UNIVERSITI PUTRA MALAYSIA

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

ZALINA BINTI ISMAIL

FP 2012 55

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

By

ZALINA BINTI ISMAIL

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

October 2012

Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Masters of Science

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

By

ZALINA BINTI ISMAIL

October 2012

Chair: Associate Professor Che Roos Bin Saad, PhD

Faculty: Agriculture

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

ZALINA BINTI ISMAIL

Oktober 2012

Pengerusi: Profesor Madya Che Roos Bin Saad, PhD

Fakulti: Pertanian

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.

ACKNOWLEDGEMENTS

Foremost, I would like to express my sincere gratitude to my supervisor Assoc. Prof. Dr. Che Roos Saad for the continuous support of my master study and research, for his patience, motivation, enthusiasm, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis. Thanks for generously providing guidance on the technical aspect of this thesis, for continuously encouraging me and pushing me to my limits to complete my thesis this semester, and for all the patience and support you gave me since day 1 of my thesis completion. I could not have imagined having a better supervisor and mentor for my master study.

I would like to express my deep and sincere gratitude to my co-supervisor, Assoc. Prof. Dr. Sharr Azni Harmin and Dr. Annie Christianus. Their wide knowledge and their logical way of thinking have been of great value for me. Their understanding, encouraging and personal guidance have provided a good basis for the present thesis. I owe my most sincere gratitude to Mr. Abdullah Abdul Rahim, for valuable advice and friendly helps. His extensive discussions around my work and interesting explorations in operations have been very helpful for this study.

I would like to extend my thanks to the staff at Aquaculture Research Station in Puchong, Selangor for their kindness in lending their hand during the experiments. Million thanks to Mr. Azmi Yaacob, Ms. Norazlina Nordin, Mr. Mohammad Syahrizan Shaharudin and Mr. Roszainal Yusop for their help.

Sincerely thanks to the Assistant Lab Officer, Mrs. Nur Shafika Maulad Abd Jalil, Mrs. Zaiton Basar and Mr. Jasni Mohd Yusoff for helping me out during the experiments.

I owe my loving thanks to my husband Shaikh Mohd Azhari Shaikh Yusof, without his encouragement and understanding it would have been impossible for me to finish this work. His support, encouragement, quiet patience and unwavering love were undeniably the bedrock upon which the past four years of my life have been built. His tolerance of my occasional vulgar moods is a testament in itself of his unyielding devotion and love. My special gratitude is due to my parent, my sisters and my mother in law for their loving support.

In my daily work I have been blessed with a friendly and cheerful group of fellow students. Sincere thanks to all my friends especially Noor Fazielawanie Mohd Rashid, Nurhidayu Al-saari, Nurul Ashikin Muhammad, Nik Md Azuadi Nik Daud, Norhidayah Mohd Taufek and Mohamad Faizul Mat Isa others for their kindness and moral support during my study. Thanks for the friendship and memories.

Last but not least I would like to express my gratitude to Ministry of Higher Education (MOHE) and Sultan Idris Education University (UPSI) for scholarship and financial support during my studies.

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 Colleges 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 degree of Masters of Science.

Members of the Thesis Examination Committee were as follows:

Mihdzar Bin Abdul Kadir, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Mohd Salleh Bin Kamarudin, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Muta Harah Binti Zakaria, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Ahmed Jalal Khan Chowdhury, PhD
Professor/Deputy Dean
Kulliyah of Science
International Islamic University Malaysia
Pahang, Malaysia
(External Examiner)

SEOW HENG SONG, PhD
Professor/ Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Che Roos Bin Saad, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Annie Christianus, PhD
Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Sharr Azni Bin Harmin, PhD
Professor
Faculty of Science & Biotechnology
Universiti Selangor (UNISEL)
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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

LIST OF TABLES

Table		Page
3.2.4	Treatment and dosage for *Anabas testudineus*	28
3.3.1	Oocytes diameter of female *Anabas testudineus* at different stage of ovulation in treatment (2, 20, 200 µg/kg)	31
3.3.2	Result of captive breeding experiments of *Anabas testudineus* treated with LHRH hormone	33
4.3.	Embryonic characteristic and duration of development in *Anabas testudineus*	42
5.3.2.1	Survival rate and Specific Growth Rate of A*nabas testudineus* larvae nursed in aquaria filled with 2 liters of water for 28 days at three stocking densities	66
5.3.2.2	Weekly mean total length (mm) of *Anabas testudineus* nursed in aquaria filled with 2 liters of water for 28 days at three stocking densities	67
5.3.2.3	Weekly mean body weight (mg) of *Anabas testudineus* nursed in aquaria filled with 2 liters of water for 28 days at three stocking densities	67
5.3.3	Water quality parameters in nursing aquaria throughout the study period	68

LIST OF FIGURES

Figure		Page
2.1	*Anabas testudineus* Production in Malaysia	6
2.2.1	Map of Asia. Circle Showing the Distribution of *Anabas testudineus*	9
2.2.4	Mature Male and Female of *Anabas testudineus*	12
2.6	Newly Hatched Larvae of *Anabas testudineus*	21
3.2.1	Genital Pore for Male and Female of *Anabas testudineus*	26
3.2.3	Germinal Vesicle Stages of *Anabas testudineus* Oocytes	27
4.3.1	The Zygote Stage of Embryonic Development in *Anabas testudineus* about 10 min after fertilization (scale bar = 0.2 mm).Vm–Vitelline membrane; Y- Yolk; Ps Perivitelline space. Figure show the dorsal views only	45
4.3.2	Cleavage Stage of Embryonic Development in *Anabas testudineus* (scale bar = 0.2 mm). Panel (1a)-2 cell; (2a)-4 cell; (3a)-8 cell; (4a)-16 cell; (5a)-32 cell; (6a)-64 cell; (7a)-128 cell. Figure show the dorsal views only	47
4.3.3	Blastula Stage of Embryonic Development in *Anabas testudineus* (scale bar = 0.2 mm). Figure show the dorsal views only	48
4.3.4	Gastrula Stage of Embryonic Development in *Anabas testudineus* (scale bar = 0.2 mm). (1)- Germ ring stage; (2)- 10% epiboly stage; (3)- 25% epiboly stage; (4)- 50% epiboly stage; (5)-75% epiboly stage; gr-germ ring; eb- epiboly; p-polster; tb- tail bud; np-neural plate. Figure show the dorsal views only	50
4.3.5	Segmentation Stage (scale bar = 0.2 mm). (1)-2 somite; (2)-5 somite; (3)-6 somite; (4)-14 somite; (5)-15 somite (6)-20 somite; p-polster; np-neural plate; kv-kupffer's vesicle; ov-optic vesicle; hb-hindbrain (rhombencephalon); fb-forebrain (prosencephalon); mb midbrain (mesencephalon); tb-tail bud; s-somite; cd-cuvierian ducts. a- shows the dorsal view; b-shows the lateral view	52

4.3.6 Hatching Stage (scale bar = 0.2 mm). Panel (1a)-Newly hatch larvae; (2a)-1st day larvae; (3a)-2 day old larvae; (4a)-3 day old larvae; (5a)-4 day old larvae; v.sbin-vt-subintestinal vitelline vein; v.crd.a-anterior cardinal vein; t-tail; pf-pelvic fin; uj-upper jaw; lj-lower jaw. a- show the lateral views only 54

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

TABLE OF CONTENTS

		Page
ABSTRACT		ii
ABSTRAK		v
ACKNOWLEDGEMENTS		viii
APPROVAL		x
DECLARATION		xii
LIST OF TABLES		xiii
LIST OF FIGURES		xiv
LIST OF ABBREVIATIONS		xvi
CHAPTER		
1	**INTRODUCTION**	1
2	**LITERATURE REVIEW**	5
	2.1 Aquaculture Industry	5
	2.2 Biology of Climbing perch *Anabas testudineus*	8
	2.2.1 Distribution	9
	2.2.2 Common Names and Taxonomy	10
	2.2.3 Morphological Characteristic	10
	2.2.4 Sexual Dimorphism	11
	2.3 Reproduction in Fish	13
	2.4 Gonad Development	15
	2.4.1 Gonad	15
	2.4.2 Ovary	15
	2.5 Hormone	17
	2.5.1 Application of LHRHa in Induced breeding	17
	2.6 Induced Breeding	19
	2.7 The Larval	20
	2.8 Effect of Stocking Density on Larval rearing	21
3	**EFFECT OF LUTEINIZING HORMON RELEASING HORMONE (LHRHa) ON REPRODUCTIVE PERFORMANCE IN CLIMBING PERCH *Anabas testudineus* (BLOCH)**	
	3.1 Introduction	24
	3.2 Materials and Methods	25
	3.2.1 Broodfish Selection	25
	3.2.2 Hormone Preparation	26
	3.2.3 Ovulatory Response	27
	3.2.4 Experimental Design	28
	3.2.5 Statistical Analysis	30
	3.3 Results	30
	3.4 Discussion	34
	3.5 Conclusion	37

4 **EMBRYONIC DEVELOPMENT OF CLIMBING PERCH *Anabas testudineus***
4.1 Introduction 38
4.2 Materials and Methods 39
4.2.1 Broodfish Selection 39
4.2.2 Hormonal Injection 39
4.2.3 Experimental Design 40
4.2.4 Observation on Embryonic development 40
4.2.5 Larval development 41
4.3 Results 41
4.3.1 Zygote Period 45
4.3.2 Cleavage 46
4.3.3 Blastula 48
4.3.4 Gastrula 49
4.3.5 Segmentation 51
4.3.6 Hatching 53
4.4 Discussion 56
4.5 Conclusion 59

5 **EFFECT OF STOCKING DENSITY ON GROWTH AND SURVIVAL IN CLIMBING PERCH *Anabas testudineus***
5.1 Introduction 60
5.2 Materials and Methods 61
5.2.1 Broodfish Selection 61
5.2.2 Hormonal Injection 62
5.2.3 Induced Spawning 62
5.2.4 Larval Rearing 63
5.2.5 Statistical Analysis 64
5.3 Results 65
5.3.1 Breeding Performance 65
5.3.2 Survival Rate and Growth 65
5.3.3 Water Quality in Nursing aquaria 68
5.4 Discussion 69
5.5 Conclusion 71

6 **CONCLUSION AND RECOMMENDATION** 72

REFERENCES 74
APPENDICES 87
Appendix A: Feeding Schedule During Rearing period 87
Appendix B: Embryonic Development of *Anabas Testudineus* 88
BIODATA OF STUDENT 93
LIST OF PUBLICATION 94