics and wide range of food preference, this species has gained popularity for being a candidate for fish cultures in many Asian countries, especially Malaysia, Thailand, India, Philippines and Bangladesh [7, 29, 34]. *A. testudineus* is an important food fish in Southeast Asia, and usually sold as live fish in markets where it is kept alive for several days by keeping it moist [8, 30]. In Malaysia alone, the *A. testudineus* selling price is RM12 – RM15 per kilogram in markets and RM7 – RM9 per kilogram in farms [15]. For the ornamental fish industry, the retail price in aquarium shop is RM 2 – RM 6 per fish depending on its size and its appearance. According to the Food and Agriculture Organization [10], this species was also introduced to United State of America from Southeast Asia as a potential aquaculture species to the local community, with the market price fetching around US$4 per kilogram [16].
Due to its increasing demand as edible and ornamental fishes, seeds collected from nature are no longer able to satisfy the large scale commercial production. Therefore, induced breeding is the only alternative method for quality and consistent seed supply and production [33]. Various spawning agents have been reported to induce the breeding of *A. testudineus*, such as Wova-FH [32], Ovatide [34], Ovaprim [15], LHRHa (Luteinizing Hormone Releasing Hormone analogue hormone) [7, 38], and the fish pituitary gland [21]. Although various spawning agents have been tested successfully, there is still limited information on comparative study of these hormones for a better production rate. Only a few studies have demonstrated a comparative study of different concentrations of the hormones. Zalina *et al.* [38] compared 2, 20 and 200 μg $kg^{-1}$ LHRHa hormone for induced breeding of *A. testudineus*. Sing *et al.* [34] and Sarkar *et al.* [32] compared 100, 200 and 300 μg $kg^{-1}$ during their respective studies using Ovatide and Wova-FH in climbing perch. So far, no comparative evaluation between hormones has been reported.
It is also important to note that some freshwater fishes produced through induced breeding exhibit morphological abnormalities that could lead to fatality at a later stage [31]. The embryonic stages could be one of the most critical indications of environmental [9, 28],

nutritional [27] and genetic factors [37]. Such physical and biological factors responsible for larval development are important to the successful cultivation of larvae for commercial scale production [35]. Therefore, the aim of the present study is to compare the efficiency of SGnRH and LHRH analogue hormones on the reproduction and breeding behavior of the climbing perch, *A. testudineus*. Details of the different stages, i.e. embryonic, larval and juvenile development, were also observed and addressed in this study.

## 2. Materials and methods

### 2.1. Broodstock management and acclimatization

Nine healthy and sexually mature female and 18 male *A. testudineus* were obtained from a commercial farm in Bestari Jaya, Selangor, Malaysia (3º24'N &101º22'E). Sexually mature females have bodies that are slightly larger and noticeably thicker than mature males. The males are darker in coloration, and their pectoral fins are rough when mature. The genital papillae of the male are rather pointed and narrow in shape [34]. Sexually mature females display a swollen and pinkish color on their genital papilla, and their pectoral fins are smooth [34].

The fishes were transported in ice-packed plastic begs to a fish-bioassay facility at Monash University Malaysia, Selangor within 4 h of sampling. The acclimatization was carried out in a high-density polyether (HDPE) broodstock holding tank (500 L). During the acclimatization period, both females and males were fed twice a day (0900; 1700) with Hikari® floating type fish pellet (Kyorin food Ind. Ltd., Japan), according to 3 % of their body weight. Water in the holding tank was continuously filtered through an overhead filter system (Astro® AS-2099, China) at a flow rate of approximately 1120 L $h^{-1}$. Water quality, such as dissolved oxygen (DO), pH and temperature, were determined using a portable colorimeter (Harch DR/890, Colorado, US); DO at 6.8 ± 1.5 mg $L^{-1}$; pH at 7.5 ± 0.5; temperature at 27.3 ± 1 °C; ammonia-nitrogen at 1.90 ± 0.5 mg $L^{-1}$, nitrate-nitrogen at 0.32 ± 0.8 mg $L^{-1}$, nitrite-nitrogen at 0.097 ± 0.002 mg $L^{-1}$**,** and water hardness at 0.150 ± 0.025 g $L^{-1}$ Na. The photoperiod was set at 12 h: 12 h (light: dark) using automatic timer for aquarium fluorescent lamps (20-Watt, Philips, US). No mortality of fishes was observed during the acclimatization period.

### 2.2. Induced breeding using LHRHa and SGnRHa

The experiment was carried out on 18 males with body weights which varied from 63.33 – 78.98 g and 14.27 – 15.23 cm in total length; and 9 females with body weights ranging from 89.33 – 97.00 g and a total length of 16.07 – 17.56 cm. Two hormone treatments - Salmon Gonadotropin Releasing analogue hormone (SGnRHa) and Luteinizing Hormone Releasing Hormone analogue hormones (LHRHa) (Syndel Laboratories Ltd. Canada) - were used as breeding agents for the fishes in a separate tank (8 L). For hormone injections, a high efficient dosage i.e. 200 µg $kg^{-1}$ was suggested in LHRHa [38], in order to compare breeding efficiency of these two hormones. All free oozing males and ripe female were kept in the ratio of 2:1 in a separate tank for all treatments [32]. The males and females were anesthetized using 68.4 mg $L^{-1}$ Tricaine-S (MS-222, tricaine methanesulfonate) (Syndel Laboratories Ltd. Canada), and subsequently injected with SGnRHa and LHRHa hormones intramuscularly at the posterior end of the dorsal fin. Immediately after hormone injection, the brooders were released into their tank respectively. The control group, which did not receive the hormone administration, was carried out simultaneously. The control fishes were injected with 200 µg $kg^{-1}$ saline (0.85 %, NaCl) and maintained in a separate tank. The fish handling protocol was carried out according to the recommendations approved by the Monash Animal Research Platform (MARP-1) (AEC no.: MARP/2012/117). All experiments were performed in triplicates.

The effective fecundity of each female was determined by a random sampling of the representative egg samples in a 10 mL graduated measuring cylinder from the total eggs released by the females. The total number of eggs in 1 mL were then counted and multiplied with the total volume of released eggs. The fertilization, hatching rates, and survival rates were determined by randomly selecting 100 egg samples and/or 100 larvae from each treatment. For egg fertilization, only eggs with intact nucleus were considered for the calculation. The ova diameter was measured by 20 eggs in a row alongside a measuring scale under a dissecting microscope. The diameter of each egg was calculated based on the total length divided by 20 eggs. Fecundity, fertilization, hatching, survival rates and ova diameter were calculated by using the following formulas [3, 32, 38]:

Number of egg reproduced = Total eggs in 1 mL × total volume of released eggs

Fertilization rate (%) = Total fertilized eggs / total released eggs

Hatching rate (%) = Total hatched eggs / total fertilized eggs

Survival rate (%) = Total survived larvae (until Day-7) / total of larvae (Day-1)

Ova diameter = Total length of eggs (mm) / 20 eggs

### 2.3. Embryonic, larval and juvenile development

The most effective hormone was used to study the embryonic, larval and juvenile development of *A. testudineus*. For embryonic development, 50 fertilized eggs were randomly sampled at 30 min to 1 h intervals until the hatching stage (20 h). The observation and photography were carried out using a microscope (LEICA, Germany), and a digital camera (Olympus PEN E-P3, Japan). Five days after hatching, *Artemia franciscana* (Great Lake *Artemia*, Salt Lake City, Utah, USA) were provided for the larvae in a separate tank. Aeration was provided in the tanks and water was exchanged continuously, with the fresh water filtered through a CPF-15000 pressurized pond filter with built-in UV clarifiers (Grech, US). Fish larvae feeding with *Artemia* was continued throughout the 60 days. For larval and juvenile development, 20 fish larvae were randomly selected from the fish tanks. Total length measurements and images of the samples were taken under the microscope by using a micrometer (Nikon Corp., Japan), and recorded by the digital camera at 2 h intervals until the post larval stage. Continuous observation of the juvenile development was performed on a regular basis for 2 months.

### 2.4. Statistical analysis

The significance of the effects of LHRHa and SGnRHa hormones on the average number of eggs produced, average diameter of the eggs, fertilization, hatching and larval survival were analyzed using the T-test. Differences were considered significant when the *p*-value was $< 0.05$. Statistical analysis was performed using a Statistical Package for the Social Sciences (SPSS) Version 20.

## 3. Results

### 3.1. Comparative induced breeding using LHRHa and SGnRHa

Female broodstock, treated with 200 µg $kg^{-1}$ Luteinizing Hormone Releasing Hormone analogue hormone (LHRHa), showed a better overall breeding performance in terms of latency period, average number of eggs produced and hatching rates compared to the fish group treated with the same amount of dosage of Salmon Gonadotropin releasing analogue hormones (SGnRHa) (Table 1). Male fishes displayed mating behavior after being treated with the hormones. Copulation latency was observed in the first 9 hours in the fish group treated with LHRHa, while an hour delay in copulation was observed in the group treated with SGnRHa. The average number of eggs produced by the female in the LHRHa group was significantly higher ($P < 0.05$) than those produced in the SGnRHa group, accounting for 2773 and 393 eggs per individual respectively (Table 1). However, the eggs were generally smaller (0.87mm in diameter) than those eggs produced by the female using SGnRHa hormone (1.04mm in diameter). The egg fertilization rates in both groups were not significantly ($P > 0.05$) affected by the types of hormones (83.60 – 83.83%). Nevertheless, that is a different case in the hatching rate, noting that the larval hatching rate is the second most important element in the effectiveness of the hormone after the total egg reproduced. The present result shows that the larval hatching rate in the fish group treated with LHRHa had a higher efficiency ($P < 0.05$) at 97.12 % compared to SGnRHa with a mere 42.50% (Table 1). In terms of larval survival rates, no significant difference was found in the groups treated with different type of hormones. The results show that the larval survival rate was recorded as more than 90% in the two different groups.

**Table 1:** Induced breeding of *Anabas testudineus* by Luteinizing Hormone Releasing Hormone (LHRHa) and Salmon Gonadotropin Releasing (SGnRHa) analogue Hormones

| Treatment | Average weight of female (g) | Average length of female (mm) | Average weight of male (g) | Average length of male (mm) | Latency period (h) | Average no. of egg produced (no. $mL^{-1}$) | Average diameter of egg (mm) | Fertilization (%) | Hatching (%) | Larval survival (%) | Remark |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 95.60±21.23 | 17.56±1.24 | 78.98±4.32 | 15.23±1.22 | Nil | Nil | Nil | Nil | Nil | Nil | No spawning |
| LHRHa | 89.33±25.38 | 16.47±0.55 | 63.33±5.05 | 14.95±0.89 | 9 | $2773±200^{a}$ | $0.87±0.03^{a}$ | $83.6±15.9^{a}$ | $97.17±2.3^{a}$ | $93.5±6.2^{a}$ | Complete spawning |
| SGnRHa | 97.00±24.00 | 16.07±1.76 | 71.50±8.83 | 14.27±0.85 | 10 | $393±110^{b}$ | $1.04±0.03^{b}$ | $83.83±15.4^{a}$ | $42.5±3.6^{b}$ | $98.2±1.0^{a}$ | Complete spawning |

### 3.2. Embryonic, larval and juvenile development

#### 3.2.1. Fertilized egg

The observation of different biological development stages was performed using the LHRHa hormone. The diameter of the fertilized eggs ranged from 0.80 – 0.84 mm. The eggs were spherical in shape and the center of the fertilized ovum was yellowish in color. The outer egg membrane layers were clear and pearl-like in appearance; and separated the rest of the egg by a small perivitelline space. The adhesiveness of the egg membrane was apparent at this stage (Fig. 1 a).

#### 3.2.2. Formation of the embryo

The first cleavage occurred at approximately 2.30 h after fertilization. The eggs continued further from the 2-cell stage to 4-, 8-, 16-, 32-, 64- until 128-cells within 4 hours. The eggs entered the early gastrula stage at 6 h (Fig. 1 b). Proliferation of blastoderm was started on the yolk sac. Differentiation of the embryonic tissue began in the late gastrula stage at around 12 h (Fig. 1 c).

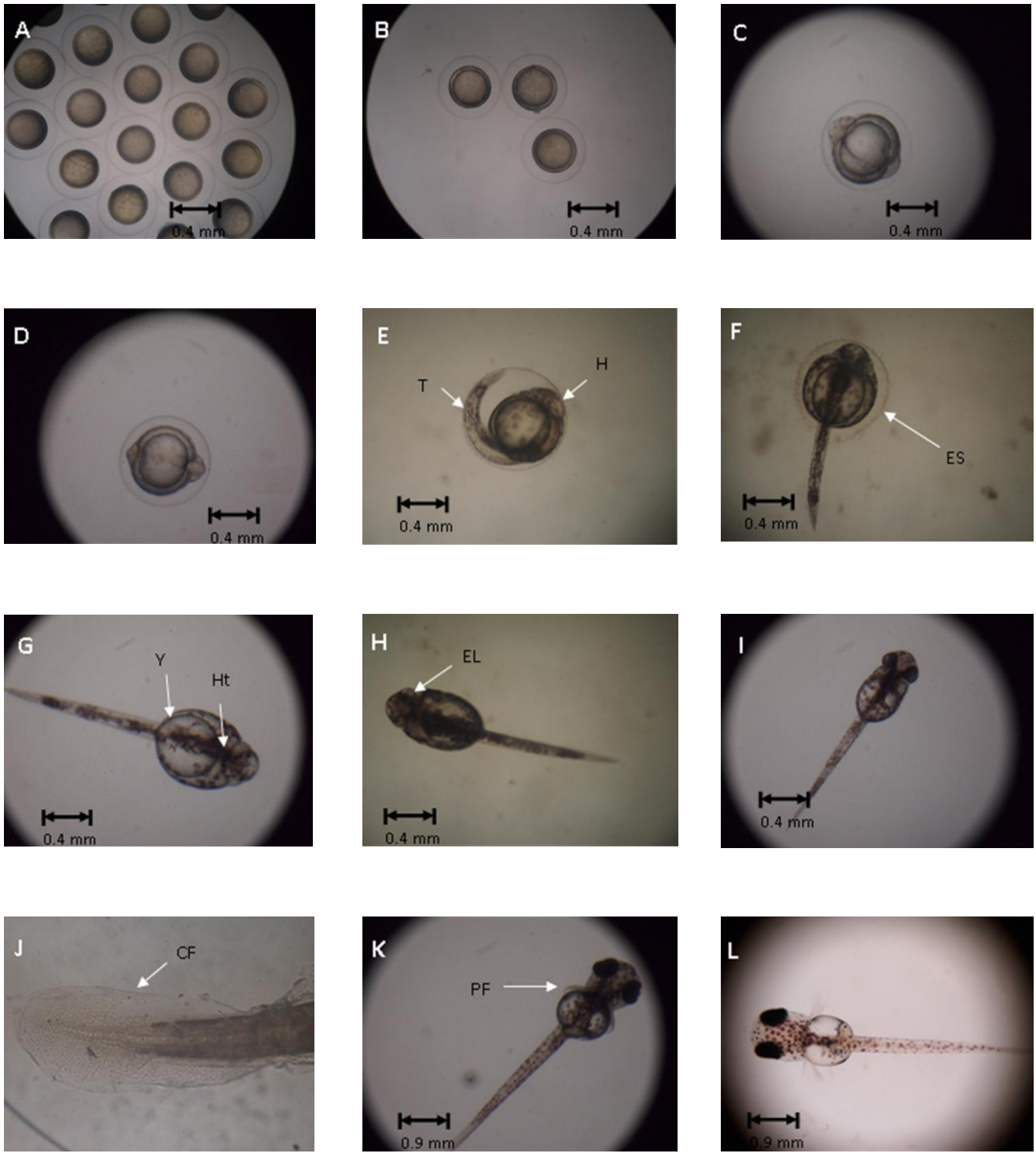
#### 3.2.3. Differentiation of embryo

Bilaterally paired blocks of somites were distinguishable in the early neurula stage (Fig. 1 d). The anterior protuberance formed a head and the posterior part elongated to form the tail. The rudimentary eye vesicles appeared on both sides of cephalic end and Kupffer's vesicle enlarged. Formation of optic bud, brain and optic vesicle, heart and blood vessels was observed at 17 h (Fig. 1 e). The tail part was detached from the yolk and showed some slight movement (Fig. 1 e). Before hatching, a frequent embryonic twitching was observed. This was due to the embryo's attempt to rupture the perivitelline membrane. In successful cases, the egg membrane is broken down from the caudal region and the hatchling emerges from egg capsule at 18 h (Fig. 1 f).

#### 3.2.4. Hatching and post larval development

The newly hatched larva (0.7 – 1.1mm in total length) was transparent with a brownish star- or branch-shaped melanophores distributed across the body. The eyes were un-pigmented, the mouth and fins were under developed, and the digestive duct was thin and tightly attached to the yolk. The diameter of the yolk sac was around 0.5 mm. A functional heart with blood circulation was noticed running from the anterior to the posterior tail of the body. The head and yolk sac appeared to be bulb-shaped when viewed from above (Fig. 1 g). Five to six hours later, pigmented eyes had developed and were sensitive to the direction of light. The larva inhabited the bottom of the tank and swam occasionally using its under-developed tail (Fig. 1 h). A few hours later, a thin membranous fin fold surrounded the caudal region extending up to the yolk sac (Fig. 1 i & j). The larva swam rapidly and dwelled at the edge of the tank with the head and belly upside down. The pectoral fin started to form at the posterior part of the operculum on Day-2 (Fig. 1 k). Blood circulation was clearly observed in the notochord, brain and tail. The blood corpuscles were reddish in color indicating the development of hemoglobin as the mouth began to develop. The melanophores were intensified and scattered on the head, yolk sac, tail and fins (Fig. 1 l). The yolk sac was greatly reduced in size by Day-3 and the mouth was completely developed with upper and lower jaws. The total body length ranged from 6.03 mm (Fig. 1 m). The differentiation of alimentary canals such as the

esophagus, stomach and intestines were nearly completed. At this stage, the larva was starting to feed on exogenous food stuff such as yeast or phytoplankton (Fig. 1 n). The melanophores extended from the head along the trunk to the caudal fin. The soft fins (dorsal, anal and caudal) were clearly defined at these stages. Rays were faintly noticeable at the end of caudal region (Fig. 1 o & p). The reserved yolk was completely absorbed in the further differentiation. Pigmentations were more concentrated in the anterior region, but the density was greatly reduced (Fig. 1 q). The size of the stomach was larger than the intestines and other internal organs appeared as heart-shaped when viewed laterally and the length of the larvae ranged from 8.01 mm (Fig. 1 r). In the post larval stage, the melanophores started to disappear and the body turned a yellowish color (Fig. 1 s). The dorsal and anal fin rays were hardening (Fig. 1 t), and the juvenile was morphologically similar to the adult except for their color, which will become darker with age (Fig 1. u).



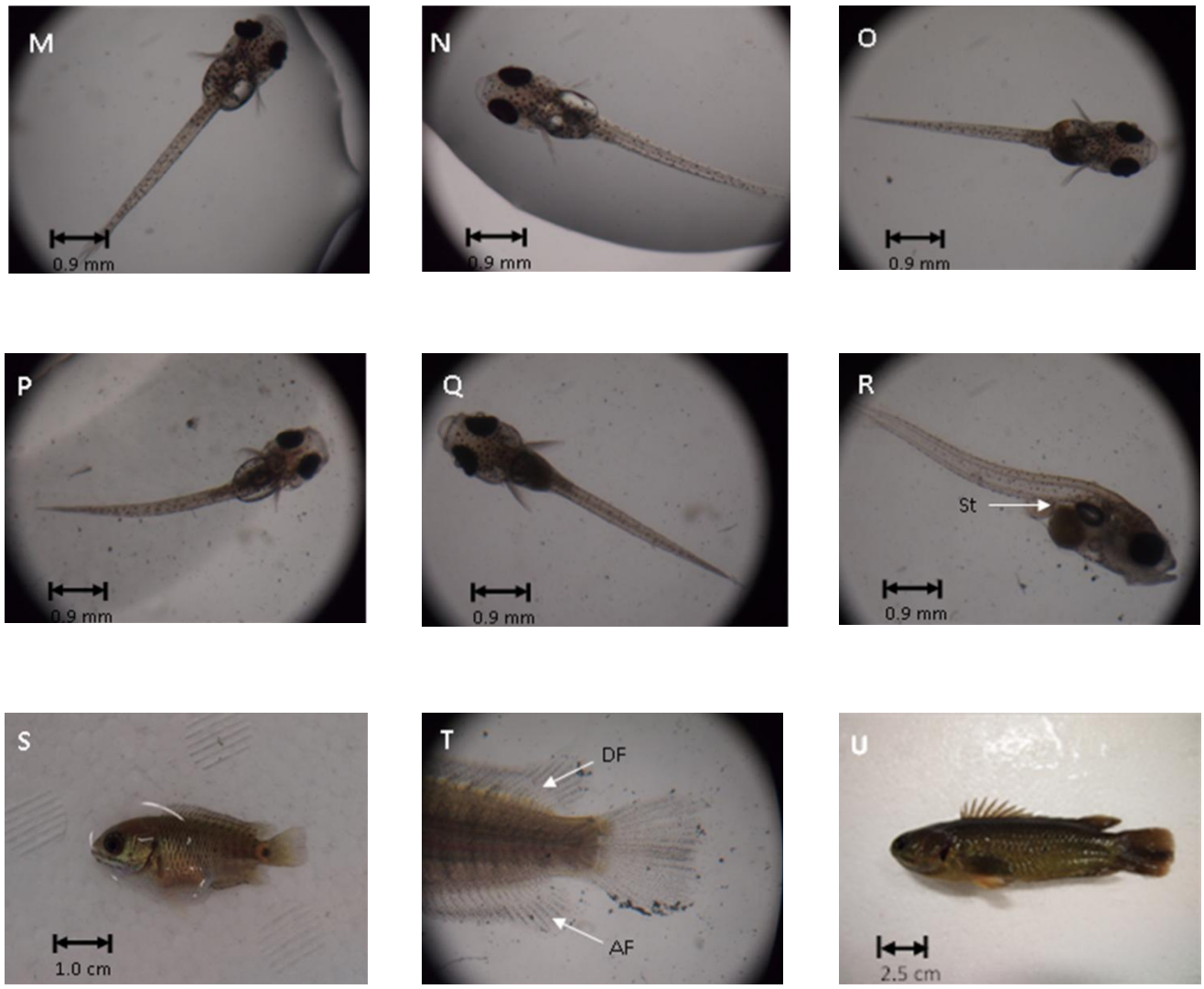



**Fig 1:** Embryonic, early larval development and adult stage of *Anabas testudineus*. (a) Fertilized eggs at 2 h; (b) Early gastrula stage at 6 h; (c) Late gastrula stage at 12 h; (d) Early neurula stage (Head and tail formation) at 13 h; (e) Somite stage (Formation of optic bud, brain and optic vesicle, heart and blood vessels) at 17 h; (f) Pre-hatched larvae at 21 h; (g) Hatched larvae at 24 h; (h) Settlement stage at 30 h; (i) Free-swimming stage at 36 h; (j) Formation of caudal fin (Free-swimming stage); (k) Larvae at 48 h (Pectoral fin formation); (L) Larvae at 60 h (Pigmentation intensified and scattered); (m) Larvae at 72 h (Mouth fully developed); (n) Larvae at 96 h (Yolk size reducing); (o) Larvae at Day-5; (p) Larvae at Day-7; (q) Larvae at Day-12 (Yolksac completely absorbed); (r) Well-developed gastrointestinal system (at Day-12); (s) Juvenile stage; (t) Complete development; (u) Adult stage of male *A. testudineus*. Labels indicate (T) Tail; (H) Head; (ES) Egg shell; (Y) Yolk; (Ht) Heart; (EL) Eye lens; (CF) Caudal fin; (PF) Pectoral fin; (St) Stomach; (DF) Dorsal fin, and (AF) Anal fin. Scale bars indicate various measurements (in mm or cm).

## 4. Discussions

Our study indicated that *Anabas testudineus* broodstock administrated with 200 µg $kg^{-1}$ Luteinizing Hormone Releasing Hormone analogue hormone (LHRHa), showed a better breeding performance when compared to the Salmon Gonadotropin Releasing analogue Hormone (SGnRHa). The hormones LHRHa and SGnRHa have been used in the artificial spawning of many teleost species for many decades [20]. However, the effectiveness of these synthetic hormones is usually dependent on the species and the way they are administered e.g. dosage, time and type of injection. In the present study, adult stages of *A. testudineus* were used to compare the effectiveness of LHRHa with SGnRHa. The present results show that a higher number of eggs and hatching rate were observed in *A. testudineus* administrated with LHRHa. Males and females demonstrated mating behavior 9 h after hormone injections. Similar results were reported in Zalina *et al.* [38]. The authors demonstrated that *A. testudineus* broodstock treated with LHRHa reduced the latency period from 11 h (2.0 µg $kg^{-1}$ of LHRHa) to 9 h (200 µg $kg^{-1}$ of LHRHa). However, when the fishes were administrated with 200 µg $kg^{-1}$ LHRHa, the average amount of eggs produced by a female was found to be lower (2773±200 eggs) when compared to their study (30499±7935 eggs). The egg fertilization rate in the present study also reported to be 12.8% lower than what was reported by these authors (96.04%). Surprisingly, our study showed that the egg hatching rate was much higher (97.17%) than the results reported by the authors (56.52%). The variation of these results may be attributed to different handling methods performed in the two studies. In the present study, hormone administrated brooders were placed together until complete spawning was accomplished, while the stripping method was used to collect eggs and sperm for fertilization as reported in Zalina *et al.* [38]. Alternatively, it may be related to the strains, conditions and size of the brooders in the different geographical areas [36], or attributed to

individual parental investment which, moderated by the food availability, which may have occurred during the spawning period [28]. The egg diameter of 0.87±0.03 mm observed in the present study was similar as that reported by Morioka *et al.* [22] (0.86 – 0.92 mm) using LHRHa as the breeding hormone. The egg diameter was recorded at 1.04±0.03 mm when using SGnRHa and greater than that reported by Sarkar *et al.* [32] (0.60–0.84 mm) using the SGnRHa containing hormone.

Studies showed that several other hormones, such as murrel GnRH [13], *Channa punctatus* GnRH [12], Wova-FH [32], Ovaprim [15] and Ovatide [34] were used in *A. testudineus* to induce breeding. However, some hormones required multiple administrations at least twice to complete maturation and ovulation. The available commercial products such as Ovatide and Ovaprim contain SGnRHa and dopamine (a brain neurotransmitter inhibitor). The GnRH elicits the release of stored gonadotrophins from the pituitary, while the dopamine inhibitor serves to remove other inhibition of GnRH release. Ovaprim yields 80% of egg fertilization and 60% of their hatching, while pituitary gland extract results in 45% fertilization and 25% hatching in *Clarias batrachus*. Nevertheless, a better performance was noticed in *A. testudineus* whereby egg fertilization reached 73.11% and 92.06% of egg hatching [5]. Studies showed that the latency period on mating behavior using Ovaprim was reported at 10 – 12 h in *A. testudineus* [17]; 21 h in *Channa striatus* [11] and 10 – 14 h in *Labeo rohita* [24]. When Ovatide was used in *A. testudineus* breeding, complete spawning was observed at 0.3 ml $kg^{-1}$ of fish, the average egg produced per g of female was recorded at 505.84±7.6, with 90.2% egg fertilization and 92.3% of hatching rate [34]. However, there is a possibility that the brooders will face mortality due to Domperidone's stress contained in the Ovaprim and Ovatide. This compound could decrease the levels of hemoglobin in fish blood, which results in acute anemia if taken in excessive dosage [6]. Therefore, precaution in the application of these products should be taken. The present study showed that LHRHa may be a better candidate as it is more potent than the SGnRHa.

The development of embryonic, larval and juvenile stages in *A. testudineus* that were administrated LHRHa were also addressed in the study. The first cleavage occurred at 2.30 h after the eggs were fertilized, which was an hour later when compared to Zalina *et al.* [38] and Klimogianni *et al.* [18]. According to Jalilah *et al.* [15], the blastomeres were formed after 30 min of fertilization. The formation of the blastomeres was only noticed after 6 – 12 h in this study. Yolk absorption was completed by day-12 in the present study, which was 5 days later than that reported in Morioka *et al.* [22]. The intake of exogenous food did not seem to occur when the yolk was completely absorbed. The larvae started to feed on *Artemia* 2 days prior to the complete absorption of yolk. The energy intake shift from endogenous to exogenous largely depends on the species, development and survival during the larval stages [19, 23]. This was also observed in a number of freshwater fishes, including *A. testudineus* [22].

The larvae of *A. testudineus* had a rapid development of the eyes, mouth and alimentary tracts during the yolk sac stage like those reported in rabbitfish, *Siganus guttatus* [4]. These advantages make it possible for the larvae to feed before the yolk is completely absorbed. Due to the size of its mouth which is only suitable to certain livefeed, the climbing perch larvae is considered a fish species that is hard to rear in the early stages [2]. The food supply is extremely important to achieve a high survival and growth rate in the larval stage. Mass mortality can occur anytime if the food supply is inadequate [14]. Different species and development stages require a different sequence of food during the initial larval stages. In most hatchery practices, the feeding regime for freshwater fishes would mostly begin with smaller zooplankton to larger food items such as rotifer, *Moina* or other zooplankton to artificial diets. Nevertheless, the use of *Artemia* nauplii as starting food item showed promising results in the laboratory rearing scale of the present study. The full development of the jaw and mouth of the *A. testudineus* larvae in 72 h facilitated the feeding process after hatching. Several studies have shown that freshwater fish larvae developed their mouth in a short period of time after hatching. Ogunji and Rahe [25] found that the mouth of crossing Indian catfish, *Heteropneustes longifilis*, opened after only 3 – 4 h of hatching, while Puvaneswari *et al.* [28] reported that the mouths of *H. fossilis* opened after 36 h of hatching. In Malaysian Mahseer, *Tor tambroides*, the upper and lower jaws of the larvae were fully developed after 7 days of hatching [3]. Similar to that reported in Amornsakun *et al.* [2], the authors found that the climbing perch only started to feed on zooplankton at 3 – 15 days-old. After 16 days-old, the larvae was classified as in the juvenile stage and continued to receive an artificial diet as their main food source. Commercial feed pellets (nutritional value: protein, 39%; fat, 7.5%; fiber, 2.4%; ash, 9.9% and phosphorus, 1.6%) were given as a single food source to the juvenile stage of *A. testudineus* in the study. The grow-up stage to adult phase took around 2 months to reach a sexually mature individual.

## 5. Conclusion

The present study concludes that induced breeding of the climbing perch *A. testudineus* using 200 µg $kg^{-1}$ LHRH analogue hormones has a better performance in terms of a shorter latency period, higher average number of egg produced and hatching rates as compared to the SGnRHa hormone. Furthermore, our study provides information on the morphological development during the early life stages of this important species. From an aquacultural viewpoint, these results contribute some basic guidelines for the comparative study of different hormones and important details on embryonic, larval and juvenile development in climbing perch.

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