UNIVERSITI PUTRA MALAYSIA

A STUDY ON THE INDUCED SPAWNING OF CATFISH (CLARIAS GARIEPINUS)

ANAND ASVESTER UJAN

A STUDY ON THE INDUCED SPAWNING OF CATFISH (*Clarias gariepinus*)

By

ANAND ANAK ASVESTER UJAN

A Project Report Submitted in Partial Fulfillment of the Requirement for the Degree of Bachelor of Bioindustry Science in the Faculty of Agriculture and Food Sciences Universiti Putra Malaysia Bintulu Campus

2007

DEDICATION

Especially to;

My Dad,

Asvester Ujan ak. Daya

My Mom,

Semalau ak. Balom

Beloved My Brother and Sister,

Mary Sugi Asvester , Tony Chaong Asvester,Soda Asvester and Clinton Jemut Asvester

"Thank you for love and support"

and

Special Thanks to My Dear,

Kelly Ak Bantin

"Haleluya……Puji Tuhan……"

ABSTRACT

A study to determine the dosage for hormone ovaprim to be injected to the African catfish (*Clarias gariepinus*) injection frequency that is suitable and efficient was carried out in Universiti Putra Malaysia Campus Bintulu, Sarawak for a duration of seven weeks. In the experiment 27 of catfish broodstock (18 males and 9 females) were used and injected using the hormone ovaprim. A female broodstock (full dose) which have injected will be paired with 2 males broodstock injected (half dose) and then kept in blue plastic tanks which holds up to 500 liters of water. The mating process occurred in 12 hours after injection. There were three dosages of ovaprim tested on each parent catfish (0.25 ml/kg, 0.5 ml/kg and 1.0 ml/kg). For each dose, three replications were done. The results showed that with 1.0 ml/kg dosage that have been injected on the African catfish broodstock achieved, 64.8%-73.2% fertilization rate. Number of eggs produced was also high is 354,000 to 424,500 eggs. Using a dosage of 0.5 ml/kg, average resulted is 55.1%-72.9% fertilization rate. While the dosage 0.25 ml/kg gave the lowest value with a fertilization rate of 53.2%-55.4%. From this study a high dosage (1.0 ml/kg) rate is the most suitable dose for the breeding of catfishes.

ABSTRAK

Kajian menentukan kadar dos hormon ovaprim yang disuntik pada ikan keli Afrika (*Clarias gariepinus*) untuk menentukan kadar dos yang sesuai dan efisien telah dijalankan di Hatcheri Universiti Putra Malaysia Kampus Bintulu, Sarawak selama tujuh minggu. Dalam kajian ini, sebanyak 27 induk ikan keli Afrika (18 jantan dan 9 betina) telah diperlukan dan di suntikkan dengan hormone ovaprim. 1 ekor induk betina (dos penuh)yang telah disuntik akan dipasangkan dengan 2 ekor induk jantan (setengah dos) dan akan diletakkan di dalam tangki plastik biru yang berkapasiti 500 liter. Proses persenyawaan berlaku dalam tempoh 12 jam. Bagi hormon ovaprim, terdapat 3 dos telah diuji ke atas induk ikan (0.25 ml/kg, 0.5 ml/kg dan 1.0 ml/kg). Untuk setiap dos, tiga replikasi disediakan. Keputusan kajian menunjukkan bahawa pada dos 1.0 ml/kg yang di suntik pada ikan keli Afrika mendapati kadar persenyawaan yang tinggi iaitu 64.8%-73.2%. Kadar pengeluaran telur juga didapati adalah tinggi dengan purata 354,000-424,500 biji telur. Diikuti dengan dos 0.5 ml/kg memberikan keputusan yang sederhana dengan kadar persenyawaan 55.1%-72.9%. Sementara dos 0.25 ml/kg memberi nilai yang terendah sekali dengan kadar persenyawaan 53.2%-55.4%. Oleh itu pada kadar dosage yang tinggi (1.0 ml/kg) adalah dos yang effisien dan sesuai untuk disyorkan pada ikan keli Afrika.

ACKNOWLEDGEMENTS

I would like to express my thanks and appreciation to all whose have assisted me in the completion of this research project. Many thanks and gratitude to my supervisor Mr. Aizam Zainal Abidin who have guided me. May god bless him forever. I also would like to express my thanks to Mr. Kamil Latif and Dr. Osumanu for helping and guiding me throughout the duration of this study.

I would also like to thank all the hatchery staff of Universiti Putra Malaysia Bintulu Campus, Sarawak for helping me in my research especially Mr. Jackson, Mr. Ayum Balai and Mr. Bolhari Hasan. They had assisted me in many ways. Where have help me for my daily work. Their contribution have immensely made this research done successfully.

I also would like to express my thanks to my friends Mr. Augustin Jetie, Mr. Alex Jukie, Mr. Kevin Muyang Tawie, Mr. James Chula, Mr. Mensan Blayong, Mr. Benedict Thomas, Mr. Andrew Iling, Mr. Warawit, Mr. Ahmad Murad, Mr. Maxwell Kabu, Mr. Shairul, Mr. Razak and Kak Aida for the contribution and support during my study in Universiti Putra Malaysia Bintulu Campus. May god bless all.

APPROVAL SHEET

I certify that this research project report entitled **"A Study on the Induced Spawning of Catfish *(Clarias gariepinus)*"** has been examined and approved as a partial fulfillment of the requirement for the degree of Bachelor of Bioindustry Sciences, Universiti Putra Malaysia Bintulu Campus.

Name: En. Aizam b.Zainal Abidin
Faculty of Agriculture and Food Science
Universiti Putra Malaysia Bintulu Campus
(Supervisor)

Name:Prof. Dato. Nik Muhammad Bin. Nik Abdul Majid
Dean
Faculty of Agriculture and Food Science
Universiti Putra Malaysia Bintulu Campus

Date: 4/5/2007

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATION

1	**ml/kg**	Mililiter per kilogram
2	**kg**	Kilogram
3	**g**	Gram
4	**cm**	Centimeter
5	**°C**	Degree Celcius
6	**mm**	Milimeter
7	**min**	Minutes
8	**sec**	Second
9	**SAS**	Statistical Analysis System
10	**HSD**	Tukey's Studentized Range
11	**ANOVA**	One-Way Analysis of Variance
12	**%**	Percentage
13	**T1**	Dose 0.25 ml/kg Treatment
14	**T2**	Dose 0.5 ml/kg Treatment
15	**T3**	Dose 1.0 ml/kg Treatment

CHAPTER 1

INTRODUCTION

Catfishes have consumer preference and their culture systems are yet to be established in many countries of Asia. The culture of catfish in Malaysia is limited exclusively to freshwater catfishes. Marine or brackish water catfish culture is non-existent mainly because the hatchery and culture techniques is not available. This fish is a good to be cultured, because of its high growth rate, disease resistance, ability to take up oxygen from the air, etc., yet most local people were not aware of the potential of this fish. There are several species of catfish which is popular in the Malaysian aquaculture industry. The keli kayu (*Clarias batrachus*), keli bunga (*Clarias macrocephalus*) and keli Afrika (*Clarias gariepinus*) are the most important followed by the patin (*Pangasius sutchii*) and baung (*Mystus nemurus*).

Morphologically, catfish have few characteristic that differentiate them from each other. The catfish does not have scales and are usually equipped with pectoral spines. Catfishes have a smooth skin, greenish brown on their upper body and of lighter colour on the lower parts of the body. Their body is quite long and compact while the head is quite flat and their dorsal part is rough. The catfish cultured in Malaysia are categorized into three families namely the Bagridae, Pangasidae and Siluridae. The Bagridae comprises of catfishes such as baung or river catfish. Siluridae includes catfish such as the walking catfish, the *Clarias sp*. There are two species of *Clarias*, namely the *Clarias batrachus* and *Clarias macrocephalus*. These two species differ in the shape of the occipital bone covering the head region. The

culture of Pangasidae is solely represented by *P. sutchi* while Bagridae is only represented by *M. nemurus*.

Catfish culture began in the early 1960s with the culture of the keli kayu (*Clarias batrachus*) on a small scale, mostly involving the fattening of stock procured from rice growing areas. In the early 1970s, the catfish culture in Thailand was plagued with severe disease problems. Since 1981, the Freshwater Fisheries Research Centre, Batu Berendam, Melaka, has undertaken research in the induced spawning of *C. macrocephalus*. In 1982, this institute succeeded in spawning *C. macrocephalus* using homoplastic and heteroplastic pituitary extract.

At about the same time, the African catfish, *C. gariepinus*, was introduced in to Malaysia became popular. This fish is one of the most suitable species for aquaculture in Africa. Since the 1970s it has been considered to hold great promise for fish farming in Africa. This exotic catfish, which was introduced into this country sometime in the early 1980s, was a fast grower, highly resistant to diseases and readily accepted by the local people. The African catfish is an air breathing fish and well adapted to adverse ecological conditions. They normally inhabit swamps, marshy and derelict waters. These water bodies are usually shallow with heavy silt of decaying vegetation and organic load with poor nutrient release. Besides, these water-bodies have low pH, oxygen and primary productivity. On the contrary they have high carbon dioxide, hydrogen sulphide , methane and free ammonia, and this type of adverse environment is quite insensitive to the above air breathing slow growing, hardy omnivorous fishes. CIFA has standardized the following technologies on the breeding, seed production, larval rearing and grow out system of

this precious species. Some success has been achieved with the artificial hybridization of clariid catfish at interspecific and intergeneric levels. At the interspecific level, *Clarias gariepinus* (Burchell) has been hybridized with *C. fuscus* (Lacepede) in China (Zheng *et al.*, 1988) and with *Clarias macrocephalus* (Gunther) in Thailand (Jantrarorai *et al.*, 1994). The African catfish, however, could not match the demand or preference by local consumers for the local catfish. The African catfish retailed for about RM4.00/kg as compared with the *C. batrachus* or *C. macrocephalus*, both of which commanded market price in the region of RM6.00/kg.

The culture of Pangasid catfish began sometime in the early 1980s following the success of hatchery fry production in Thailand. Initially, culture was undertaken in earthen ponds. Cage culture of *P. sutchi* in reservoirs proved to be a more acceptable culture technique for the fish, and was rapidly taken up by the industry. The breeding season of *P. sutchi* is mid-April to mid-June, and September and December- January (Thalathiah *et al.,* 1983). The culture of *M. nemurus* is an even more recent development, beginning only in the mid 1980s. At first, *Mystus* fry was procured entirely from the wild especially rivers, lakes and reservoirs.

Nowadays breeders and fish farmers are interested to invest in this catfish breeding business because of its short breeding time, high marked price and high profitability. Most catfish can attain sexual maturity in captivity. This has provided great impetus to the industry as mass production has been made possible with the application on induced breeding techniques. The availability of fry is a prerequisite to developing a sustainable industry. Research in developing induced breeding catfish, namely *C.*

macropcephalus using pituitary extract has been reported in Thailand (Tongsanga *et al.*, 1963; Sidthimunka *et al.*, 1968) and in the Philippines (Carreon *et al.*, 1973).

As farmers and entrepreneurs become more experienced, and the technique of induced breeding have become more accessible to them, production has subsequently increased. It was estimated that a total of about 5 million catfish fry comprise the *Clarias* species. This production figure is expected to double in the next 5 years due to increasing demand for live catfish locally and abroad, especially in Hong Kong, Singapore and Taiwan.

PROBLEM STATEMENT

Syndel's introduction of Ovaprim in 1987 marked a landmark in improving the ease of application and reliability of the product throughout the world. Ovaprim contains this peptide and a dopamine antagonist. Ovaprim is the most widely used induced spawning product in the world and has been used in virtually every country. Usually, the usage of higher dose (1.0 ml/kg) on fish results better effect in the production of egg (female) and milt (male) compared to lower dose (0.25ml/kg). Some of the broodstock do not react positively when injected with lower doses of ovaprim. Besides that, the major factor in the success of fish spawning is the availability of strong and healthy mature brooders as their health and conditions will affect the quality of eggs or fry produced. As it is the start of the production chain, quality of broodstock is of almost importance in ensuring the success of spawning. The conditions and behaviour of the fish during the domestication process will be closely monitored to minimise stress to the fish as such stress will inhibit their sexual maturity for a certain time. The hormone was tested in Universiti Putra Malaysia Bintulu Campus in order to test its effectiveness in the local climatic and environmental conditions.

1.1 OBJECTIVES

The objectives of this study are:

1. To determine the efficiency of ovaprim hormone for the induced breeding of catfish (*Clarias gariepinus*) in Universiti Putra Malaysia Bintulu Campus.
2. To determine the suitable dosage of the hormone to be injected into the catfish (*Clarias gariepinus*) breeding.

CHAPTER 2

LITERATURE REVIEW

2.1 Fish Reproduction

The most significant advancement in the field of aquaculture during recent decades is the development of techniques to induce reproduction in fish. Those techniques have allowed farmers to gain profit through fish breeding and to manipulate the timing of production to suit production cycles (Mittelmark and Kapuscinski, 2004).

Inducing the reproduction on fishes have been done on many species of fishes including the *Clarias* sp is one of the commercialized fishes and profit can be very promising. The freshwater catfish, *Mystus montanus* which accounts about 10% of commercial landings of air breathing fish in Tamil Nadu, India, is one of the most highly priced fish due to its tender flesh, few bones, and good taste (Jerdon, 1849). Through the statements above, the technology of inducing reproduction can increase profit to *Clarias* breeders (Arockiaraj *et al.*, 2003).

The spawning biomass is employed routinely in stock assessments of fishes as indicators of reproductive potential. The size at first maturity of *Mystus montanus* was 10-11 cm (8-12 g) in male and 13-14 cm (14-16 g) in female, respectively (Arockiaraj *et al.*, 2004).

2.2 Induced Spawning with Hormone Injection

Before this, many researcher doing the research about catfish. One of the research is about the effects of maturational hormone treatment on spermatogenesis of hybrid catfish. The hybridization between two different species *(Clarias macrocephalus x Clarias gariepinus*) for the same group of fishes may produce fertile hybrid F1. In order to improve the spermatogenesis, a series treatment with single hormone of ovaprim, ovaplant, human chorionic gonadotropin (hCG), carp pituitary gland (cPG) and combinations of cPG with ovaprim or hCG were tested. Histological observation showed 25% of total observed testes producing a few sperm, but backcross with eggs of *C. macrocephalus* still gave negative results (Abol-Munafi *et al.*, 2006).

Other research is about induced spawning of the striped murrel *Channa striatvs* using pituitary extracts, human chorionic gonadotropin, luteinzing hormone releasing hormone analogue and ovaprim. The latency period was the highest in pituitary-injected fish but fertilization percentage was the least. The synthetic hormone ovaprim could be recommended for induced spawning in murrel since it produced better results in terms of fertilization and hatching (Hanifa *et al.*, 2000).

The ovopel treatment induced much better effects of spawning, it is particularly important that the application of ovopel to females of greater body weight resulted in a yield of eggs of much better quality (Brzuska, 2001).

In the induced spermentation and milt management in *Pangasius bocourti* (Sauvage, 1880), natural milt production in *P. bocourti* is low but can be rapidly enhanced by a single hCG injection. Because of wide fluctuations in milt production between males, milt from two males should be collected in order to provide sufficient volume for fertilizing eggs from one female (Cacot *et al.*, 2003).

A reliable source of seed supply is the key to success of finfish aquaculture in any species. The increased used of potent synthetic GnRH agonists during the last decade in the induced spawning of a variety of farmed fishes has provided substantial control over seed production. The potency of a GnRH agonist is mainly dependent on its biological stability and is determined by (a) its increased resistance to enzymatic cleavage due to a position 6, which provides a configuration matching the docking site on the receptor ; and sometimes (b) the increased hydrophobicity of some analogs, which enhances interactions with the membrane-bound receptor. In addition, the initial dose of the administered GnRH agonist, fish species, water temperature and stage of gonadal maturity also have a profound effect on the potency (Alok *et al.*, 1999).

Thyroid hormones are known to exert a direct influence on transcriptional regulation of GnRH peptides and on the streoidogenic response of testicular tissue of gonadotropins. Catfish GnRH (cf GnRH) is a crucial neuromodulator involved in regulation of the various aspects of reproduction like spermatogenesis, synthesis of sex steroids and regulation of courting and spawning behaviour in catfish (Kumar *et al.*, 2005)

Pangasius sutchi did not respond to spawning induction using single injection of an analog of luteinising releasing hormone (LRH-A). However, when injected with two sequences of injections at varying dose levels, 33% ovulated with a stimulatory dosage of 20 *u*g and resolving dose of 30 *u*g LRH-A/kg. Dosages lower and higher produced negative results. Trials using LRH-A in combination with homoplastic pituitary extract (HPE) showed promising results (Thalathiah *et al.*, 1986).

2.3 Natural Reproduction

While aspects of the reproductive biology of other *Clarias spesies* are beginning to worked out (Ezenwaji, 1992), breeding in *C. albopunctatus*, including the environmental factors operating and influencing the catfish during spawning and early juvenile migration in the Anambra flood river system, has not been investigated (Ezenwaji, 1998).

The following five factors were considered as potential physical factors involved in inducing the spawning of *S. biwaensis* : daily precipitation, water temperature of Seta River, water level of the river, change of water level of the river relative to the water level of the previous day. Spawning activity was mainly observed from 2200 to 0430, but it continued up to 0600 when it was vigorous. The water temperature on days when spawning occurred ranged from 17.7°C to 30.0° C (Maehata, 2001).

2.4 Mortality Rate

The fertilization, hatching and larval survival rates of parents and crossbreds of *H. longifilis* are important factors to be taken into account when appraising the

crossbreds aquaculture potential. The average fertilization and hatching rate were very high in all genetic groups (Nguenga *et al.*, 2000).

The review of fish genetics and breeding research in Vietnam, using cold shock to induce triploid catfish gave preliminary results of 36.2% larval survival and 48.1% Triploids. Growth and survival of triploid (3n) catfish from fry to adult stages are normal as those of 2n catfish. After 5 months of rearing in grow-out ponds, the final weight of triploid fish was 16.4% higher than that of normal fish (Thien *et al.*, 2001).

CHAPTER 3

METHODOLOGY

The experiment was conducted at Universiti Putra Malaysia Bintulu Campus Hatchery unit. *Clarias* reaches sexual maturity very fast and it is possible to use fish weighing between 500 g - 1 kg (8 months-1 year old) grown in intensive systems. Healthy males and females were selected by external morphological characteristics and hand stripping (Billard *et al.*, 1984). Three dose levels were tried of ovaprim: 0.25 ml/kg, 0.5 ml/kg and 1.0 ml/kg body weight for females and half dose for males. This fish was induced to spawn by a single intramuscular injection of hormone. Each breeding set consisting of two males and one female was released into a breeding tank after injection. Spawning was observed 12 h after injection. The step of catfish induced spawning were described below ;

3.1 Injection of Female Spawners

Selected mature females with a well distended, swollen abdomen from which ripe eggs can be obtained by slightly pressing the abdomen toward the genital papilla. The method of administering the ovaprim hormone solution is by intra-muscular injection into the dorsal muscle. Females are injected in the evening and are kept separated from the males in the tank 500 liter. The step of injection of female spawner is described in Figure 1. Fill the syringe, insert the needle 2.5-3.0 cm long and 0.6-7 mm diameter and empty the syringe again into the mortar. Then cover the head with a wet towel in order to keep it quiet during injection, when this is possible insert the needle 2-2.5 cm at angle of 30-45° into the dorsal muscle in the direction of the tail. This procedure has to be followed always, as the needle often gets

blocked if the pituitary material is not completely crushed and it is unpleasant for the fish and annoying for the operator to resolve this problem once the needle is inserted into the fish. After the injection finger-rub the injected area, so that the suspension will be distributed evenly trough out the muscles. Put back the fish in the tank (500 liter) and wait about 12 hours until all eggs have maturated and ovulated in the ovary.

Figure 1: Injection of spawners

3.2 Collection of milt

The males of catfish cannot be stripped and consequently the sperm can only be obtained by sacrificing a male. The male is killed on it back and open the body cavity with pair of scissor (Figure 2). Do this carefully without damaging the inner organs. After that, pull the intestine aside to make yellow-pink testes visible. Between the testes and the urogenital papilla several wormlike lobes can be noticed. They do not contain milt and cannot be used. Entirely remove the two testes without squeezing them and dry them with a piece of filter paper. 30-60 seconds after

contact with water, the milt will lose its activity. It is important that the testis do not come into contact with water. Most of the ripe milt is located in the cream-coloured lobes. The surgically must make with a pair of scissors small incisions in the lobes and squeeze the milt out. Take care that fingers are dry during the preparation of the milt.

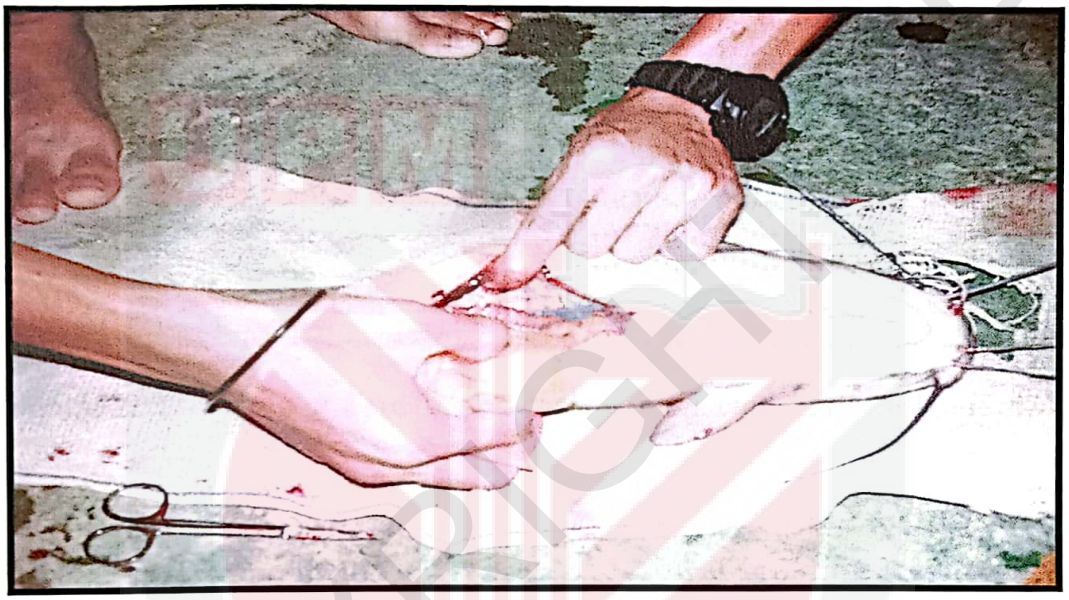

Figure 2: Collection of Milt from Male Spawners

3.3 Stripping of Female Spawners

Stripping of the female spawner is carried out by gently pressing their abdomen with a thumb from the pectoral fin towards the genital papilla. Ovulated eggs will flow out easily in a thick jet from the genital vent and are usually collected into a dry plastic container. An average of 4012 ± 100 eggs were spawned by each female. Do the strip gently the fish till some blood appears. This is often a sign that the ovary is empty. The mingling from the blood with the eggs should be prevented. Estimate the weight of the egg mass (1 g egg mass contains about 700 eggs) to obtain the number

of eggs that can be fertilized. Do not use more than 200 g of the collected eggs for the fertilization.

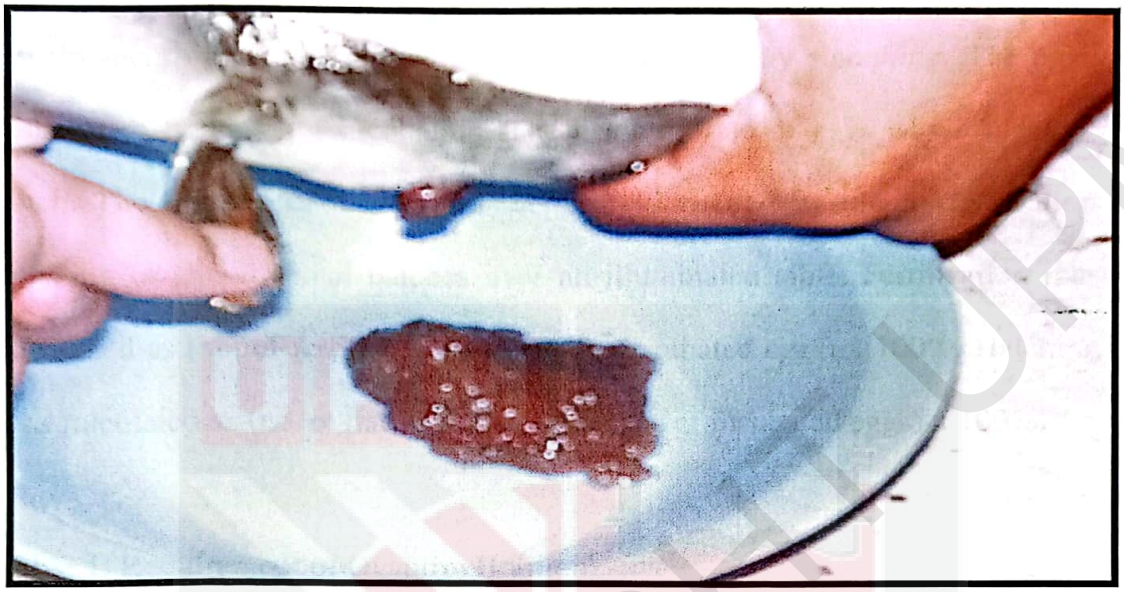

Figure 3: Stripping of Female Spawners to Collected Eggs for the Fertilization

3.4 Fertilization of Eggs

The artificial fertilization of eggs should be squeeze the freshly dissected testes and distribute the droplets evenly on top of the egg mass. The same method should be applied if stored chilled milt were used. The eggs and the semen were mixed using a feather for 5-10 minutes (Figure 3). After the mixing have completed, the eggs were washed using clean water or rain water and then kept in the tank for hatching.

3.5 Incubation of Eggs

The development process from fertilized egg to hatching is dependent upon water temperature. The higher the water temperature the faster the eggs will hatch but the suitable temperature is 27°C-29°C. During this time the eggs should be regularly monitored. Healthy developed egg will have a transparent green-brownish colour. If

all eggs turn a white colour, the batch should be discarded. White eggs, which are always present amongst the developing ones, should be removed to avoid the development of fungi. Removing can be easily done by siphoning. The eggs will hatch after 12 hours of incubation. The hatching rate of eggs was evaluated after hatch. The number of hatched larvae was determined by direct observation and counting of experimental batches over an illuminated table. Fertilization rate was calculated as (no. of fertilized eggs/total no. incubated eggs) x 100%. Hatching rate was calculated as (no. of hatched larvae/total no. of incubated eggs) x 100%.

Table 1: Requirement of Ovaprim Hormone

Dosage of hormone	♀	♂
0.25 ml/ kg	3	6
0.5 ml/ kg	3	6
1.0 ml/ kg	3	6
Total	9	18

Note : ♀ = female ♂ = male

3.6 Experimental Design and Statistical Analysis

Fertilization rate and hatching rate were compared using a one-way analysis of variance (ANOVA) followed by Tukey's Studentized Range (HSD) test to determine significant differences ($p \leq 0.05$) among means of treatment for the treatment with. The statistical software used was the Statistical Analysis System (SAS).

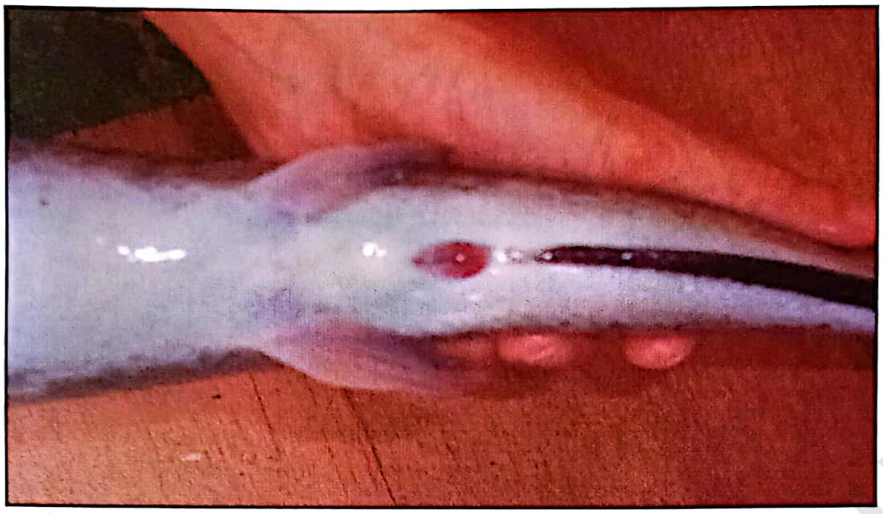

Figure 4: Female African Catfish

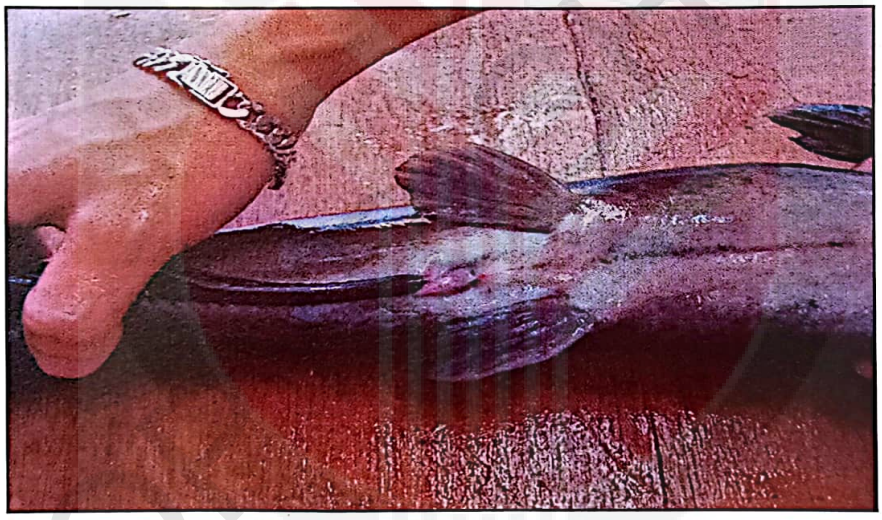

Figure 5: Male African Catfish

CHAPTER 4

RESULTS

4.1 Ovulation Comparison Based on the Three Dosages Used

The results of the breeding trials of *Clarias gariepinus* are summarized in Table 2. It was observed that early spawning (latency period is 12 hour) occurred in the fishes injected with the ovaprim dosages of 0.25 ml/kg, 0.5-ml/kg and 1.0 ml/kg body weight respectively. The three dosages resulted in complete spawning. Generally, at the 1.0 ml/kg dosage produced the highest number of eggs which ranged from 354,000-424,500 eggs and the fish weight was between 900 g-1.0 kg. This dosage resulted in the highest compared with other treatments. At 0.5 ml/kg dosage produced 315,000-343,500 eggs with the female fish weight ranging from 800 g-1.0 kg. When the dosage was 0.25 ml/kg produced 107,000-310,500 eggs with the female fish weighing from 500 g-1.0 kg.

Table 2: Average Eggs Produced by the Female Fish According to 3 Ovaprim dosages

Treatment (Dose)	Replication	Weight of female	Mean
0.25 ml/kg	1	500.0 g	310,500
	2	1.0 kg	107,000
	3	600.0 g	297,000
0.5 ml/kg	1	1.0 kg	315,000
	2	900.0 g	337,500
	3	800.0 g	343,500
1.0 ml/kg	1	900.0 g	424,500
	2	900.0 g	409,500
	3	1.0 kg	354,000

Figure 6: Mating Process

Figure 7: The Eggs and the Semen were Mixed Using a Feather

Table 3: Fertilization Rate (%)

Treatment	Replicate (%)			Total	Average (%)
	1	2	3		
T1 (0.25 ml/kg)	60.0	54.5	50.5	165.0	55.0
T2 (0.5 ml/kg)	65.0	59.9	73.7	198.6	66.2
T3 (1.0 ml/kg)	72.4	73.5	70.7	216.6	72.2

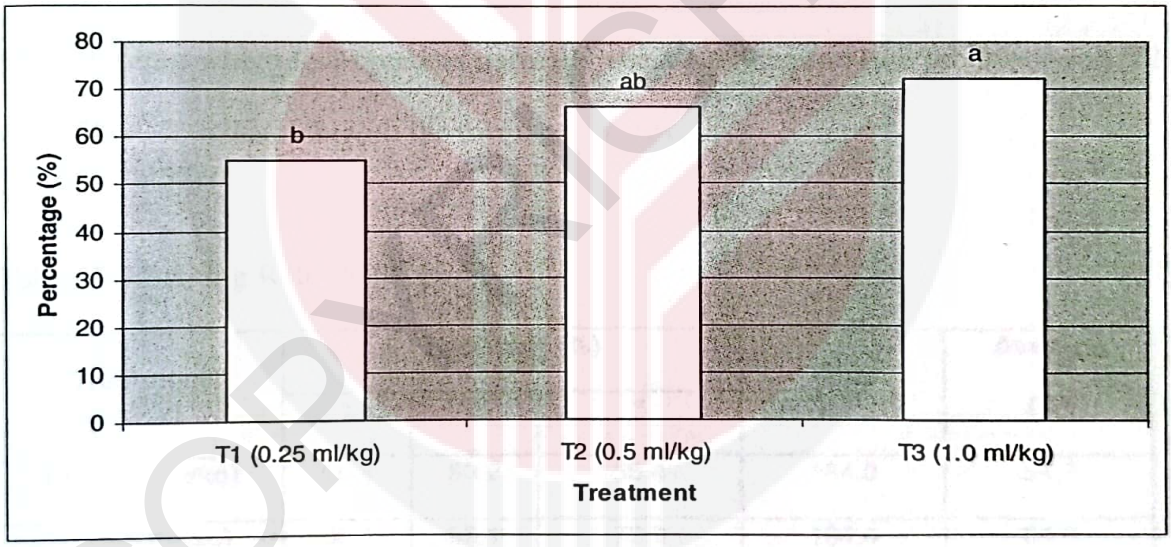

Figure 8: Fertilization Rate (%)

Note: Means with the same letter are not significantly different.

4.2 Comparison among Fertilization at 3 Dosages Used

After the eggs from the female broodstock are being mixed with the sperm from the male for about 15 minutes, the egg undergoes fertilization. Treatment 3 (T3) Table ; Figure 8 (1.0 ml/kg) produced the highest fertilization rate between 70.70% to 73.50% compared to the other treatments. Overall, the total means by using (1.0 ml/kg) T3 was 72.20 % Tukey's test was performed it was found that T3 showed significant difference (P≤0.05) with T1. T1, which was 0.25 ml/kg was the lowest dosage injected into African catfishes. At this level, fertilization rate produced were at a range of 50.50% to 60.00% with a mean of 55.00%. For T2, it showed no significant difference (P≤0.05) with T1 and T3. T2 were the intermediate with a rate of 0.50 ml/kg which fish breeders normally used. Fertilization rate produced was T2 were 59.90% to 73.70% with the mean of 66.20%.

Table 4: Hatching Rate (%)

Treatment	Replicate (%)			Total	Average (%)
	1	2	3		
T1 (0.25 ml/kg)	55.4	53.2	55.4	164.0	54.7
T2 (0.5 ml/kg)	66.4	55.1	72.9	194.4	64.8
T3 (1.0 ml/kg)	73.2	69.5	64.8	207.5	69.2

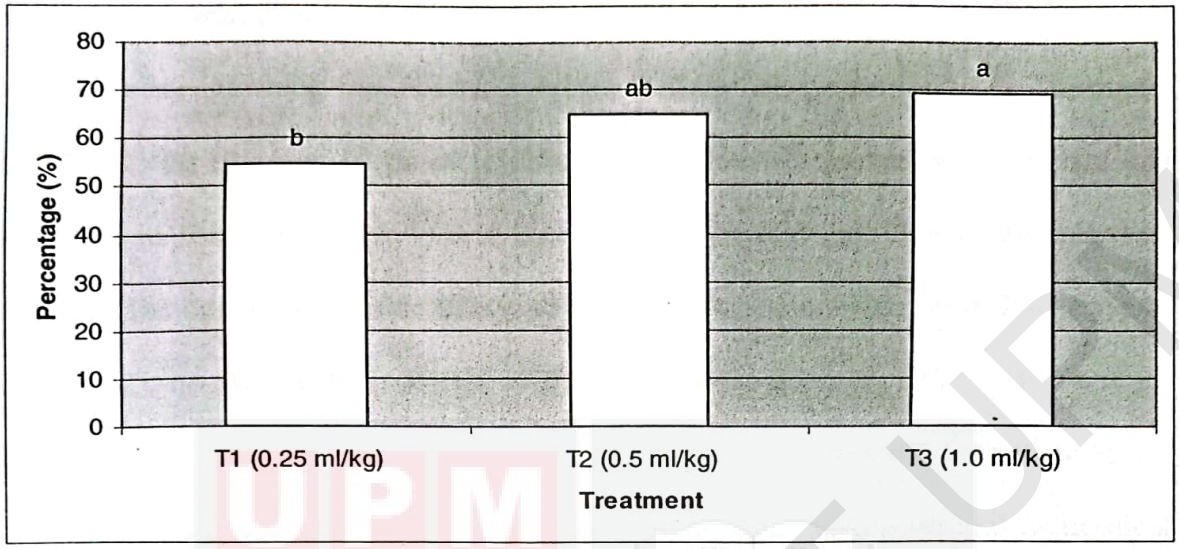

Figure 9: Hatching Rate (%)

Note: Means with the same letter are not significantly different.

4.3 Comparison among Hatching at 3 Dosages Used

The changes in color of eggs and other characteristics were noticed during the embryonic development. The eggs were grayish-white in color, spherical in shape at the beginning (0–2 days), elongated shape and distinctly visible form of larvae. Figure 9 and Table 4 shows hatching rate at 3 different level of ovaprim. At T3, dose 1.0 ml/kg produced high hatching rate at the ranged of 64.80% to 73.20% with the mean was 69.2%. In addition, T3 was found significantly difference ($P \leq 0.05$) with T1 at a dosage of 0.25 ml/kg. At T1, the hatching rate was the lowest compared to T2 and T3. The hatching rate for T1 were at a range of 53.20% to 55.40% while the mean was 54.7%. For T2, which had a dose of 0.50 ml/kg, there were no significant difference ($P \leq 0.05$) for T1 and T3 using Tukey's test. T2 produced a total mean of 64.80% with an average hatching rate of between 55.10% to 72.90%.

4.4 Development Stages of *Clarias gariepinus*

In this study, spawning was observed within 12 hour after injection of the hormone. Fertilized egg (Figures 10.1); of *Clarias gariepinus* were adhesive, demersal and spherical in form. The yolk sphere contained no oil globule. Due to the adhesive nature of the egg, considerable debris adhered to the capsule of the egg. The grayish-white egg capsule was transparent, while the yolk was greenish or brown in colour. The developmental stages of *Clarias gariepinus* were divided into 4 stages : embryo, hatchling, larva and post-larva with each stage having typical anatomical and physiological features. The Ontogenic events in the early development of C. gariepinus is shown in Table 5.

Early Embryonic Development of *Clarias gariepinus*

Table 5: Ontogenic Events in the Early Development of *Clarias gariepinus*

Age (hours)	Ontogenic events
0.20 – 1.0	2 – 16 cell stage.
1.0 – 1.5	Morula stage.(Figure 10.5)
1.5 – 2.0	Morula second stage.(Figure 10.6)
2.0 – 2.5	Blastula stage.(Figure 10.7)
3.0 – 5.0	Germinal ring formed; embryonic shield formed; more than half of yolk invaded.
7.0	Gastrula stage.(Figure 10.8) Yolk invasion two-thirds complete.
8.5	Yolk invasion complete; yolk – plug stage almost complete.
10.0	Embryonic rudiment distinct.(Figure 10.9) 2 – 3 myomeres ; eye vesicles demarcated.
12.0	7 – 8 myomeres ; heart rudiment visible ; demarcation of brain.
14.5	12 – 17 myomeres ; head and tail differentiated.(Figure 10.10)
17.75	Entire space inside egg occupied by embryo; heart beat visible; tail beginning to separate from yolk; blood circulation commenced; embryo making frequent movements.
22.0-24.0	Hatching begins.(Figure 10.13)

Figure 10: Developmental Stages of *Clarias gariepinus*

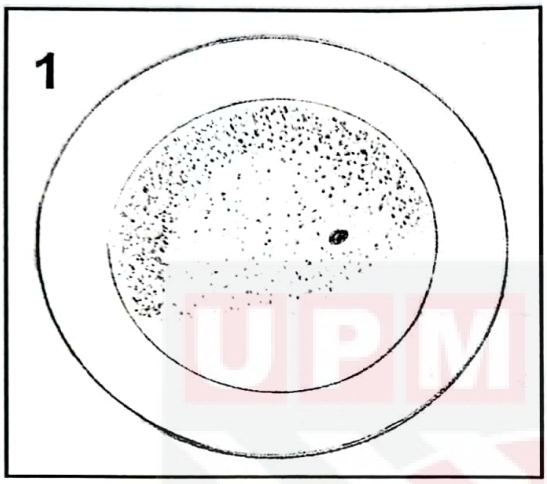

Figure 10.1: Fertilized Egg

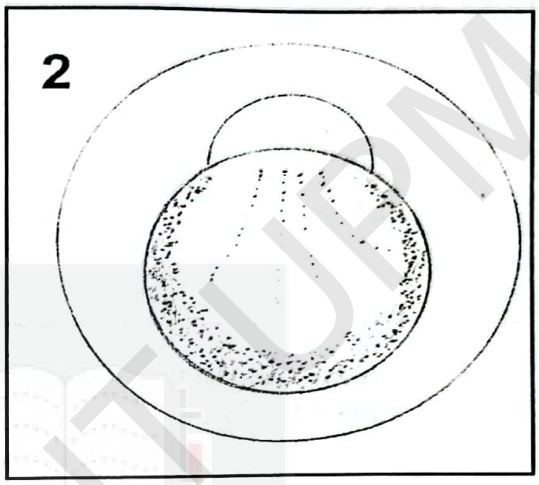

Figure 10.2: The Egg Enlarges (20 min)

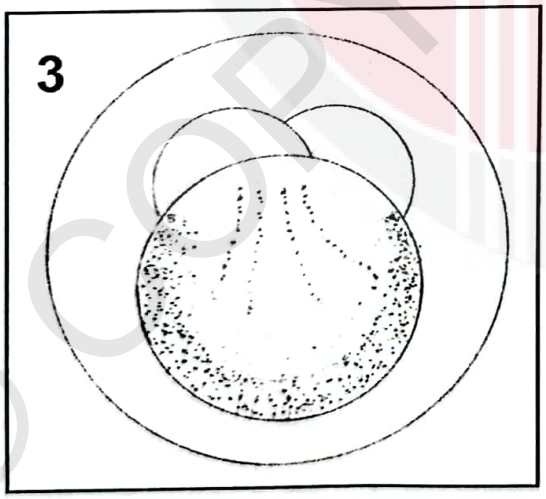

Figure 10.3: First Mitotic Division (22 min)

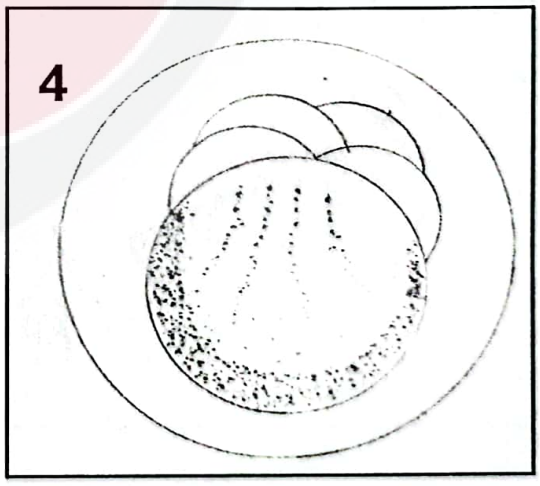

Figure 10.4: 4 Cells Stage (25 min)

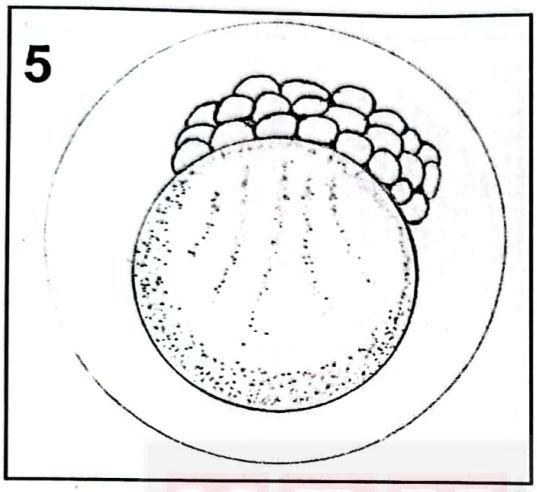

Figure 10.5: Morula Stage (early stage)
(1.0-1.5 hour)

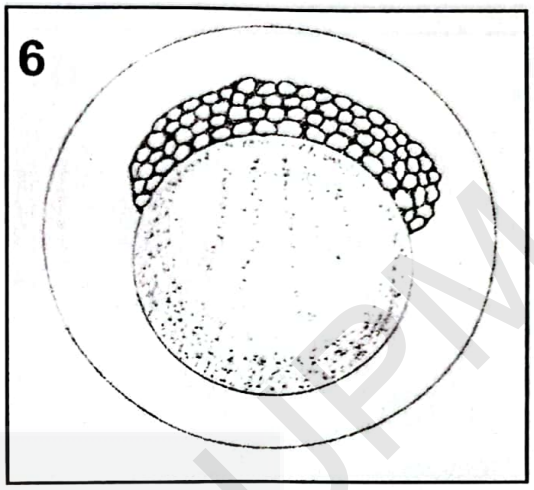

Figure 10.6: Late Morula Stage
(1.0-2.0 hour)

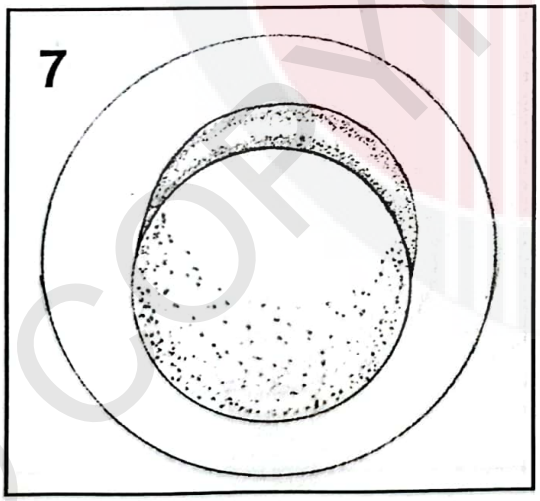

Figure 10.7: Blastula Stage
(2.0-2.5 hours)

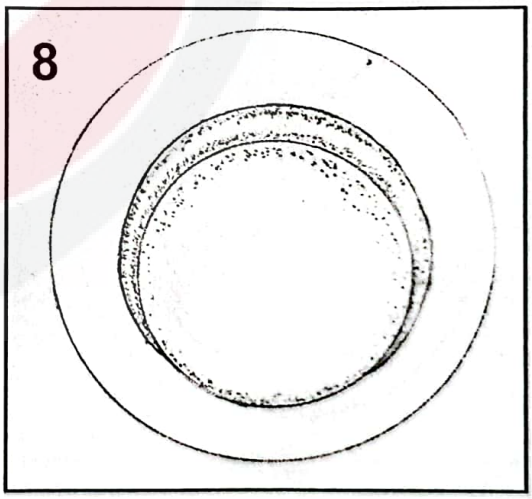

Figure 10.8: Gastrula Stage
(7 hours)

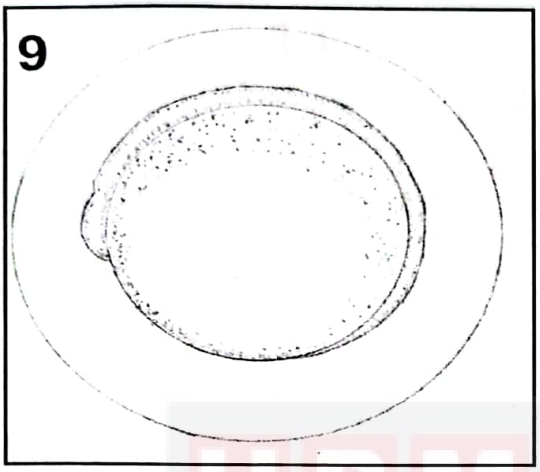

Figure 10.9: Ten-hours Old Embryo

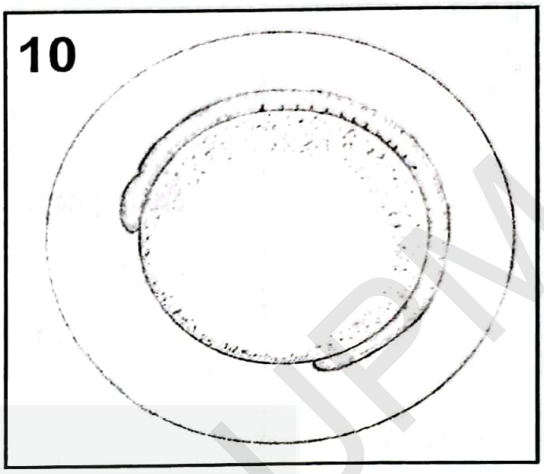

Figure 10.10: Fourteen-hours Old Embryo

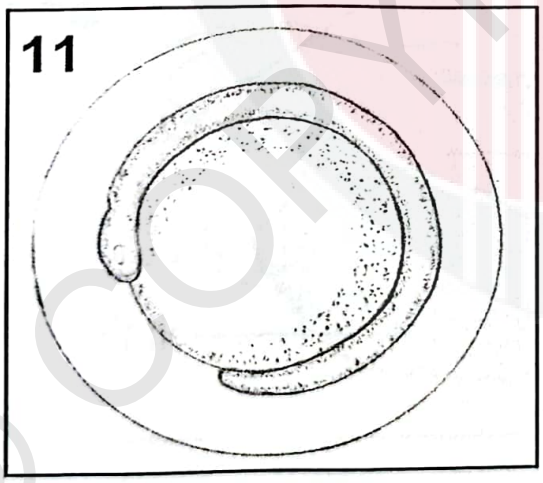

Figure 10.11: Eighteen-hours Old Embryo

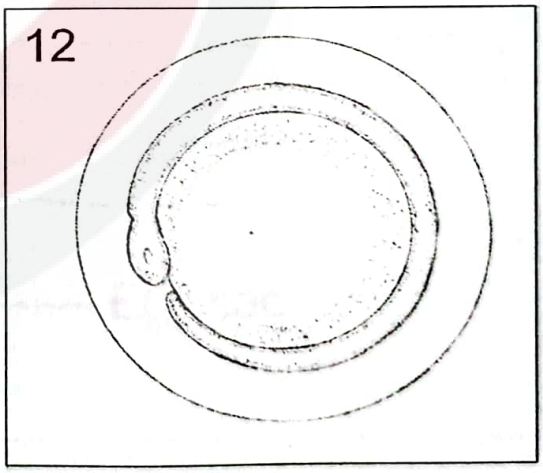

Figure 10.12: Twenty-two-hours Old

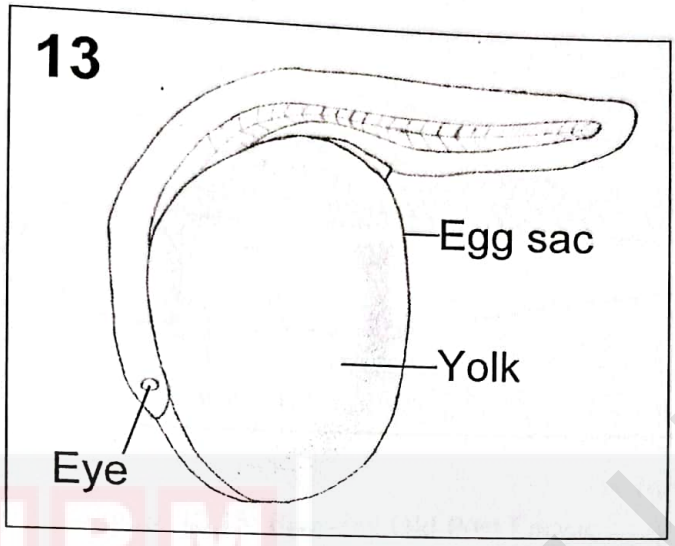

Figure 10.13: Newly Hatched Larva

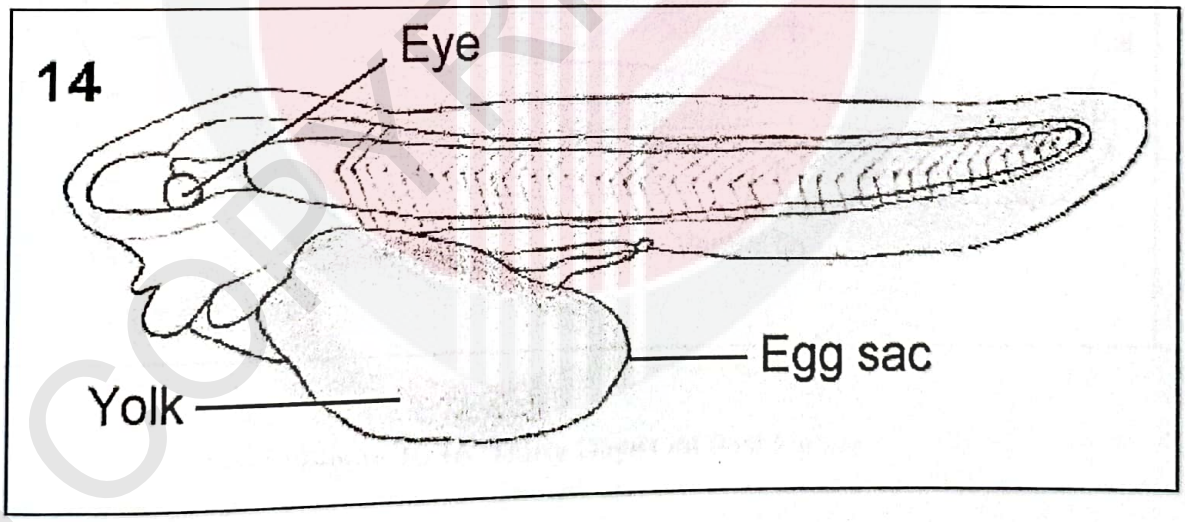

Figure 10.14: Forty-eight-hours Old Larva

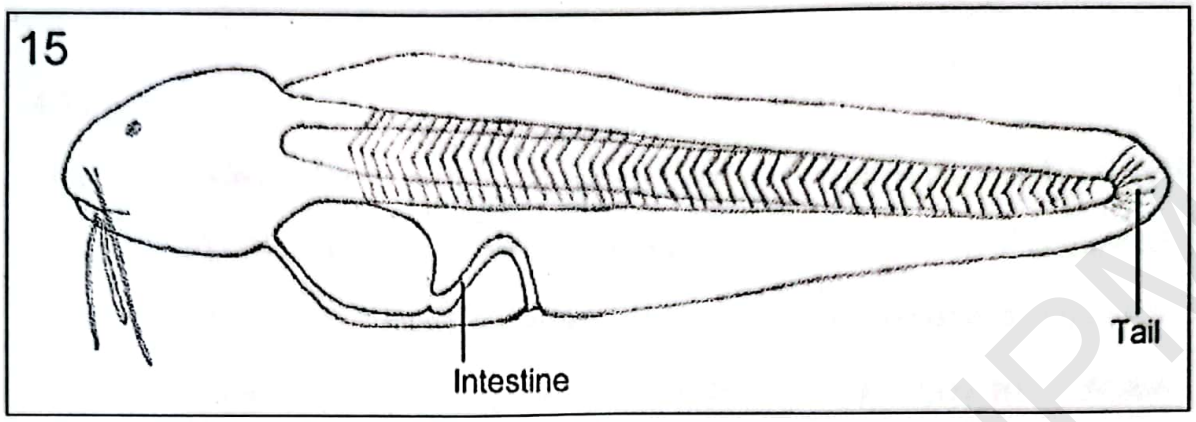

Figure 10.15: Five-day Old Post Larvae

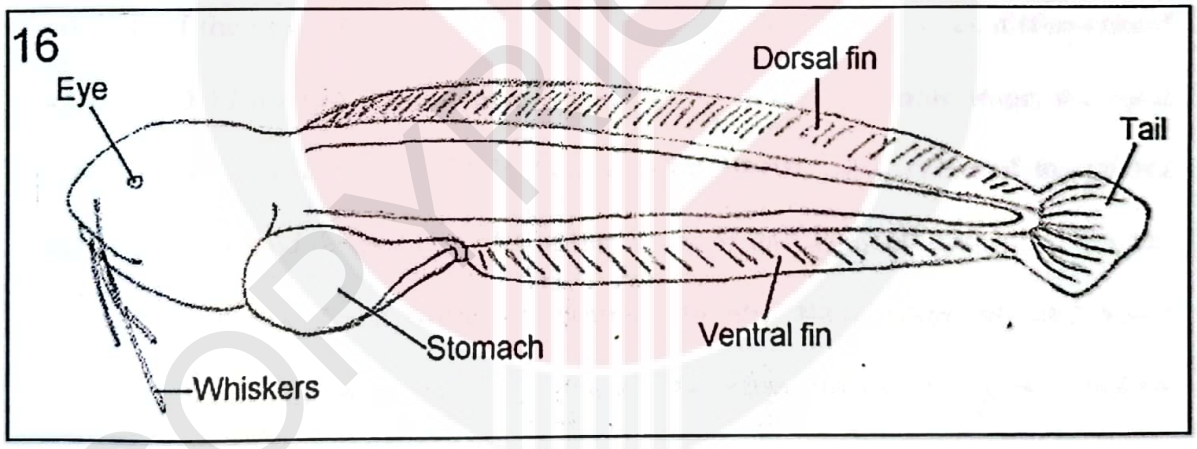

Figure 10.16: Thirty Days Old Post Larvae

4.5 Embryo

The time required to develop from the first cleavage to formation of an embryo was 1 hour. The embryonic development of *Clarias gariepinus* was usually completed within 22.0-23.0 h after fertilization. The first cleavage commenced 20 min after fertilization when the blastodisc divided into two blastomeres (Figure 10.3). Within another 5 min, the four-cell stage was obvious (Figure 10.4). The eight-cell stage was reached after 10-15 min. Sixteen blastomeres were noticeable within the next 15-20 min. The 32-cell stage was attained in the next 20 min and the number of cells doubled (64-cell stage) in the following 10 min. The morula stage (Figure 10.6) was visualized within 1.5 hour after fertilization. By about the seventh hour, the head and tail ends of the embryo were distinguishable (Figure 10.8). Myomeres differentiated between 10-12 hour of development (Figure 10.9). In the 15-somite stage, the optic vesicles appeared and in the 14-16 hour (Figure 10.10), the number of myomeres increased to 20 and the position of Kupffer's vesicle and a pulsating mechanism, the forerunner of the heart were discernible. In the final stage of embryonic development, the growing embryo occupied the entire previtelline space, and by about 1.5-2.0 hour before hatching, it exhibited frequent twitching movements (Figure 10.11). After a pause of about 30 sec, this frequent movement suddenly culminated in a violent jerk breaking the previtelline membrane, and the hatchling emerged tail first (Figure 10.12).

4.6 Hatchling

Hatchlings (Figure 10.13) of *Clarias gariepinus* showed a laterally compressed transparent body, characterized by the presence of an almost round yolk sac, occupying about 45% of the total length. Chromatophores were completely absent. The mouth, alimentary canal, and gills were not yet differentiated. The primitive streak of the notochord was quite prominent; about 25-27 myomeres were distinct and another 7-8 apparent in the tail region. The tip of the tail was rounded and the fin fold was differentiated, but not very distinctly. Newly hatched larvae were not active and generally remained resting on their sides at the bottom of the container.

4.7 Larva

A relatively broad space appeared between the head and anterior margin of yolk in 2.0-3.0 hours old larvae. This space facilitated the accommodation of the developing heart. Buccal invagination was apparent in 6.0-8.0 hours old larvae, and the alimentary canal formed as a straight tube emerging from the posterodorsal aspect of the yolk sac. Barbels appeared in the form of tiny protuberances in 1 day old larvae. The upper and lower jaws was formed, and the lower jaws showed occasional movements. The urinogenital opening was distinct and situated just posterior to the anal opening in 2 day old larvae (Figure 10.14). The pectoral fin bud appeared as a moderate elevation. Intestinal coiling of the alimentary canal was noticeable. The yolk was exhausted by the end of the third day of development, and larvae commenced exogenous feeding even before completion of yolk absorption.

4.8 Post- Larvae

In 5 day old larvae (Figure 10.15), streaks denoted rudimentary rays, which appeared in the caudal fin. The pectoral fin was differentiating and was in the form of a flap just behind the operculum; at this time, sidewise movement of the larvae commenced. The completely absorbed, and larvae began wandering in search of food. Ten day old post larvae were endowed with eight branched rays in the dorsal fin and seven-eight in the caudal fin, and at this stages, the outline of brain in the cranial cavity could clearly be seen under a microscope. The phenomenon of aerial respiration began on the seventh day of development.

CHAPTER 5

DISCUSSION

Past research had been done to determine the effectiveness of ovaprim hormones at different level of doses (0.25 ml/kg, 0.5 ml/kg and 1.0 ml/kg) on the fertilization of African catfish (*Clarias gariepinus*). It was also to determine the appropriate level of dose to be applied on the African catfish for breeding process. Induced spawning and hatching of *N. notopterus* by injecting ovaprim hormone have been reported but details are not available (Anoymous, 2002). This study, however is to confirm the results obtained in local conditions at Universiti Putra Malaysia Bintulu Campus.

According to the survey that have been done, the African catfish that were injected by three different doses showed a latency period for 12 hours. The latency period available in the literature is 6-25 hours for *Channa punctatus* (Banerjii, 1974), 22-25 hours for *Heteropneutesfosslis* (Kohli and Goswani,1987), 14 hours (Rao *et al.*, 1989) for *Clarias gariepinus*. Spawning was completed at a dosage medium (0.5 ml/kg) high dosage of (1.0 ml/kg) and ovaprim injected low-dosage (0.25 ml/kg) respectively. The catfish showed aggressiveness after 10 hours of injection of ovaprim. This show a result that the three doses of different hormone levels have induced the male and female catfish for breeding process (Alok *et al.*, 1993) and single injection of LHRHap resulted in successful induction of spawning in *H. fossilis* after 14-18 hours and in *C. batrachus* after 18-21 hours (Manickam and Joy, 1989).

In the eggs production process of the female African catfish, the results of the three dose of ovaprim produced different rates as shown in table 2 on high level of dose (1.0 ml/kg) that have been injected to the female produced high number of eggs production where egg average number is 354,000-424,500. In *Heteropneustus fossilis* the dosage of ovaprim was given 0.3–0.7 ml/kg body weight and the number of eggs spawned increased with increasing dosage up to 0.7 ml/kg (Haniffa *et al.*, 2000). In Malaysia, the normal dosage used is 0.5 ml/kg. With this dosage the breeder can produce a moderate number of eggs (315,000-343,500). However, compare to the dosage of 0.25 ml/kg dose the parent female catfish could not as much eggs. This showed that a high of dose that have been tested on female catfish is the best dose in producing eggs in the large number compare to two other dose that are tested together. Singh *et al.* (2002) showed that number of ovulated eggs was significantly higher in higher dose of Ovaprim tested in catfish *Heteropneustes fossilis*.

Different doses of ovaprim also affects the milt production on the parent male catfish. It also determines the quantity and quality of milt that have been produced. On a highest level (1.0 ml) and medium level (0.5 ml) of doses can also increases the sperm production of the male catfish, the effect of this male catfish can produce large amount of milt. This will definitely increase the fertilization rate and hatching of eggs during the incubation. Based on the present experiments the ovaprim dose of 1 ml/kg body weight for female and 0.5 ml/kg for male can be recommended. Nandeesha *et al.* (1990) and Haniffa *et al.* (1996) have applied different dosages (0.3–0.6 ml/kg body weight) of ovaprim selected for induced spawning in carps and

murrels. In the lower levels of dosage, production of milt are less. Some of the broodstock do not react positively when injected with lower doses of ovaprim.

When compared with other hormones, ovaprim proved to have better returns for fish farmer. The use of ovaprim can increase the percentage of fertilization and hatching rate respectively (Table 3 and 4). In terms of fertilization and hatching, ovaprim yielded better results (Nandeesha *et al.*, 1990 ; Alok *et al.*, 1993). From the results, obtained the highest percentage of fertilization was observed when a dosage of 1.0 ml/kg (70-73.5%) and 0.5 ml/kg (59.9-73.7%). In the species Mrigal injected with ovaprim, 90% fertilization was observed by Azad and Shimray (1991). Higher percentage in fertilization will influence percentage of hatching rate to be higher. From the results, it is recommended to use 1.0ml/kg dose to produce large number of eggs.

After the eggs have been fertilized, it will undergo 4 stages of development (embryo, hatchling, larva, post-larva) in the breeding tank. Changes in the pattern of the entire structure of an organ or of a specific organ in relation to the environment are decisive for evaluating the developmental patterns of a species (Balon, 1999). Since the egg envelope is thick, transparent, and sticky, observation on the development of *Clarias gariepinus* is difficult. Changes in structure emphasize the thresholds between embryonic, larval, and post larval development from the onset of cleavage, or at the time of organogenesis, respectively (Kovac, 2000; Carlos *et al.*, 2002).

The yolksac of *Clarias gariepinus* was fully resorbed by the third day. Disappearance of the yolk sac was observed on the third day in *Clarias lazera*

(Panjionghua and Zhengwenbiac, 1987) *C. fuscus* (Panjionghua and Zhengwenbiac, 1982), *C. batrachus* (Landge, 1995), *Channa striatus* (Alikunhi, 1953), and *Mystus macropterus* (Wang *et al.,* 1992). *Clarias gariepinus* larvae gradually changed to an orange colour in the early post-larval stage. Similar colour changes (purple red) were noticed in *C. striatus* larvae in the late post- larval stage (Yackob and Ali, 1992).

The mouth and the tip of the notochord in *C. gariepinus* were directed upwards on the fourth-sixth day. In *C. striatus, C. marulius,* and *M. macropterus* the tip of the notochord turned upwards on the fifth-seventh, ninth-thirteenth and second day respectively (Khan, 1926; Parameswaran and Murugesan, 1976; Wang *et al.*,1992). In the present study, the air breathing habit developed 7-9 days after hatching. Similar reports are available in the literature for *Anabas testudineus* (12-13 days; Munshi and Hughes, 1992), and *H. fossilis,* and *Clarias* sp (5-8 days; Dehadrai, 1972). In this study, similar stages of embryonic development occurs (Table 5).

Fish farmers are much less familier with the culture of catfish species because of the lack of breeding and feeding techniques and non-availability of seeds from the wild (Meehan, 2002). Despite this small-scale operations have been attempted for *M. gulio* and *M. oculatus* (Hunter and Kimbrell, 1980) and the successful culture of larger catfishes e.g., *Heteropneustes fossilis* and *C. batrachus*, have also been achieved in the past (Marguiles, 1997). The high fecundity, short embryonic period, fast development of sense organs and air-breathing habit of *C. gariepinus* suggest that is a suitable species for commercial culture.

CHAPTER 6

CONCLUSION

In this study, the results showed clearly demonstrate the possibility of using synthetic fish hormone Ovaprim for effective induced spawning and seed production of *Clarias gariepinus* at Universiti Putra Malaysia Campus Bintulu. It is recommended that the seed of *Clarias gariepinus* could be produced in captivity through scientific management of eggs, larvae and hatchlings. The successful development of protocols for captive breeding is likely to pave way towards commercialization of the technology, which may introduce an exciting entrepreneurial area. The present dosage of 1.0 ml/kg body weight for female and 0.5 ml/kg body weight for male of Ovaprim exhibited encouraging results for the induced spawning and hatching and may be used as a standard doses in future breeding of *C. gariepinus* under local conditions.

REFERENCES

Abol-Munafi, A.B.,Liem, P.T., Ambak, M.A. and Siraj, S.S. 2006. Effects of Maturational treatment on spermatogenesis of hybrid catfish (*C. macrocephalus x C. gariepinus*). Journal of Sustainability Science and Management 2006 Volume 1. p 24-31

Alok, D., Krishnan, T., Talwar, G.P., Carg, L.C. 1993. Induced Spawning of catfish Meter *Opneutes fossilis* (Bloch), using D-Lys super (6) salmon gonadotropin releasing hormone analog. Aquaculture. p 159-167

Alok, D., Talwar, G.P. and Garg, L.C. 1999. In vivo activity of salmon gonadotropin releasing hormone (GnRH), its agonists with structural modifications at positions 6 and 9, mammalian GnRH agonists and native cGnRH-II on the spawning of an Indian catfish. Aquaculture **7**: 383–392

Alikunchi, K.H. 1953. Notes on the bionomics, breeding and growth of the murrel *Ophiocephalus striatus* (Bloch). Proc. Indian. Acad. Sci. (B) **38**: 10-20

Anonymous, 2002. National Agricultural Technology Project (NATP) Report (2002-2003). National Bureau of Fish Genetic Resources, Lucknow, India. Pp 3.

Arockiaraj, A.J., Haniffa, M.A.K., Seetharaman, S. and Singh, S.P. 2003. Early development of a threatened freshwater catfish *Mystus montanus* (Jerdon). Acta Zoologica Taiwanica **14**: 23-32

Arockiaraj, A.J., Haniffa, M.A.K., Seetharaman, S. and Singh, S.P. 2004. Cyclic changes in gonadal maturation and histological observations of threatened freshwater catfish "Narikeliru" *Mystus montanus* (Jerdon 1849). Acta Zoologica Taiwanica **34**: 253-266

Azad and Shimray. 1991. First success in induced breeding of Indian and exotic carps in Manipur using Ovaprim-c. Fishing Chines. **10**: 28-29

Balon, E.K. 1999. Alternative ways how to become a definitive phenotype or a juvenile (and on some persisting linguistic offences). Environ. Biol. Fish, **56**: 17-38

Banerji, S.R. 1974. Hypophysation and life history of *Channa punctatm* (Bloch) J. Inland Fish. Soc. India, **6**: 62-73

Billard, R., Reinaud, P., HollebecQ, M.G. and Breton, B. 1984. Adancement and synchronization of spawning in *Salmo gairdneri* and *Sital trutta*. Following administration of LHRHa-combined or not with pimozide. Aquaculture **43**: 57-66

Brzuska, E. 2001. Artificial spawning of European *Silurus glanis* L.: differences between propagation results stimulation of ovulation with carp pituitary and Ovopel. Aquaculture **32**:11-19

Cacot, P., Eeckhoutte, P., Muon, D.T., Trieu, N.V., Legendre, M., Mariojouls and Lazard, Jerome. 2003. Induced Spermiation and milt management in *Pangasius bocourti* (Sauvage, 1880). Aquaculture **215**: 67-77

Carlos, A.M., Sanchez, M.C., Papp, G.S., Parra, A.D. and Ross, L.G. 2002. Observations on spawning, early development and growth of the puffer fish *Sphoeroides annulatus*. J. Aquaculture. Trop. **17**: 59-66

Carreon, J.A.,Ventura, R.F. and Almazan, G.J. 1973. Notes on the induced breeding of *Clarias macrocephalus* (Gunther). Aquaculture **2**: 5-16

Dehadrai, P.V. 1972. Workshop Report II. All India Co-Ordinated Project on " Air-Breathing Fish in Swamps. CIFRI, Patna, Bihar, India

Ezenwaji, H.M.G. 1992. The reproductive biology of four African catfishes (*Osteichthyes*: Clariidae) in Anambra River basin, Nigeria. Hydrobiologia **242**: 155–164

Ezenwaji, H.M.G. 1998. The breeding biology of *Clarias albopunctutus* Nichols & LaMonte, 1953 in semi-intensively managed ponds in the floodplain of the River Anambra, Nigeria. Ecology of Freshwater Fish. **7**: 101-107

Haniffa, M.A., Rose, T.M and Shaik, M.J. 1996. Induced of ovulation in *Channa striatus* by salmon gonadotropin releasing hormone analogue (sGNRH). Fish Chimes. **16**: 23-24

Haniffa, M.A., Mohamed, J.S. and Rose, T.M. 2000. Induced spawning of the stripped murrel *Channa striatus* using pituitary extract, HCG, LHRH- A and ovaprim. Acta. Icht. Piscat. **30**: 53-60

Hunter, J.R. and Kimbrell, C.A. 1980. Early life history of Pacific mackerel *Scomber japonicus*. Fish. Bull. **78**: 89-101

Horvath, L., Tamas, G. and Seagrave, S. 2002. The propagation and culture of African catfish (*Clarias gariepinus*). In Carp and Pond Fish Culture. Second edition. p 146-149

Jantrarotai, W., Sitasit, P. and Rajchapakdee, S. 1994. The optimum carbohydrate to lipid ratio in hybrid clariid catfish (*Clarias macrocephalus* x *C. gariepinus*) diets containing raw broken rice. Aquaculture **127**: 61-68

Jerdon, T.C. 1849. On the freshwater fishes of South India. Madras. J. Lif. Sci. **15**:302-346

Khan, H.A. 1926. Observations on the breeding habits of some freshwaters fishes in Punjab. J. Bombay. Nat. Hist. Soc. **29**: 958-962

Kohli, M.P.S. and Goswami, U.C. 1987. Spawning behaviour of a freshwater airbreathing Indian catfish *Heteropneustes fossilis* (Bloch). Matsya, **12**: 180-183

Kovac, V. 2000. Early development of *Zingel streber*. J. Fish. Biol. 57: 1381-1403

Kumar, S.S., Gupta, S.A.D, Majumdar, K.C. and Senthilkumaran, B. 2005. Effect of thyroid hormone depletion on monoamines and expression patterns of catfish GnRH in the air-breathing catfish, *Clarias gariepinus*. Fish Physiol Biochem **31**:189–192

Landge, A.T. 1995. Seed production of *Clarias batrachus*. Management of freshwater fish seed farms. Pp. 123-126

Maehata, M. 2001. The physical factor inducing spawning of the Biwa catfish, *Silurus biwaensis*. Ichthyol Research **48**: 137–141

Manickam, P. and Joy, K.P. 1989. Induction of maturation and ovulation by pimozide LHRH analogue treatment and resulting high quality egg production in Asean catfish, *Clarias batrachus* (L). Aquaculture. **55**: 201-213

Marguiles, J.J. 1997. Development of visual system and inferred performance capabilities of larvae and early juvenile scombirds. Marine and Freshw. Behav. Physiol. **30**: 75-98

Meehan, W.E. 2002. Fish culture in ponds and other inland water 1st edition, H.R publishing house, Pilani, India. Pp. 285

Mittelmark, J. and Kapuscinski, A. Induced reproduction in fish (2004). WWW. Seagrant. Umn. edu/aqua/induce.html.

Munshi, D.J.S., Hushes, G.M. 1992. Air breathing fishes of India. Oxford and IBH Publishing Co. pvt. Ltd. New Delhi. Pp. 181-208

Nandeesha, M.C., Das, S.K., Nathaniel, D.E., Varghese, T.J. and Shetty, H.P.C. 1990. Breeding of Carp with Ovaprim Standardization of Dosage and Nation wide Demonstration (Abstract), World Aquaculture Society, Halifax, Canada. Pp. 81

Nandeesha, M.C., Das, S.K., Nathaniel, D.E., Varghese, T.J., Sharma, K. and Keshavabath. 1993. Preliminary results on induced spawning of pond-raised Maser. *Tor. Khudru*. J. Aqua. Trop., **8**: 55-60

Nguenga, D., Teugels, G.G. and Ollevier, F. 2000. Fertilization, hatching, survival and growth rates in reciprocal crosses of two strains of an African catfish *Heterobranchus longifilis* Valenciennes 1840 under controlled hatchery conditons. Aquaculture Res. **31**: 565-573

Panjionghua and Zhengwenbiac. 1982. Observations on the embryonic and larval development of *Clarias fuscus*. Acta Hydrobiol. Sin. **7**: 445-454

Panjionghua and Zhengwenbiac. 1987. Observations on the embryonic and larval development of *Clarias lazera*. J.S. China Natl. Univ. **1**: 19-27

Parameswaran, S. and V.K. Murugesan. 1976. Observations on the hypophysation of murrels. Hydrobiol. Sin. **50**: 81-87

Rao, G.R.M., Janakiram, K. and Muduli, H.K. 1989. National seminar on forty years of freshwater aquaculture in India., Abstr. **111/3**: 7-9

Saidin, T. and Othman, A.F. 1986. Induced spawning of *Pangasius sutchi* (Fowler) using an analog of luteinizing releasing hormone and homoplastic pituitary extract. Asian Fisheries Society. p 687-688

Sidthimunka, A., Sanglert, J. and Pawapootanon, O. 1968. The culture of catfish (*Clarias sp*) in Thailand. FAO Fish. Rep. **5**: 196-206

Singh, D.V. Ram, R.N. and Singh, I.J. 2002. Evaluation of dose of Ovaprim for Inducing maturation and ovulatory response in the catfish, *Heteropneustes fossilis*. Indian J. Fish. **49**: 1–12

Thalathiah, S., Hamilah, H. and Ahmad, A.O. 1983. A study on the breeding aspects of *Pangasius sutchi* (Fowler) in Melaka. Paper presented at the International Conference on Development and Management of Tropical Living Aquatic Resources, 2-5 August 1983. Universiti Pertanian Malaysia Serdang, Selangor, Malaysia.

Thien, T.M., Dan, N.C. and Tuan, P. 2001. Review of fish genetics and breeding research in Vietnam. p. 91-96

Tongsanga, S., Sidthimunka, A. and Menasveta,D.1963. Induced spawning of catfish (*Clarias macrocephalus* Gunther) by pitutary hormone injection. Proc. Indo-Pacific Fish. Counc. **10**: 205-213

Wang, D.S., Zhang, Y.G. and Luo, Q.S. 1992. Observations on the larval development of *Mystus macropterus* (Bleeker) Bagridae. J. Fish. Biol. **40**: 371-379

Yackod, W.A.A. and Ali, A.B. 1992. Simple method for backyard production of snakehead *Channa striatus* (Bloch) fry. Naga. **15**: 22.23

Zheng, W.B., Pan, P.H. & Liu, W.S. 1988. Culture of catfish in China. Aquaculture **75**: 35-44

APPENDICES

APPENDIX 1

Data for the Number of Eggs Produced

Table 6: Dosage Used (ml/kg) and Number of Eggs Produced (ovulation) at Different Trials

Date	Weight			Dosage			Mean eggs produced			Mean
	Female	Male 1	Male 2	Female (Per kg)	Male 1 (Per kg)	Male 2 (Per kg)	Sample 1	Sample 2	Sample 3	
20.11.2006	500 g	500 g	500 g	0.25 ml	0.125 ml	0.125 ml	41,500	40,000	36,500	354,000
24.11.2006	900 g	500 g	500 g	1.0 ml	0.5 ml	0.5 ml	45,000	51,000	45,500	424,500
13.12.2006	900 g	1 kg	1 kg	1.0 ml	0.5 ml	0.5 ml	46,000	46,500	44,000	409,500
15.12.2006	1 kg	1 kg	1 kg	1.0 ml	0.5 ml	0.5 ml	41,500	39,000	37,500	354,000
18.12.2006	1 kg	1 kg	1 kg	0.5 ml	0.25 ml	0.25 ml	39,500	35,500	36,000	315,000
27.12.2006	900 g	1.2 kg	1.2 kg	0.5 ml	0.25 ml	0.25 ml	38,500	37,500	36,500	337,500
29.12.2006	1 kg	1 kg	1 kg	0.25 ml	0.125 ml	0.125 ml	39,000	35,500	36,000	331,500
10.01.2007	800 g	1.2 kg	1.2 kg	0.5 ml	0.25 ml	0.25 ml	39,000	38,500	37,000	343,500
14.01.2007	600 g	900 g	900 g	0.25 ml	0.125 ml	0.125 ml	35,000	34,500	35,500	315,000

APPENDIX 2

Fertilization and Hatching Rates

Trial 1 (Dose 0.25 ml/kg)

Fertilization Rate

Date	Dosage of ovaprim	Sample	No. of fertilization	Fertilization rate
20. 11. 2006	0.25 ml	1	33/53	62.26%
		2	29/41	70.73%
		3	37/55	67.27%
Mean				**60.0%**

Hatching Rate

Date	Dosage of ovaprim	Sample	No. of hatching	Hatching rate
20. 11. 2006	0.25 ml	1	22/38	57.89%
		2	26/42	61.90%
		3	28/43	65.11%
Mean				**55.4%**

Trial 2 (Dose 1.0 ml/kg)

Fertilization Rate

Date	Dosage of ovaprim	Sample	No. of fertilization	Fertilization rate
24. 11. 2006	1.0 ml	1	43/55	78.18%
		2	36/40	90.0%
		3	30/41	73.17%
Mean				**72.4%**

Hatching Rate

Date	Dosage of ovaprim	Sample	No. of hatching	Hatching rate
24. 11. 2006	1.0 ml	1	38/51	74.50%
		2	45/49	91.83%
		3	42/54	77.77%
Mean				**73.2%**

Trial 3 (Dose 1.0 ml/kg)

Fertilization Rate

Date	Dosage of ovaprim	Sample	No. of fertilization	Fertilization rate
13. 12. 2006	1.0 ml	1	58/75	77.33%
		2	51/59	86.44%
		3	48/59	81.35%
Mean				**73.5%**

Hatching Rate

Date	Dosage of ovaprim	Sample	No. of hatching	Hatching rate
13. 12. 2006	1.0 ml	1	46/55	83.63%
		2	35/45	77.77%
		3	43/61	70.49%
Mean				**69.5%**

Trial 4 (Dose 1.0 ml/kg)

Fertilization Rate

Date	Dosage of ovaprim	Sample	No. of fertilization	Fertilization rate
15. 12. 2006	1.0 ml	1	33/45	73.33%
		2	50/63	79.36%
		3	59/71	83.09%
Mean				**70.7%**

Hatching Rate

Date	Dosage level	Sample	No. of hatching	Hatching rate
15. 12. 2006	1.0 ml	1	18/25	72.0%
		2	29/40	72.5%
		3	33/46	71.73%
Mean				**64.8%**

Trial 5 (Dose 0.5 ml/kg)

Fertilization Rate

Date	Dosage of ovaprim	Sample	No. of fertilization	Fertilization rate
18. 12. 2006	0.5 ml	1	31/46	67.39%
		2	38/53	71.69%
		3	28/36	77.77%
Mean				**65.0%**

Hatching Rate

Date	Dosage of ovaprim	Sample	No. of hatching	Hatching rate
18. 12. 2006	0.5 ml	1	33/54	70.37%
		2	26/33	78.78%
		3	26/36	72.22%
Mean				**66.4%**

Trial 6 (Dose 0.5 ml/kg)

Fertilization Rate

Date	Dosage of ovaprim	Sample	No. of fertilization	Fertilization rate
27. 12. 2006	0.5 ml	1	27/38	71.05%
		2	25/40	62.5%
		3	43/65	66.15%
Mean				**59.9%**

Hatching Rate

Date	Dosage of ovaprim	Sample	No. of hatching	Hatching rate
27. 12. 2006	0.5 ml	1	16/26	61.53%
		2	20/33	60.60%
		3	29/47	61.70%
Mean				**55.1%**

Trial 7 (Dose 0.25 ml/kg)

Fertilization Rate

Date	Dosage of ovaprim	Sample	No. of fertilization	Fertilization rate
29.12. 2006	0.25 ml	1	14/25	56.0%
		2	33/54	61.11%
		3	31/48	64.58%
Mean				**54.5%**

Hatching Rate

Date	Dosage of ovaprim	Sample	No. of hatching	Hatching rate
29. 12. 2006	0.25 ml	1	9/17	52.94%
		2	26/45	57.77%
		3	16/24	66.66%
Mean				**53.2%**

Trial 8 (Dose 0.5 ml/kg)

Fertilization Rate

Date	Dosage of ovaprim	Sample	No. of fertilization	Fertilization rate
10. 01. 2007	0.5 ml	1	38/48	79.16%
		2	35/44	79.54%
		3	27/31	87.07%
Mean				**73.7%**

Hatching Rate

Date	Dosage of ovaprim	Sample	No. of hatching	Hatching rate
10. 01. 2007	0.5 ml	1	30/36	83.33%
		2	24/29	82.75%
		3	27/35	77.14%
Mean				**72.9%**

Trial 9 (Dose 0.25 ml/kg)

Fertilization Rate

Date	Dosage of ovaprim	Sample	No. of fertilization	Fertilization rate
14. 01. 2007	0.25 ml	1	19/37	51.35%
		2	37/58	63.79%
		3	23/43	53.48%
Mean				**50.5%**

Hatching Rate

Date	Dosage of ovaprim	Sample	No. of hatching	Hatching rate
14. 01. 2007	0.25 ml	1	20//37	54.05%
		2	23/35	65.71%
		3	28/43	65.11%
Mean				**55.4%**

APPENDIX 3

Data for Fertilization and Hatching Rates for 3 Different Dosages

Table 7: Fertilization Rate Based on 3 Different Dosages

Treatment (Dose)	Replication		
	1	2	3
0.25 ml/kg	60.0%	54.5%	50.5%
0.5 ml/kg	65.0%	59.9%	73.7%
1.0 ml/kg	72.4%	73.5%	70.7%

Table 8: Hatching Rate Based on 3 Different Dosages

Treatment (Dose)	Replication		
	1	2	3
0.25 ml/kg	55.4%	53.2%	55.4%
0.5 ml/kg	66.4%	55.1%	72.9%
1.0 ml/kg	73.2%	69.5%	64.8%

APPENDIX 4

Analysis of Fertilization Rates (comparisons treatment using Tukey's)

The SAS System 23:29 Friday, March 9, 2007 3

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for fertilization

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	24.47667
Critical Value of Studentized Range	4.33902
Minimum Significant Difference	12.394

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Trt
A	72.200	3	T3
A			
B A	66.200	3	T2
B			
B	55.000	3	T1

APPENDIX 5

Analysis of Hatching Rates (comparisons treatment using Tukey's)

The SAS System 23:29 Friday, March 9, 2007 6

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for hatch

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	33.48889
Critical Value of Studentized Range	4.33902
Minimum Significant Difference	14.497

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Trt
A	69.167	3	T3
A			
B A	64.800	3	T2
B			
B	54.667	3	T1

APPENDIX 6

Equipments used Breeding Technique

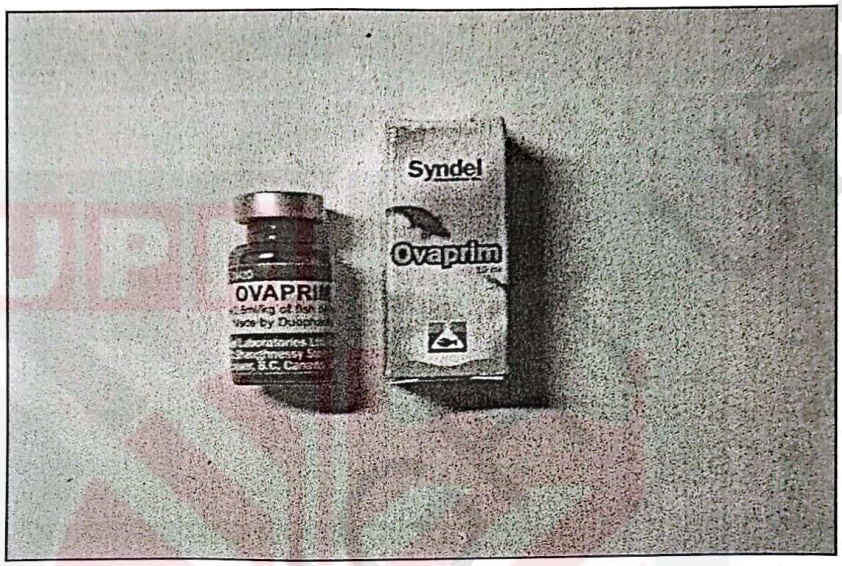

Figure 11: Ovaprim Hormone

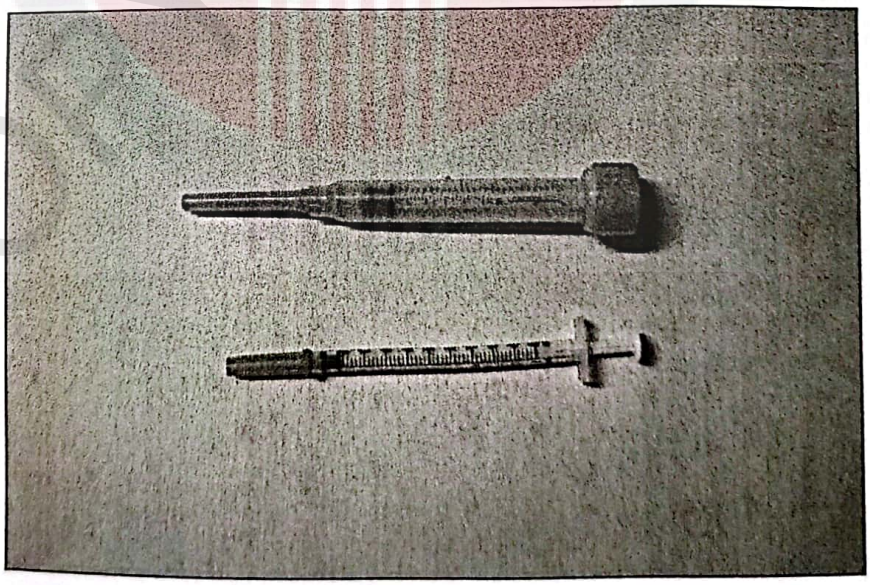

Figure 12: Syringe 1 ml and 2 ml

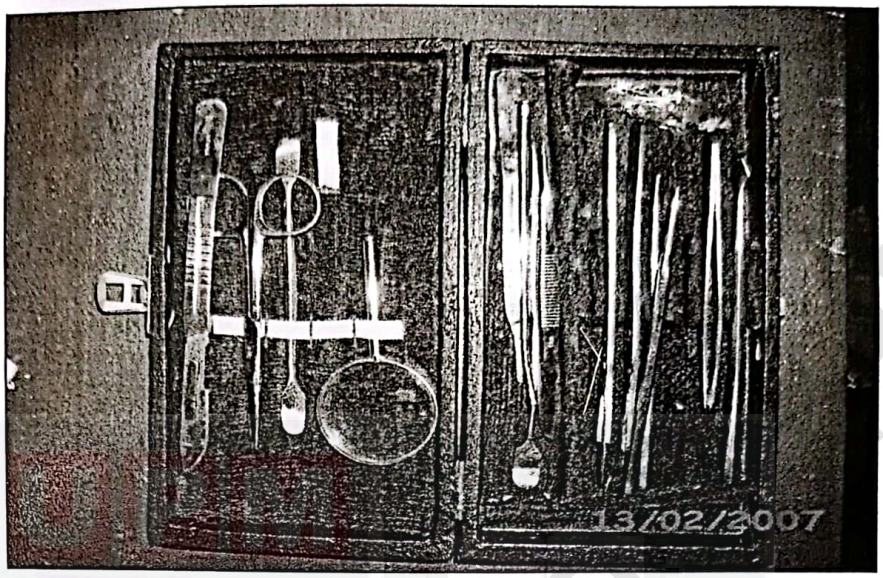

Figure 13: Surgical Instruments

Figure 14: Feather

APPENDIX 7

Eggs of *C. gariepinus* Broodstock

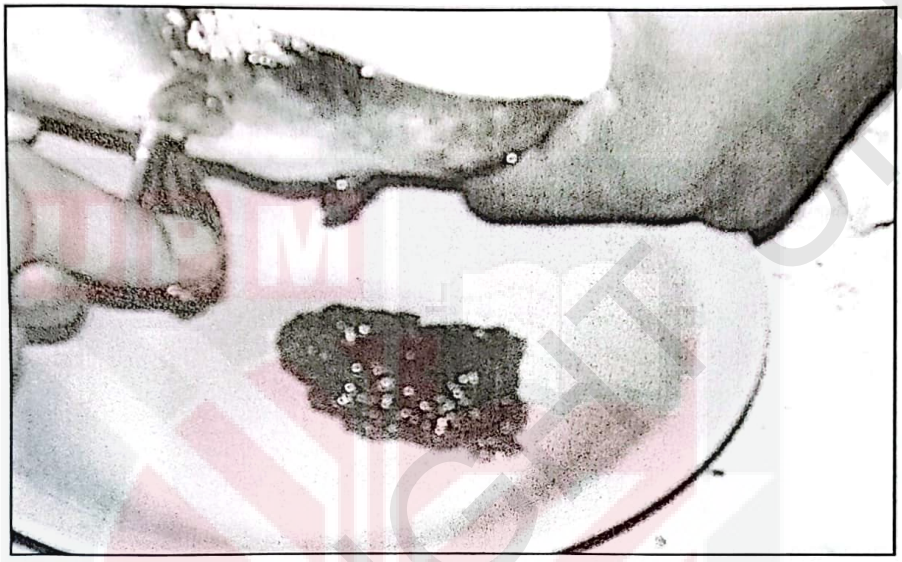

Figure 15: Stripping of Female Spawners to Collected Eggs

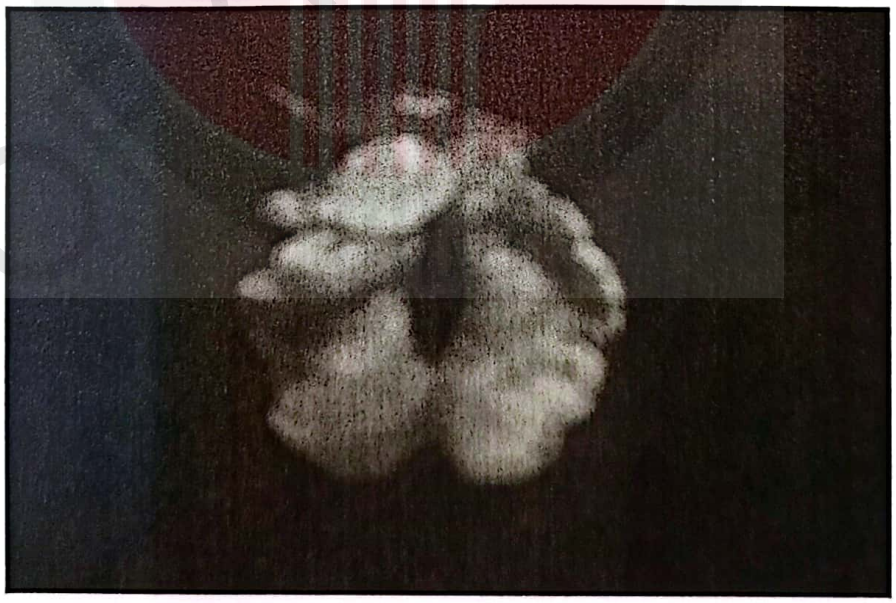

Figure 16: Milt from Male Spawners

PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled **"A Study on the Induced Spawning of Catfish *(Clarias gariepinus)*."** by the supervisor in a joint authorship. However, it has to be evaluated by the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Campus and published in form approved by the Faculty.

ANAND AK ASVESTER UJAN

Date: 30/04/2007