# EARLY DEVELOPMENT AND LARVAL REARING OF CLIMBING PERCH, *Anabas testudineus* Bloch

By

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**October 2012**

**Chair: Associate Professor Che Roos Bin Saad, PhD**

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*Anabas testudineus* or locally known as '*puyu*' is a freshwater fish species grown in Southeast Asian countries. This study was carried out from March – December 2010 at Aquaculture Experimental Station of Universiti Putra Malaysia. This fish is also known as a species that has a low survival rate during its early life stage and fry. Its Seed production and stock assessment are still poorly understood due the high mortality at first stage of development. In the rearing aspect, high food convertion ratio has been recorded when this fish is reared in hapas and earth ponds using homemade food.

The objectives of this study were to induce breed climbing perch (*Anabas testudineus*) using a commercial hormone preparation (Luteinizing Hormone Releasing Hormone analogue (LHRHa), to observe and record the morphological embryonic development of the *A. testudineus* and to determine optimal stocking density. The first experiment was conducted to

determine its the effectiveness of LHRHa as an agent to induce maturation and ovulation of *A. testudineus* with the intensity level of 2, 20, 200 µg/kg of body weight and saline as a control. The brooder were injected one time and left to spawn in the aquarium tanks in the sex ratio between male and female as 1:1. The parameters observed include fertilization rate, hatching rate, latency period, eggs production and oocytes diameter. For induced breeding, it was found that all intensity of LHRHa hormone level could enhance the fish to breed with the exception of the control group. It was observed that the fertilized eggs of *A. testudineus* were almost spherical in shape, clear pearl likes in appearance and free floating on water surface. Egg production was significantly higher in fish treated with 200 µg/kg as compared to fish treated with 2 and 20 µg/kg of body weight of LHRHa hormone while the highest hatching percentage (65.33%) was recorded in fish treated with 2 µg/kg of LHRHa hormone. There was no significant ($P>0.05$) effect between hormone level on fertilization rate and eggs diameter. The diameters of fertilized eggs ranged from 800 µm-850 µm.

For the second experiment, fertilized eggs were obtain through induced spawning and the development of embryos was monitored by sampling embryos at every 30 minutes to 1 h intervals until hatched. The first cleavage occurred at 1:30 h, epiboly began at 5 h, while the embryonic body was formed at 12 h and hatching occurred at 20 h after spawning at water temperature of 26°C.

Finally the third experiment was conducted to examine the effect of initial larval density of *A. testudineus* on growth and survival at three different

stocking densities of 35, 55 and 75 larvae/L. Newly hatched larvae of *A. testudineus* were produced by induced spawning using LHRHa hormone. Results showed that the survival and growth of *A. testudineus* larvae and fry during 28-day nursing period were stocking density dependent. The highest survival rate 75% was recorded in 35 larvae/L followed by 55 larvae/L (53%) and lastly 75 larvae/L (43%). Water quality parameters like temperature, pH, DO and ammonia ranged from 28.3±0.1°C to 28.3±0.3°C, 8.7±0.1 to 8.8±0.3, 5.7±0.6 to 5.8±0.4 ppm and 0.12±0.22 to 0.18±0.3 ppm respectively, were stable and not influenced by the stocking densities tested.

In conclusion, the use of LHRHa was proven to effectively induce maturation and ovulation in *A. testudineus* and the doses affected the eggs production and hatching rate.

Abstrak tesis yang dikemukakan kepada senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**PERKEMBANGAN AWAL DAN ASUHAN BENIH PUYU, *Anabas testudineus* Bloch**

Oleh

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*Anabas testudineus* atau nama tempatannya *'puyu'* adalah spesies ikan air tawar yang membesar di Negara-negara Asia Tenggara. Kajian ini dijalankan pada Mac-Disember 2010 di Stesen Penyelidikan Akuakultur, Universiti Putra Malaysia. Ikan ini juga dikenali sebagai spesies yang mempunyai kadar hidup yang rendah di peringkat awal kehidupan menyebabkan pembiakan anak benih tidak dapat memenuhi permintaan pasaran. Kajian terhadap hasil pengeluaran anak benih dan bekalan stok masih lagi kurang dikaji kerana berhadapan dengan kadar kematian yang tinggi di awal peringkat pertumbuhan benih. Dalam aspek ternakan, nisbah pertukaran makanan yang tinggi telah dicatat apabila ikan diternak dalam hapa dan kolam tanah dengan menggunakan makanan buatan sendiri.

Objektif kajian ini adalah untuk merangsang pembiakan climbing perch (*Anabas testudineus*) menggunakan penyediaan hormon komersial LHRHa,

untuk memerhati dan merekodkan morfologi perkembangan embrio *A. testudineus* dan mengenalpasti kepadatan stok yang sesuai untuk meningkatkan kadar hidup.

Eksperimen pertama telah dijalankan untuk menentukan keberkesanan LHRHa sebagai agen yang menpercepatkan kematangan dan ovulasi *A. testudineus* dengan kepekatan hormon 2, 20 dan 200 µg/kg berat badan dan larutan salin sebagai kawalan. Induk telah disuntik dengan satu suntikan dan dibiarkan untuk bertelur dalam akuarium dengan kadar nisbah jantina jantan dan betina ialah 1:1. Parameter yang diteliti dalam eksperimen ini adalah kadar persenyawaan, kadar penetasan, tempoh matang ikan, jumlah telur setiap ikan dan diameter oosit. Bagi pembiakan aruhan, didapati semua kepekatan hormon LHRHa boleh merangsang pembiakan ikan kecuali kumpulan kawalan. Juga didapati, telur *A. testudineus* yang disenyawakan adalah dalam bentuk yang hampir sfera, mempunyai warna keputihan mutiara dan terapung dipermukaan air. Jumlah telur setiap ikan ketara lebih tinggi dalam ikan yang disuntik dengan kepekatan hormon 200 µg/kg berbanding dengan ikan yang disuntik dengan hormon LHRHa sebanyak 2 µg/kg dan 20 µg/kg daripada berat badan. Sementara itu, kadar penetasan yang tinggi direkod pada ikan yang disuntik dengan kepekatan hormon LHRHa 2 µg/kg. Kesan antara dos hormon adalah tidak signifikan ($P>0.05$) untuk kadar persenyawaan dan diameter telur. Diameter telur yang disenyawakan berukuran antara 800-850 µm.

Bagi eksperimen kedua, persenyawaan telah dijalankan melalui pembiakan aruhan dan perkembangan embrio dipantau melalui persampelan embrio pada selang masa 30 minit ke 1 jam sehingga menetas. Pembahagian pertama sel telur berlaku pada 1:30 jam, epiboli bermula pada 5 jam, badan embrionik terbentuk pada 12 h dan penetasan berlaku pada 20 jam selepas bertelur pada suhu air 26°C.

Eksperimen ketiga ialah mengkaji kesan kepadatan larva semasa asuhan terhadap pertumbuhan dan kadar hidup *A. testudineus* pada tiga kepadatan stok berbeza iaitu 35, 55 dan 75 larva/L. Larva *A. testudineus* yang baru menetas dihasilkan daripada pembiakkan aruhan menggunakan hormon LHRHa 2 µg/kg. Hasil kajian menunjukkan bahawa kemandirian dan pertumbuhan *A. testudineus* dalam 28 hari tempoh asuhan adalah bergantung kepada kepadatan stok. Kadar hidup paling tinggi, 75% dicatatkan pada 35 larva/L, diikuti oleh 55 larva/L (53%) dan akhir sekali 75 larva/L (43%). Parameter kualiti air seperti suhu, pH, DO dan ammonia adalah 28.3±0.1°C hingga 28.3±0.3°C, 8.7±0.1 hingga 8.8±0.3, 5.7±0.6 hingga 5.8±0.4 ppm dan 0.12±0.22 hingga 0.18±0.3 ppm tiap satunya adalah stabil dan tidak dipengaruhi oleh kepadatan stok yang diuji.

Kesimpulannya, hormon LHRHa telah menunjukkan kesan afektif untuk merangsang kematangan dan peneluran pada *A. testudineus* dan kadar dos juga menunjukkan kesan pada jumlah telur setiap ikan dan kadar penetasan.

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I certify that a Thesis Examination Committee has met on 4 October 2012 to conduct the final examination of Zalina binti Ismail on her thesis entitled "Early Development and Larval Rearing of Climbing Perch, *Anabas testudineus* Bloch" in accordance with the Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Master of Science.

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## DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

ZALINA BINTI ISMAIL

Date: 4 October 2012

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## LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| BW | - | Body weight |
| DO | - | Dissolved oxygen |
| DOF | - | Department of Fisheries |
| FAO | - | Food and Agriculture Organization |
| FOM | - | Final oocytes maturation |
| FSH | - | Follicular Stimulating Hormone |
| GSI | - | Gonadosomatic Index |
| GtH | - | Gonadotropin Hormone |
| GV | - | Germinal Vesicle |
| IM | - | Intramuscular injection |
| LH | - | Luteinizing Hormone |
| LHRHa | - | Luteinizing Hormone Releasing Hormone analog |

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# CHAPTER 1

# INTRODUCTION

Aquaculture is the farming of freshwater and marine organisms such as finfish, mollusks, crustaceans and aquatic plants (Parker, 2012). It involves cultivating aquatic populations under controlled or semi controlled conditions, and can be contrasted with commercial fishing, which harvests wild fish. For centuries, fish have been caught from sea, river and lake. Marine fish have been caught to the maximum level by the fisherman and this contributes to over exploitation. The demand of fish for food and ornamental aquaria is steadily increasing. Natural fish populations have declined during the last several decades because of environmental degradation and over-fishing (FAO, 2004). Due to this, aquaculture must be implemented seriously to supply and replace the caught fish protein demanded by the world population.

Induced spawning is very important in order to expand production of fish seed to meet the increased demand by fish farmers. Many fish spawn in environments that are nearly impossible to be simulated in a hatchery. Induced spawning by hormonal treatment is one of the ways to produce larvae or fry for stocking. Hormone induced spawning is the only reliable method to induce reproduction in most fishes. Hormone induced spawning of fish has been used for almost 60 years (Rotmann *et al.*, 1991). Surprisingly, the same procedures, with only minor modifications, have been used to spawn an entire range of fishes from the ancient sturgeon and paddlefish to

carp, catfish, salmon, sea bass, redfish, snook, and mullet (Rotmann *et al.*, 1991). In addition, induced spawning also can be used to produce hybrids that are different from the parent species, synchronize reproduction of large numbers of fish for simultaneous spawning, thereby simplifying production and marketing of the fish and produce fry outside the normal spawning season for maximum hatchery production (Rottmann *et al.*, 1991). By applying this technique, farmers can provide fish when the price and market demand is greatest, and maximize the survival of fry under controlled hatchery conditions.

Studying the morphological and ecological features of eggs and larvae is important as it is a key issue for improving seed productivity as well as stock assessment (Morioka *et al.,* 2009). Information on early embryonic and larval development is of critical importance in understanding the basic biology of a particular species and their dietary needs and environmental preferences (Borcato *et al.*, 2004; Koumoundouros *et al.*, 2001). Furthermore, studies on embryonic and early larval development are imperative and consequential to the successful rearing of larvae for large scale seed production and aquaculture (Rahman *et al.*, 2004; Khan and Mollah, 1998). Fish embryonic development consists of seven stages leading to hatching. These stages are the zygote period, cleavage period, blastula period, gastrula period, segmentation period, pharyngula period, and finally hatching. There is a scarce literature of climbing perch (*Anabas testudineus*) on early embryonic development stage especially in Malaysia. Studies by Jalilah *et al.* (2011), Hughes *et al.* (1986), Singh and Mishra (1980) and Moitra *et al.* (1986) are

not that detailed and recent study on growth and morphology development on larvae have been reported by Morioka *et al.* (2009).

Various hormone applications have been performed on *A. testudineus* to stimulate maturation and ovulation such as ovaprim (Bhattacharyya and Homechaudhuri, 2009), LHRHa (Superfact) combination with a dopamine inhibitor (Motillium) (Morioka *et al.*, 2009), Wova-FH (Sarkar *et al.*, 2005) and heteroplastic pituitary gland extract (Moitra *et al.*,1986). All of these attempts have been successfully. However, a commonly used hormone (Superfact + dopamine inhibator), ovaprim and Wova-FH for larval production have some limitations due to the factor of high cost and their unsuitability to be injected in brood stock which can causing death. It is important to identify a hormone that is easy to be administered by farmers, more effective in little dose and can produce high production in order to reliably supply of larvae.

Luteinizing hormone releasing hormone (LHRHa) is the name of a mammalian hormone that has been employed successfully to induce the reproductive hormonal. In recent years, synthetic analogues of LHRHa have been developed that are far more effective. As they are purer and are not rapidly metabolized by fish, hormonal analog remains active for a longer period. The use of LHRHa for spawning induction has several advantages over the traditional hypophysation technique. The LHRHa is a small peptide molecule. Thus it can be synthesized into its native form and also into altered forms (analogues) with slow rates of degradation. A lower dose is required when using analogue forms (Zohar *et al.*, 1989). LHRHa has been

successfully used for maturation and spawning of various fish including seabass and rabbit fish (Harvey *et al.*, 1985) and Atlantic salmon (Crim *et al.*, 1984).

Although efforts have been directed towards the morphological and ecological features of larval and juveniles stages, as key issues for improving seed productivity as well as stock assessment, these have not had the expected success (Chaco´n and Rosas, 1995), due to factors such as the high mortality during the first stages of development caused by the lack of knowledge involved in the development process. Therefore, morphological development can provide behavioural information as a part of ecology for larval and juvenile *A. testudineus* using laboratory reared specimen's data during the first weeks with the aim to develop techniques for the intensive culture of the species and to promote optimal management and conservation. Thus hypothesis of this study was use of LHRH hormone will increase the percentage of successful spawning in *A. testudineus*. Thus, the objectives for this work were:

1) To induce breed of *Anabas testudineus* using commercial hormone preparations (LHRHa).
2) To observe and record the embryonic development of *Anabas testudineus*.
3) To determine the effect of different stocking density on the growth and survival of *Anabas testudineus* larvae.