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SEX STEROID PROFILES, GONADAL HISTOLOGY AND EARLY DEVELOPMENT OF Clarias batrachus LINNEAUS IN CAPTIVITY

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By

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Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

SEX STEROID PROFILES, GONADAL HISTOLOGY AND EARLY DEVELOPMENT OF *CLARIAS BATRACHUS* LINNEAUS IN CAPTIVITY

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Clarias batrachus is a local species in South East Asia and is the most popular in terms of aquaculture and aquarium trade among Asian species. Its excellent nutritional profile and ability to perform in water environments with depleted water quality condition has made it a good candidate for aquaculture. It could accommodate high density stocking and ensure high yield per unit area. The fish is threatened and is getting extinct due to overexploitation of water resource, habitat loss, and unguarded introduction of exotic species such as *Clarias gariepinus* and indiscriminate use of agrochemicals particularly pesticides in the paddy fields which is the popular natural breeding ground of the species. This has made it increasingly difficult to obtain the seed of the species from the wild. Aquaculture is the only platform upon which the extinction of this promising species can be arrested and the fact that hatchery-bred fish has to be induced to spawn due to lack of spontaneous spawning in captivity has made the study of the reproductive biology of this species a necessity. Consequently, reproductive hormonal profile and gonadal development of *C. batrachus* were investigated at the University Putra Malaysia Aquaculture Research Station in Puchong, Selangor, Malaysia from February 2012 to January 2013. The brood stocks for the study were obtained from the wild at Negeri Sembilan. Commercial Enzyme Linked Immunosorbent Assay (ELISA) was used to assay plasma sex steroid hormones, 17β-estradiol, 11-ketotestosterone and testosterone. The range values of 17β-estradiol, 11-ketotestosterone, male and female testosterones were 111.68 ± 3.62 pg/ml (December) to 695.30 ± 8.94 pg/ml (October), 6.61 ± 0.24 pg/ml (September) to 80.27 ± 5.29 pg/ml (August), 26.67 ± 0.98 pg/ml (September) to 209 21 ± 19.49 pg/ml (October) and 7.02 ± 0.06 pg/ml (February) to 49.99 ± 2.17 pg/ml (October) respectively. The monthly values of 17β-estradiol, 11-ketotestosterone and testosterone were significantly different ($P<0.05$) and the profiles of the steroid hormones have exhibited several peak levels during the study period implying the ability of the fish to spawn several times in a year. At the same time several stages of oocyte

development were always found in the ovary while evidence of continuous spermatogenesis was detected in the testes proving that *C. batrachus* is a non-seasonal breeder. Induced breeding using different doses (0.7, 1.0, 1.3, 1.6 and 1.9 ml/kg BW of fish) of synthetic hormone, ovaprim was investigated. Fecundity, ovulation rate, fertilization rate, hatching rate and survival rate were the parameters used to judge the performance of the various doses and 1.0 ml of ovaprim per kg body weight of female fish was found to guarantee the best performance in the induced breeding of *C. batrachus.* The shortest latency period (12hours) on record was recorded in this study on all tested doses of ovaprim. The impact of ovaprim on the plasma sex steroid levels were investigated at different doses and time-intervals (0.7, 1.0, 1.3, 1.6 and 1.9 ml/kg BW at 0, 6, 12, 18 and 24 hours) and saline solution was used as control. The result revealed that, ovaprim was effective in elevating plasma sex steroid levels. Embryonic and larval development of the fish was also evaluated. The developmental events were found to be in conformity with what was reported by several scholars on catfish. However, hatching was observed to be accomplished within 24-30 hours post fertilization. Some individuals were noticed to have absorbed their yolk-sac on the third day but majority fully utilized the yolk-sac on the fourth day. This characteristic has implication on the survival and management of the larva of this species if early mortality and cannibalistic tendencies are to be controlled. The outcome of the study has shown that *C. batrachus* is a non-seasonal breeder which can breed several time in a year.

Abstrak tesis dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PROFIL HORMON REPRODUKTIF DAN PEMBIAKAN

***Clarias batrachus* (Linneaus, 1758)**

Oleh

LAWALI ALKALI ARGUNGU

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Malaysia adalah salah satu negara Asia yang mengalami kekurangan *Clarias batrachus* yang terancam. Oleh itu, profil hormon reproduktif dan perkembangan gonad *C. batrachus* telah dikaji dari Februari 2012 hingga Januari 2013. Asai Imunoserapan Berkait Enzim (ELISA) komersil digunakan untuk mengukur 17β-estradiol, 11-ketotestosteron dan testosteron. Nilai tertinggi untuk 11-ketotestosteron dalam ikan jantan adalah 80.27 ± 9.16 pada bulan Ogos 2012 dan 17β-estradiol dalam ikan betina adalah 698.30 ± 17.40 pg/ml pada bulan Oktober 2012. Nilai tertinggi testosterone untuk ikan jantan dan betina adalah pada bulan Oktober 2012 pada 209.21 ± 33.76 pg/ml dan 49.99 ± 3.75 pg/ml masing-masingnya. Nilai bulanan 17β- estradiol, 11-ketotestosteron dan testosterone menunjukkan perbezaan yang ketara ($p>0.05$). Profil hormon steroid memberikan beberapa puncak sepanjang tempoh kajian mencadangkan kemampuan ikan untuk membiak sebanyak beberapa kali dalam tempoh setahun. Pada masa yang sama, pemerhatian histologi dibawah mikroskopi cahaya menunjukkan kehadiran semua peringkat oosit di dalam ovari, manakala spermatogenesis yang berterusan jelas kelihatan di dalam testis menunjukkan bahawa *C. Batrachus* adalah spesis yang membiak tanpa musim. Prestasi pembiakan *C. batrachus* dinilai menggunakan dos ovaprim yang berbeza (0.7, 1.0, 1.3, 1.6 dan 1.9 ml/kg berat badan ikan) dan larutan salin digunakan sebagai kawalan. Satu betina dan 2 jantan digunakan untuk setiap kumpulan rawatan (N = 54). Ovulasi adalah 0, 67, 100, 100, 100 dan 100%; fekunditi 0, 14792, 29469, 27367, 28519 dan 32522/kg berat badan; persenyawaan 0, 66, 86, 84, 88 dan 90%; penetasan 0, 92, 96, 97, 96 dan 98%, dan kemandirian 0, 54, 85, 79, 76 dan 76 % untuk rawatan kawalan, 0.7, 1.0, 1.3, 1.6 dan 1.9ml/kg berat badan masing-masingnya. Semua parameter ini menunjukkan perbezaan yang ketara ($P<0.05$) untuk semua kumpulan rawatan. Ovaprim dengan dos 1ml/kg berat badan ikan merupakan dos

yang paling kos-efektif untuk pembiakan aruhan *C. batrachus*. Tempoh kependaman adalah 12 jam untuk semua dos yang telah diuji. Ini merupakan tempoh kependaman yang paling singkat pernah direkodkan untuk spesis ini dengan mengunakan agen aruhan. Impak ovaprim ke atas steroid seks plasma dikaji pada dos dan selang tempoh (0.7, 1.0, 1.3, 1.6 dan 1.9 ml/kg berat badan pada 0, 6, 12, 18 dan 24 jam) yang berbeza, dan larutan salin digunakan sebagai kawalan. Keputusan menunjukkan bahawa ovaprim adalah efektif untuk meningkatkan steroid seks plasma. Embrionik dan peringkat perkembangan larva dipantau di bawah mikroskop yang disambung kepada kamera digital. Penetasan diperhatikan berlaku dalam tempoh 24-30 jam selepas persenyawaan. Beberapa individu didapati menyerap kuning telur sepenuhnya pada hari ketiga tetapi majoritinya pada hari keempat. Ciri ini memberi implikasi ke atas kemandirian dan pengurusan larva untuk mengelakkan kematian pada peringkat awal dan kecenderungan kanibal ikan. Hasil kajian menunjukkan bahawa *C. batrachus* berupaya membiak dalam beberapa kali dalam satu jangka masa.

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APPROVAL

I certify that a Thesis Examination Committee has met on 11 July, 2014 to conduct the final examination of Lawali Alkali Argungu on his thesis entitled "Sex Steroid Profiles, Gonadal Histology and Early Development of Clarias Batrachus Linneaus in Captivity" 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 Doctor of Philosophy degree.

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This is to confirm that:

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LIST OF ABBREVIATIONS

ANOVA	= Analysis of variance
BW	= Body weight
E_2	= 17β- esatradiol
ELISA	= Enzyme linked immunosorbent assay
FSH	= Follicle stimulating hormone
G	= Group
GnRH	= Gonadotropin releasing hormone
GnRHa	= Gonadotropin releasing hormone analogue
GSI	= Gonadosomatic index
GTH	= Gonadotropin
GTH 1	= Gonadotropin 1
GTH II	= Gonadotropin II
H & E	= Haematoxylin and Eosin
11-KT	= 11- ketotestosterone
LH	= Luteinizing hormone
LHRH	= Luteinizing hormone releasing hormone
LHRHa	= Luteinizing hormone releasing hormone analogue
N	= Number
OD	= Ova diameter
Pg/ml	= picograms per milliliter
SC	= Spermatocyte
SD	= Spermatid
SEM	= Standard error of mean
SG	= Spermatogonium
SZ	= Spermatozoa
T	= Testosterone

CHAPTER I

INTRODUCTION

1.1 Background of the study

Clarias batrachus is indigenous to Asia and better known in aquarium trade and aquaculture than all other Asian species (Ng & Kottelat, 2008). Khan et al. (2000) noted that the species has been distributed widely and could be found in Malaysia, Pakistan,Thailand, Sri Lanka (Ceylon), eastern India, , Myanmar (Burma),Bangladesh, Indonesia, Philippines and the Singapore. Its introduction in Europe and America through commercial ornamental fisheries and fish farming was reported by Masterson(2007). The qualities of the species such as hardiness, relative promising growth habit, excellent nutritional profile, efficient food conversion, good market value and having better taste and more tenderous flesh than African catfish, *Clarias gariepinus* have removed doubts about its suitability for Aquaculture.

Regrettably, this economically important species is threatened and has started disappearing in some parts of it native environment a situation that is worsening it vulnerability to extinction (Wiecaszek & Krzykawski, 2010). Factors such as habitat loss, over exploitation of the wild resources, Indiscriminate used of agrochemicals and unguarded introduction of exotic species such as *Clarias gariepinus* are implicated for the present plight of *C. batrachus* (Ahmad et al., 2012; Binoy, 2010; Goswami, 2007; Sahoo et al., 2010).

In captivity, inadequate supply of quality seeds and near total reliance on seeds from the wild which is unnecessarily cumbersome, unreliable and uneconomical (Sahoo., 2005) and against the principles of resources conservation, constitute a stumbling block for the successful culturing of *C. batrachus*. To encourage more cost-effective and stable proliferation of the species many attempt were made to induce the breeding of the fish (Hossain et al., 2006; Sahoo et al., 2008; Sahoo et al., 2007; Sahoo et al., 2005; Sharma et al., 2010) using both natural and synthetic inducing agents. Despite the tremendous efforts for artificial propagation of *C. batrachus* it is still seriously facing threat of extinction in some parts of its original environment.

1.2 Statement of the problem

Clarias batrachus holds a lot of promise as an aquaculture candidate and one of the most economically important native freshwater fishes in Malaysia, quite attractive, hardy and rugged with good taste appeal, medicinally valuable and has enormous popularity among consumers. It is worrisome to note that despite many successful attempts to induce the breeding of this wonderful catfish (Hossain et al., 2006; Sahoo et al., 2008, 2007, 2005; Sharma et al., 2010), the supply of the seeds of the species is still very unstable in its native environment and constitutes a major challenge in the aquaculture industry. So far, no adequate study has been carried out on the reproductive hormonal profile, gonadal development and their relationship with the induced breeding of the species. This information will create a better platform for a more redeemable breeding management and workable understanding of the reproduction of *C. batrachus* in Malaysian's ecological environment.

1.3 Significance of the study

The study is envisaged to facilitate the optimization of reproduction conditions and fry production in the culture of *C. batrachus.* The investigation will give opportunity for an accurate prediction of the time of spawning of this commercially important fish species. The single most important drawback of large-scale commercial production of good number of fish species is the deficiency of quality seed of uniform size, free of diseases, parasites, and pests at the time of stocking in the culture enclosures. These basics requirements are hardly satisfied where the seed is obtained from the wild (Mylonas & Zohar, 2001). The outcome of these investigations is envisaged to address this fundamental shortcoming in respect of the culture of *C. batrachus*. It will permit fish farmers or culturist to plan production program well in advance. The nurseries can be stocked with seed of uniform age, size and quality. The study will help in arresting the extinction of the species by bringing its production back to glory through induced breeding in captivity. This may encourage the promotion of culture-based techniques. The availability of the popular fish in the market will significantly bring down the price of the commodity which will go a long way in supporting the food security program of the Malaysian government.

The outcome of the research may provide enabling bases for sterility study on the species through triploidy and hybridization.

1.4 Objectives of the study

The main objective of the current study was to facilitate optimization of the reproduction of *C. batrachus* through data generation that will guarantee better control of the induced breeding program and understanding of the reproduction of the species. This was achieved through the following specific objectives.

1. To determine plasma sex steroid hormones 17β-estradiol, testosterone and 11ketotestosterone in male and female brood stocks of Malaysian walking catfish *C. batrachus.*

2. To observe gonadal changes of female and male fish during the reproductive cycle for *C. batrachus.*

3. To induce breed C. *batrachus* using different doses of synthetic hormone, ovaprim as inducing agent.

4. To determine the effect of ovaprim on the sex steroid hormone levels of *C. batrachus*.

5. To observe and describe the embryonic and larval development of *C. batrachus*

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