EFFECT OF PITUITARY EXTRACTS OF CARP, *CLARIAS* AND OVAPRIM HORMONE ON THE FECUNDITY AND FERTILITY OF THE COMMON CARP, (*CYPRINUS CARPIO*)

BY

GOMINA OZOHU RAMATU (Bsc Zoology)
MSC/SCIEN/00197/2008-2009

A THESIS SUBMITTED TO THE SCHOOL OF POST GRADUATE STUDIES, AHMADU BELLO UNIVERSITY ZARIA, IN PARTIAL FULFILLMENT FOR THE AWARD OF MASTERS OF FISHERIES (MSC.FISHERIES), DEPARTMENT OF BIOLOGICAL SCIENCES, FACULTY OF SCIENCE, AHMADU BELLO UNIVERSITY ZARIA, NIGERIA.

JUNE, 2011

DECLARATION

I declare that the work in the thesis entitled **"EFFECT OF PITUITARY EXTRACTS OF CARP, *CLARIAS* AND OVAPRIM HORMONE ON THE FECUNDITY AND FERTILITY OF THE COMMON CARP (*CYPRINUS CARPIO*)",** has been performed by me in the Department of Biological Sciences under the supervision of Prof. J. Auta and Dr. S.A. Abdullahi. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

____________________	________________	________________
Name of student	Signature	Date

DEDICATION

This research work is dedicated to Almighty Allah, the owner of heaven and earth, his prophet Mohammed (SAW) and to my lovely family.

CERTIFICATION

This thesis entitled **"EFFECT OF PITUITARY EXTRACTS OF CARP, *CLARIAS* AND OVAPRIM HORMONE ON THE FECUNDITY AND FERTILITY OF THE COMMON CARP (*CYPRINUS CARPIO*)"**, written by Gomina Ozohu Ramatu meets the regulations governing the award of degree of Masters in Fisheries (M.Sc. Fisheries), Ahmadu Bello University Zaria, and is approved for its contribution to knowledge and literary presentation.

________________________ ________________________

Prof. J. Auta Date
Chairman, supervisory committee

________________________ ________________________

Dr. S.A. Abdullahi Date
Member, supervisory committee

________________________ ________________________

Prof. J. Auta Date
Head of Department

________________________ ________________________

Prof. A.A. Joshua Date
Dean, School of Post Graduate Studies
Ahmadu Bello University, Zaria

ACKNOWLEDGMENT

My gratitude goes to Almighty Allah for His love, guidance, protection and inspirations towards the successful completion of this course. My sincere appreciation goes to my able supervisor Prof. J. Auta, who suggested the topic, gave me relevant materials and offered encouragements throughout the period of this work.

My special gratitude goes to my second supervisor Dr. S.A. Abdullahi who devoted his time to go through my manuscript making useful constructive criticisms, suggestions and encouragements that led to the successful completion of this work. May Allah continue to reward you all abundantly, (Amin).

I also register my sincere acknowledgment for the academic and moral support of Dr. A.K. Adamu, Prof. S.J. Oniye, Dr. M.L. Balarabe and Dr. I.S. Ndams.

My sincere appreciation goes to the technical staff of the Hatchery Unit, Mr Luka Ibrahim and Mr. Steven for their contribution especially Mr. Luka (my able breeder) for his un relentless effort during my lab work, as well as his encouragements and patience. Indeed you contributed immensely towards the success of this work. I also acknowledge Mr. Edwin and Mr. Bilyaminu of the Rock Water Fish Farm in Jos.

My heartfelt appreciation goes to my parents Alhaji U.Z. Gomina and Hajiya Hadiza E. Gomina for being there for me at all times, you both gave me the foundation and the wings to fly, you encouraged me to further my education and contributed immensely to what I am today, May Allah continue to bless you and reward you in manifolds. To my siblings Halima (mummy Aisha), Sayyidi Sanusi, Ameena, Mustapha, Ibrahim and Altine, I love you all. May Allah continue to strengthen our bond.

Finally my unreserved appreciation goes to the love of my life, my darling husband Alh. Abdulrazaq Ismaila and my lovely daughter Fadeelah .A. for all your encouragements, love, support and prayers during my frequent absence from home. I love you. May Almighty Allah continue to strengthen our bond and shower his endless blessings upon us, Amin.

ABSTRACT

Homoplastic and heteroplastic hypophysation of *Cyprinus carpio* was investigated under controlled hatchery conditions. 80 brood stocks consisting of 30 males and 50 females of *Cyprinus carpio* and 10 *Clarias gariepinus* of mean weight of (1.42 ± 0.035) kg were obtained from Rock Water fish farm in Jos. 4mg, 5mg and 6mg/kg body weight of acetone dried pituitary extracts of Carp and *Clarias,* and 0.4ml, 0.5ml and 0.6ml/kg body weight of ovaprim were used to determine the dosage that will give the best *output* in fecundity, fertility and hatchability in *Cyprinus carpio.* Standard procedures of pituitary extraction, stripping and mixing of egg and milt were used. All the fish administered Carp pituitary extract (control) spawned and showed significant difference ($P < 0.05$) in fecundity, fertility and hatchability in *Cyprinus carpio* and spawning was found to be dose dependent. However there was no spawning in the fish hypophysized with *Clarias* pituitary hormone. All the fishes injected with ovaprim spawned, though spawning was not found to be dose dependent, the result showed that ovaprim at 0.5ml/kg body weight of fish had significant effect ($p<0.05$) when compared to carp pituitary extract and produced the best output in terms of fecundity, fertility (93.5%) and hatchability (90.1%) in *Cyprinus carpio.* CPE and Ovaprim induces ovulation, fertilization and hatching in *Cyprinus Carpio* at any of the three dosages of CPE (4mg, 5mg and 6mg/kg body weight) or Ovaprim (0.4ml, 0.5ml and 0.6ml/kg body weight.

TABLE OF CONTENTS

CHAPTER TWO

CHAPTER THREE

CHAPTER FOUR

CHAPTER FIVE

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

APPENDICES

CHAPTER ONE

1.0 INTRODUCTION

1.1 General Introduction

Aquaculture, the rearing of aquatic organisms under controlled or semi controlled conditions has expanded rapidly world-wide over the past two or three decades. (Billard and Marcel, 1996).

Fasasi, (2003) estimated the demand-supply gap of fish in Nigeria as 1.0 million tonnes while fingerling demand-supply gap was estimated to be over 500 million. This high demand for fish fingerling in the phenomenal growing aquaculture industry has stimulated the need for artificial propagation of warm water fishes; hence the only dependable source has to be through induced breeding.

Induced breeding according to Lam, (1982) involves the induction of final oocyte maturation (germinal vesicle migration and breakdown) or ovulation in female and induction of spermiation in the male, this is followed by artificial fertilization or the fish are left to spawn naturally. Apart from being able to obtain quality fish seed, induced breeding can also be used to develop strains superior to their genitors by the method of selective breeding and hybridization. These techniques permit the incubation and hatching of eggs and rearing of fry under well protected weather independent conditions. Thus, the spawning of fish is seasonally independent when induced, thereby resulting to out of season supplies of fry and fingerlings. The techniques of induced breeding of fish are manifold all which are aimed at producing large quantity of spawn, fry and fingerlings for utilization in culture or restocking water bodies or water courses.

Hormone induced spawning has been in use for almost 60 years (Rottman *et al*; 1991) and is still in use. Various hormones including Deoxycortiscosteroid Acetate (DOCA) (Adigun *et al*; 1983); Leutenizing Hormone Releasing Hormone (LH-RH); (Bruzska and Adamek, 1999) Human Chorionic Gonadotropin (HCG), (Haniffa *et al*; 2000); Crude pituitary extracts (Ofojekwu *et al*; 2001) as well as Ovaprim (Audu, 2009) have been used to induce spawning in different fish species.

The pituitary gland or hypophysis of fish is a distended lump of tissue attached to the hypothalamus. It has 2 parts i.e. the adenohypophysis and the neurohypophysis. The adenohypophysis produces a variety of hormones which includes the gonadotropins, somatotropins, corticotropin, prolactin, thyrotrophic and melanotropin. (Brian and Carolsfeld, 1993).

The gonadotrophic hormones stimulate the production of egg and sperm and are released in response to gonadotropin releasing hormone (GnRH) secreted by the hypothalamus. (Brian and Carosfeld, 1993)

The high effectiveness of Salmon releasing hormone is due to its higher affinity for binding sites in the pituitary. Among the various analogues of Salmon releasing hormone, D-Arg, Trp, Leu, Pro, Net, has been found to be highly effective and this particular analogue is used in Ovaprim. (Habibi *et al*; 1989). Many years of research, investigating hormonal triggers for ovulation and spermiation in fish went into the development of ovaprim. Ovaprim developed by Syndel laboratories ltd Canada, contains an analogue of Salmon gonadotropin releasing hormone (sGRh) and dopamine antagonist (Nandeesha *et al*; 1990).

The common Carp (*Cyprinus carpio*) is one of the earliest known fish to have been subjected to domestication. Their origin have been traced to South East Asia, where they evolved about 2,500 years ago, but are now found in fresh water worldwide with more than 2000 species grouped in approximately 340 genera (Billard and Marcel, 1996).

1.2 Statement of the Research

- The successful large scale cultivation of any organism for human consumption demands that the resource be easily renewable as it is clearly disadvantageous to cultivate any organism when the supply of the young cannot be easily replenished.
- The major constraint for the expansion of carp culture is the non-availability of quality fish seed.
- Many fish-farm owners prefer to buy fish seeds whose genetic trait is unknown rather than producing their own fish seed and this result in scarcity of fish seeds.
- The fish farmers are also faced with the problem of not knowing the required dosage of hormone that will effectively induce breeding in the common carp.

1.3 Justification

- Fish is highly nutritious, contains high levels of poly unsaturated fatty acids (including omega III fatty acids) which lowers blood cholesterol level.
- Fish has also been found to contain Unidentified Growth Factor (UGF) and Unidentified Immune Factor (UIF).
- The Common carp have a wide geographic distribution and they have many advantages for fish culture;

    i. They possess the ability to withstand a wide range of temperature and oxygen levels.

    ii. They are tolerant to low dissolved oxygen concentration,

    iii. They respire anaerobically over short periods.

    iv. They are universal receptors of feed

    v. Their milt necessary for fertilization is easy to strip without sacrificing the fish.

- Artificial fertilization by induced breeding through hormone treatment has the following advantages:

    i. Better rate of fertilization and hatching,

    ii. Protection against enemies and unfavourable environmental conditions.

iii. Better conditions for growth and survival

iv. Spawning enclosures are not required.

v. Handling of fertilized eggs is easier.

vi. Milt can be used efficiently when it is scarce by (dilution or preservation)

vii. Mixing of gametes for genetic improvement is easier.

viii. Interspectific or intergeneric hybrids can be produced

1.4 Aim

The aim of this research work is to determine the effect of using crude pituitary extracts from same species (homoplastic) and from different species (heteroplastic) as well as ovaprim on induced spawning of the common carp (*Cyprinus carpio*).

1.5 Objectives

- To determine the most effective dosage of carp pituitary extracts, that will stimulate the best fecundity and fertility in *Cyprinus carpio.*
- To determine the dosage of *Clarias* pituitary extracts that will yield the best fecundity and fertility in *Cyprinus carpio.*

- To determine the dosage of Ovaprim that will yield the best fecundity and fertility in *Cyprinus carpio.*
- To determine the dosage of the various hormones that will give the best hatching rate in *Cyprinus carpio.*

1.6 Research Hypotheses

- Carp pituitary extracts has no effect on fecundity and fertility of *Cyprinus carpio*.
- *Clarias* pituitary extracts, has no effect on fecundity and fertility of *Cyprinus carpio*.
- The varying doses of Ovaprim have no effect on fecundity and fertility of *Cyprinus carpio*.
- There is no significant difference on hatchability of *Cyprinus carpio* from the various hormone doses.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Pituitary Gland

Recent advances in the science of fisheries culture techniques have resulted in an increased understanding of the crucial role of endocrinology in aquaculture. Assessment of variations in gonadotrophic potency of various preparations in relation to phylogenic origin, sex, season and problems of dosage as well as bioassay techniques have been investigated (Ita, 1989; Madu, 1989).

The role of pituitary is that of an intermediary between the central nervous system and the gonads. The pituitary gland or hypophysis of fishes is a distended lump of tissue attached to the hypothalamus a region of the base of the brain. It is made up of two parts, the adenohypophysis and neurohypophysis. The adenohypophysis produces a variety of hormones including gonadotropin, growth hormones, corticotrophin prolactin, thyrotropin and melanotropin (Brian and carosfeld, 1993). While the neurohypophysis produces melanocyte stimulating homone, and acts as bridge between the hypothalamus and the adenohypophysis.

The part of the cranium where the pituitary gland is located is known as the sella tunica. The gland is usually embedded in fatty tissue and is connected to the brain in some fishes. While in most fishes, the infundibulum ruptures and the gland is left behind on the base of the skull. (Woynarovich and Hovarth, 1980)

2.2 The Gonadotrophic Hormone

The gonadotrophic hormone, which is one of the hormones produced by the adenohypophysis of the pituitary, stimulates the production of egg and sperm and is released in respect to Gonadotropin Releasing Hormone (GnRH). This causes a rise in plasma gonadotropin, released from the pituitary cells, whose receptors are stimulated by GnRH.

Gonadotropins regulate the formation and maturation of gametes indirectly through steroid hormones produced by the gonad itself, with any number of variations in the pattern of events depending on seasonality, cyclic rematuration an even location of developing oocytes within the ovary. (Brian and Carosfeld, 1993).

Maturation of the fish gonads which is a process where tiny incipient sex cells develop together with accessory tissue, into large organs with mature sperm and eggs that can be spawned, occurs once in a fish's life or many times a year, depending on species and conditions.

Gonadotropins influence the production of all the various stages of maturation and depending on the maturity of fish, the gonad will be more or less responsive to gonadotropin.

Historically, artificial supplementation has been the most effective and generally applicable means of inducing maturation. More recently, however, analogues of GnRH which act one step further up the hormonal chain by inducing release of the fish's own gonadotropins, are promising to be even more widely applicable and reliable (Brian and Carosfeld, 1993).

2.3 Hormone Induced Spawning

Induced spawning according to Lam (1982) involves induction of final oocyte maturation (germinal vesicle migration and breakdown) and or ovulation of the female and the induction of spermiation in male, which is followed by artificial fertilization or fish are left to spawn naturally.

The wide spread use of induced breeding techniques in fish culture has permitted the breeding of a number of species that do not ordinarily spawn under confinement e.g. The big head carp, silver carp, cat fishes e.t.c. unlike the free spawners which include *Punctius*, Common carp and tilapia that can spawn freely in captivity. This is one of the major contributions of induced breeding techniques since its first application in Brazil in 1935.

Fish fingerling production, involves a series of breeding and feeding activities which result in the mass production of fish seeds under controlled environmental conditions, usually in a hatchery. (Adebayo and Popoola, 2008). Various hormones are used to induce spawning in fish which could be synthetic or non-synthetic. The hormone is meant to hasten the ripening of the eggs with few hours, when the genitals of the breeders are fully ripe and are in the resting phase.

A number of hormones which have been demonstrated by several authors include Crude pituitary extracts, Human Chorionic Gonadotropin (H.C.G), Deoxycorticosteriod Acetate (DOCA), Luteinizing Hormone Releasing Hormone (LH-RH) as well as Ovaprim.

2.4 Crude Pituitary Extracts

Crude dried or fresh preparations of whole pituitary glands obtained from similar (homoplastic) species or different (heteroplastic) fish species have been used to induce spawning in fish. Examples of this include Carp pituitary Extracts, *Clarias* pituitary extracts, Salmon pituitary extracts, as well as pituitary, extracts of frogs have been used.

Ofojekwu *et al*; (2001) induced common carp *Cyprinus carpio* with piscine and non piscine pituitary extracts i.e. carp and frog respectively. The researchers administered three doses of the pituitary extract at 2.5, 3.0 and 4.0 mg/kg body weight of the recipient fish. They reported that all the nine fish injected with carp pituitary extract (CPE) spawned while one fish among the group administered with Frog pituitary (FPE) spawned. Three fish spawned among those injected with the combination of CPE and FPE. The researchers further reported that the number of eggs spawned increased with increasing dosage of the hormones.

Salami *et al*; (1994) reported a similar increase in egg output due to administration of HCG and CPE in *Clarias gariepinus*, Madu *et al*; (1989) also reported that CPE was the most effective hormone for induced spawning of *Clarias angularis.*

Chattopadhyay, *et al*; (2002) stimulated spawning in an entirely different species, *Pangasius sutchi,* with 1.5 mg/kg body weight of CPE as resolving, dose. The resolving does was given 6 hours after the stimulatory dose. The result indicated that in all the trials, the fish responded positively and ovulated within 5 to 6 hours after the resolving dose, and fertilization rate ranged from 95- 100%.

Bruzska, (2001) worked on the effect of CPE and ovopel as ovulation stimulators on the spawning of a related species, European catfish *(Silurus glanis).* He used 0.4mg and 3.6 mg doses of CPE, and 0.2 pellets and 1 pellet of ovopel after 24 hours. The result showed that ovulation stimulators did not significantly ($P > 0.05$) affect the weight of the obtained eggs though the effect of the stimulators were highly significant ($P < 0.05$) with respect to the percentage of live embryos after 24, 48 and 56 hours of incubation. Oriji *et al*; (1997) used a dosage of 7mg/kg bodyweight of acetone-dried CPE to induce maturation and ovulation in a closely related species *Heterobranchus bidorsalis.* Obi and Popoola, (1994) studied the effect of pituitary extract of *Oreochromis niloticus, Cyprinus carpio* and *Clarias gariepinus* on induced spawning of *Clarias gariepinus* and reported that fish injected with pituitary extract from *Oreochromis niloticus* had partial spawning while fish injected with Carp pituitary extract and Clarias pituitary extract spawned satisfactorily. The researchers reported a high performance in carp pituitary extract and concluded that "Carp is a universal donor therefore its comparable performance in not unexpected".

Omoregie *et al*; (1998) compared the efficacy of pituitary extracts of carp, Tilapia and *Clarias* on common carp and reported that treatment with Tilapia pituitary extract alone did not lead to egg production by the common carp. This result showed that none of the six fishes administered Tilapia pituitary extract spawned. Only one spawned among those administered the *Clarias* pituitary extract alone while all the six fish given CPE spawned positively.

2.5 Human Chorionic Gonadotropin (HCG)

In recent years, Human Chorionic Gonadotropin (HCG) has received some attention as a substitute for pituitary extracts. HCG, a hormone secreted by the human placenta during pregnancy has been found to be effective in induced spawning in fish. The effectiveness of HCG, overcome the problem of inadequate supply and high cost of pituitary glands as well as the problem of variability in the potency of the pituitary.

This purified human gonadotropin, mimics the endogenous gonadotropin. It is available in ampulses as primonyly or pognyl. Since, there is a direct correlation between blood levels of gonadotropin and readiness to spawn, HCG has been used very successfully in inducing spawning by augmenting serum gonadotropin hormone level in animals that are clearly matured. (Okpanachi, 1999). HCG is administered at a dose of 1-4IU/g of fish body weight (1000-40001U/kg body weight) using distilled water as carrier.

Garcia-Alonso and Vizziano (2004) worked on the induction of oocyte maturation in the white croaker *(Micropogenieas furnieri)* using HCG in Paraguay. The workers injected 22 fully grown females of 100, 300, 500 and 7501U/kg body weight. They observed that HCG has a clear and repeated stimulatory effect when doses of 100, 300 and 5001U/kg body were applied. Eyo, (1997) induced *Clarias gariepinus* with 2.0, 3.0, 4.0 and 5.Omg/ l00g body weight of crude HCG and reported that spawning increased with increase dosage of crude HCG. The researcher observed that groups of fish treated with 4.0 and 5.Omg/ l00g body weight spawned profusely and yielded large number of ripe ova upon stripping. The males gave large quantities of whitish milt in all doses except 2.Omg/ l00g body weight dose. Similar results were reported for Sciaenidae species such as *Ungulates* in which oocyte maturations were stimulated by doses from 100-5001U/kg body weight. (Thomas *et al*; 1995).

Colura (1987) reported the stimulation of *Sciaenops ocellatus* by 500-6001U/kg body weight using HCG. Middaugh and Yoakum (1974) however, observed no reaction when two females were injected with 750IU/kg body weight of HCG. Manob *et al*; (1998) also reported on induced spawning using HCG, four species of fish namely: *Clarias, Pangasius, Rohu* and *Puntius* were tested. The workers finally recommended doses of 2000-8000IU/kg of brooder as appropriate for *Clarias* and *Pangasius.* According to the workers, HCG had no effect on *Puntius* and *Rohu.*

In a different report, Selvaraj (2007) reported a significant (P< 0.05) increase in Gonado-Somatic Index (GSI) of male and female stripped Murrel, *Channa striatus* due to HCG implant.

2.6 DeOxyCorticosteroid Acetate (DOCA)

This is a synthetic hormone which acts mainly as a stimulus for egg discharge, thus inducing pre-ovulation. Males are therefore not usually administered with DOCA but with pituitary extract as substitutes. DOCA is soluble in oil media such as groundnut oil, ethyloleate or prophenyl glycol. The recommended dosage of DOCA for fishes in a single or double dose is 50mg/kg body weight for females and should be administered intraperitonealy.

Ufodike *et al*; (1986) reported successful results with DOCA at 5mg/100g fish body weight on ovulation and fertilization of *Clarias gariepienus* in Panyam fish farm Plateau state. Adigun, (1982) carried out an experiment on induced spawning of *Clarias lazera* using acetone dried carp pituitary extract, Deoxycorticosteriod Acetate (DOCA) and HCG. He found that carp pituitary extract and DOCA to be effective in induced spawning of *Clarias lazera.*

2.7 Leutenizing Hormone-Releasing Hormone (LH-RH)

Leutenising Hormone Releasing Hormone (LH-RH), a mammalian hypothalamic peptide, has the capacity to release gonadotropin from pituitary gland. Chinese report on successful use of mammalian based LH-RH analogue (D-Ala, Pro, Net) for induced breeding of carps created worldwide interest on the use of LH-RH for breeding various species of fish. A major breakthrough in fish breeding research was the finding that dopamine acts as an inhibitor, when LH-RH was used alone without pituitary extract, spawning failure clearly indicated that dopamine blocks the action of LH-RH on the secretion of gonadotropin. Thus blocking of dopamine action, with some antagonists like pimozide, potentiates the action of LH-RH resulting in successful spawning (Chang and Peter; 1983).

Haniffa *et al*; (2000) used four different hormones to induce spawning in another species, the stripped murrel, *Channa striatus*. The researchers used CPE, HCG, LH-RH analogue and Ovaprim. Spawning was complete in the medium (10 + 100mg/kg body weight) and high dosages (10 + 150mg/kg body weight) of CPE whereas in the low dosage (10 + 5mg/kg body weight) spawning was partial.

2.8 Ovaprim

Many years of research investigating hormonal triggers for ovulation and spermiation, result into development of Ovaprim. Among the various analogues of Salmon releasing hormone, D-Arg, Try, Leu, Pro, Net has been found to be highly effective and this particular analogue is what is used in Ovaprim. (Habibi *et al*; 1989).

Ovaprim developed by Syndel laboratories ltd. Canada, contains an analogue of Salmon gonadotropin releasing hormone (SGRh) and dopamine antagonist. (Nandeesha *et al*; 1990).

More recently Ovaprim has been used by induced breeders the world over and has been yielding good results. Ovaprim itself, has been used successfully in the Australian eel-tailed catfish (*Neosilurus ater*) (Cheah and Lee, 2000); red-tailed tinfoil barb, *Barbonymus altuse* (Mohammad *et al;* 1994); The Thai Nahseer, *Tor tambroides* and *T. douronensis* (De Silver *et al*; 2004); walking cat fish, *Clarias batrachus* (Sahoo *et al;* 2005), rainbow sharks, *Epalzeorhynchos frenatum,* (Hill *et al*; 2005); Clown knife fish, *Chitala chitala*, (Sarkar *et al*; 2006); and a number of ornamental fish species.

Khan *et al;* (1992) reported the successful spawning of rohu and mrigal with Ovaprim at Fish Hatchery Islamabad, Pakistan. Nandeesha *et al*; (1990) used Ovaprim to induce breeding in Indian major carps. Goudie *et al*; (1992) tested it on channel cat fish *Ictalurus punctatus* in the USA. Cheah and Yeo (1994) induced spawning in the walking catfish, *Clarias batrachus* with the hormone in Malaysia.

In another study Haniffa and Srindhar (2002), induced spawning in spotted Murrel, *Channa punctatus* using HCG and Ovaprim. Doses of 1000IU to 3000IU/kg body mass of HCG and 0.1ml to 0.7ml/kg of Ovaprim were administered in a single dose. Result showed that increase in dosage of the hormones, produced elevated values in egg output. Spawning was complete among fishes injected with 0.5ml Ovaprim and 3000IU HCG. Nandeesha *et al*; (1993); Francis (1996) and Haniffa (1996) reported that the dosage of Ovaprim for induced spawning in Carp and Murrel ranged between 0.3ml/kg and 0.6ml/kg body weight.

Cheah and Lee (2000) induced twenty gravid female catfish (*Neosilurus ater*) with 0.25, 0.5, 0.75 and 1.00ml/kg body weight of Ovaprim and twenty males with 0.5ml/kg body weight of Ovaprim at the same time as the females were treated. The workers observed that the ovulatory response was positive when the fish were administered 0.5, 0.75 or 1.0ml/kg body weight of Ovaprim. According to them *N. ater* requires lower dosages for induced ovulation as compared to the other cat fishes. Cheah *et al*; (1993) reported that ovulation in *C. batrachus* was achieved only when Ovaprim was administered at the level of at least 0.7ml/kg body weight of Ovaprim.

In Nigeria, Nwokoye *et al*; (2007) investigated induced propagation of African clariid catfish, *Heterobranchus bidorsalis* using Ovaprim and Pituitary Extract obtained from *H. bidorsalis*. A total of 60 gravid females and 20 mature males were injected in 10 spawning trials over 70 days. The outcome of the research showed that Ovaprim performed significantly better ($p < 0.01$) in almost all the parameters investigated. The workers opined that Ovaprim is cost effective and therefore recommended its use.

Very recently Audu and Ofojekwu (2010) compared the effect of carp pituitary extracts and Ovaprim on induced breeding of *Clarias gariepinus* using 4mg, 5mg and 6mg/kg body weight of CPE as well as 0.4ml, 0.5ml and 0.6ml/kg body weight of Ovaprim. He reported that all the females induced with CPE and Ovaprim spawned in all the dosages and Ovaprim at 0.5ml/kg body weight gave the best output in the fecundity, fertility and hatchability of *Clarias gariepinus* with ($P < 0.05$) level of significance.

Okoro *et al*; (2007) induced oocyte maturation with 0.5ml/kg of Ovaprim in *Clarias gariepinus*. The researchers reported that availability of other hormones in the form that can be used for induced breeding in Nigeria is not as easy as obtaining Ovaprim which can presently be purchased from fish breeding agents. According to them, Ovaprim can be stored on the shelf and remain potent for a long time without the use of refrigerating agent. It is also sold in liquid form and this makes it easy to measure out for use in a calibrated syringe. This is unlike the case when using powdered pituitary materials that require electricity to power a sensitive weighing balance for accurate measurement. They therefore recommended the use of Ovaprim even in the rural communities in Africa.

2.9 The Common Carp (*Cyprinus carpio*)

2.9.1 Origin

In Nigeria, common carp was introduced in 1952, which coincided with one of the first attempts to culture fish commercially at the Panyam fish farm in the central Jos Plateau, region. The area had an elevation of 1,400m with a near perfect climate for carp breeding and rearing, and with seasonal temperatures very similar to parts of Europe.

The common carp broodstock were brought to Panyam fish farm from Central Europe as 100g fingerlings by K.K. Zwilling an Austrian in charge of the government farm. They consisted of 50% fully scaled and 50% mirror varieties, derived principally from the Dinkel Bala strain produced in Bavaria Germany Breeding of these fishes was very successful and their progenies were soon to be found throughout Nigeria where fish ponds were constructed.

2.9.2 Biology

Common carp belongs to the kingdom Animalia, phylum Chordata, class Osteichthyes, order Cypriniformes, family Cyprinidae, genus Cyprinus and species *Cyprinus carpio*. It is one of the few species that have a global distribution.

It has a moderately compressed body with fairly large scales. The mouth is more or less protractile, no adipose fin or scales on the head and it has a forked tail.

The post larvae feed mainly on Zooplankton including the nauplii of Daphnia, Moina and Cyclops. The adults feed mainly on decayed organic matter and benthos such as Tricoptera, mollusks, chironomids and ephemeroptera. The feeding habits of adults include burrowing into pond embankments, they gulp in mud in which the digestible matter is filtered out and the rest egested out, carps also thrive well on artificial diets.

The growth rate under culture is a function of several factors. It has a wide range of temperature tolerance. The carp starts breeding when water temperature is about 20^0C. The males have highly developed testes which forms about 20 – 30% of the entire weight (i.e. Gonadosomatic index is high). They spawn on submerged vegetation, in freshly inundated rivers and also in very large ponds. Artificial and semi artificial propagation are widely employed because it responds very well to induce breeding technique.

Plate i: Common carp (*Cyprinus carpio)*

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Broodstock Collection and Management

A total of 50 matured male and female *Cyprinus carpio* and 20 matured male and female *Clarias gariepinus,* weighing between 1.2 – 1.4kg were obtained from Rock water fish farm in Jos. They were conveyed in oxygenated polythene bags to the departmental fish pond where they were acclimatized for 3 weeks.

During this period of acclimatization, the bloodstocks' were fed on highly nutritious feed containing about 25% crude protein. The fish were fed two times daily at the rate of 5% body weight as recommended by Madu, (2008) and 24 hours prior to the breeding exercise, the fish were starved.

Plate ii: ***Cyprinus carpio*** **Transported in Oxygenated Polythene Bags**

Plate iii: ***Cyprinus carpio*** **Broodstocks**

3.2 The Hatchery

The biological science hatchery of Ahmadu Bello University Zaria was used in conducting this experiment. The hatchery is a flow through / re-circulatory system made up of four components. These components are the culturing chambers, two macro filtration chambers, a sterilization chamber and a sedimentation / water reservoir.

With these components in the hatchery, the municipal tap water which is the source of water for the hatchery could safely be used for breeding experiment.

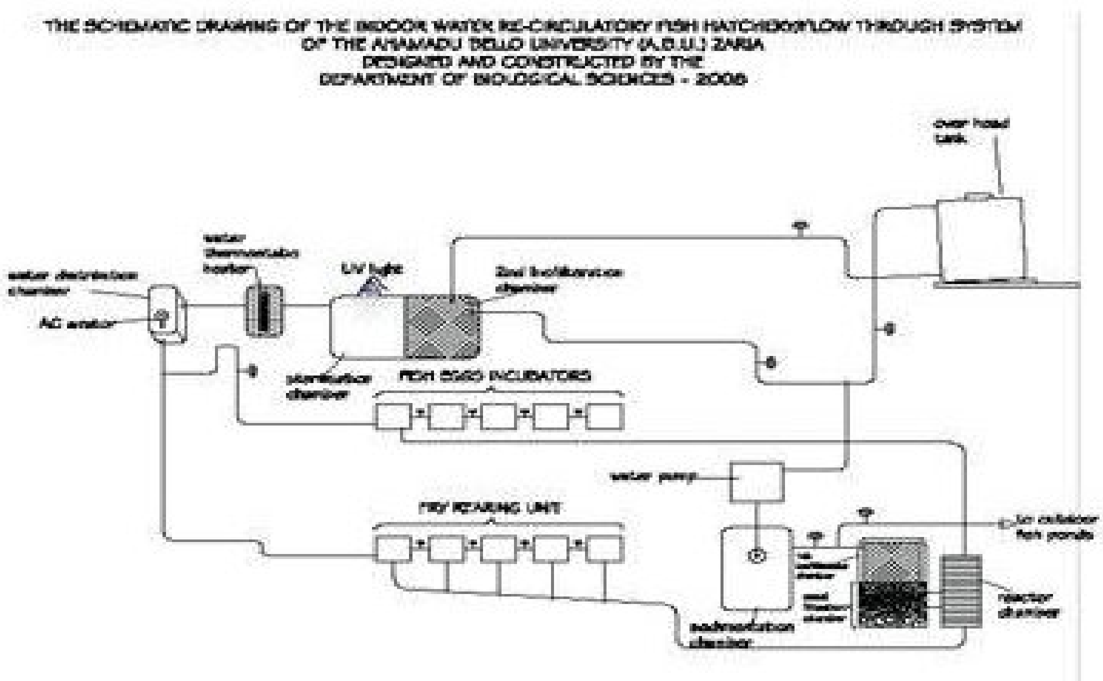

Fig. I Schematic Diagram of the Hatchery.

3.3 Experimental Design

The experimental designs employed consist of 3 treatments A, B and C. Each treatment had three concentrations and each concentration was replicated. Each replicate had three brood fishes (2 males and a female).

Treatment A: Carp pituitary extract alone (control).

Treatment B: *Clarias* pituitary extract

Treatment C: Ovaprim

The females of treatment A were injected with carp pituitary extracts where replicates 1, 2 and 3 consisted of 4mg, 5mg and 6mg / kg body weight of carp pituitary extracts respectively. The females of treatment B were injected with *Clarias* pituitary extracts where replicates 1, 2 and 3 consisted of 4mg, 5mg and 6mg/kg body weight of *Clarias* pituitary extracts.

The females of treatment C were injected with Ovaprim where replicates 1, 2 and 3 consisted of 0.4, 0.5 and 0.6mg/kg body weight of Ovaprim.

Table 1: Design of the Experiment Showing the Doses of Administered Hormones.

Treatment	Hormone	Replicates		
A	Carp pituitary extracts	4mg/kg body weight	5mg/kg body weight	6mg/kg body weight
B	*Clarias pituitary extract*	4mg/kg body weight	5mg/kg body weight	6mg/kg body weight
C	Ovaprim	0.4mg/kg body weight	0.5mg/kg body weight	0.6mg/kg body weight

3.4 Extraction of Pituitary Glands

Pituitary extracts of Carp and *Clarias* were obtained from both sexes of mature Carp and *Clarias.* The pituitary gland located inside the skull at an angle of 45^0 to the fish eye, was extracted using the methods of Shehadeh *et al*; (1975). The donor fish was weighed and was held near the head with one hand, and with a dull knife, the fish was given a blow on the head at the skull.

A triangular cutting was then made on the dorsal side of the head and the cut portion removed to expose the brain pan. The pituitary gland was then carefully collected from the ventral side of the brain using a small spatula. Freshly collected pituitary gland from the donor carp, *Cyprinus carpio* as well as that of *Clarias gariepinus* was immediately placed in acetone in separate specimen bottles and labeled accordingly. After spending 35 hours in acetones, the glands were removed and dried on a filter paper at about 22°C for 24 hours. The desiccated glands were then stored in tightly covered glass bottles and placed in a desiccator.

Plate iv: Carp Pituitary Gland

Plate v: *Clarias* Pituitary Gland

3.5 Preparation of Extract for Injection

Pituitary glands were taken from the glass ware and weighed. 4.00, 5.00 and 6.00mg of the acetone dried pituitary glands were used for each kilogram weight of fish to be induced. The weighed pituitaries were homogenized in water containing adequate volume (2ml) of physiological saline solution (0.6%). A period of 30 minutes was allowed for the active ingredients of the hormone to dissolve out of the gland. The extract was then centrifuged and the supernatant drawn into a 5ml hypodermal syringe leaving the solid tissue of the brain at the bottom of the centrifuge tube (plate ix).

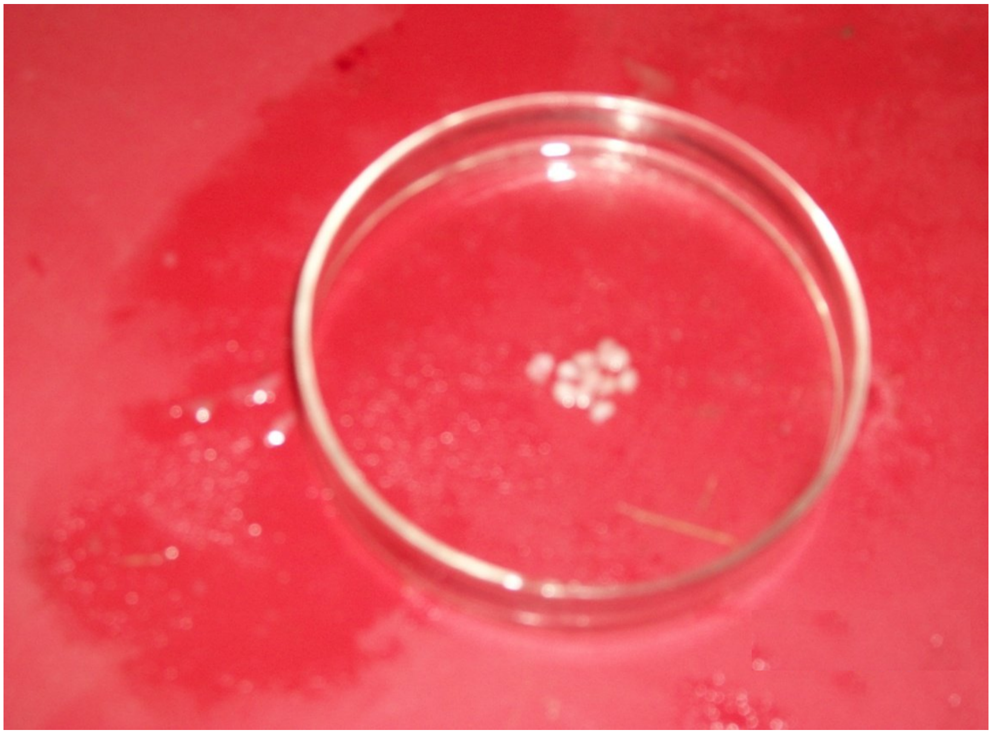

Plate vi: Extracted Pituitary Glands

Plate vii: Marcerated Pituitary Gland

Plate viii: Centrifuge Machine

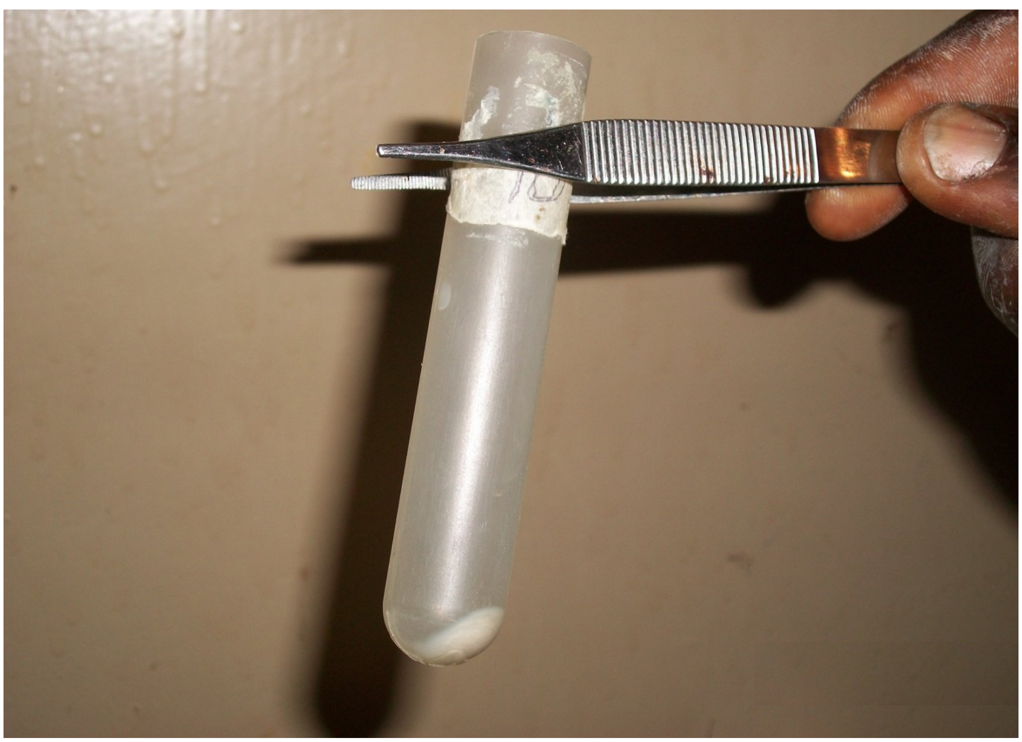

Plate ix Centrifuged Carp Pituitary

3.6 Administration of the Hormone

The priming and final dose method of administration was adopted in this experiment. The priming dose was 20% of the total dose. The priming dose influences the completion of final stages of ripening. The final dose consisted of 80% of the total dose. The inducements were administered intramuscularly above the lateral line and below the anterior end of the dorsal fin, just underneath the skin (Plate x).

After the fish was injected and before the needle was withdrawn, the site of injection was pressed slightly to prevent the suspension from running out. The interval between the first and the second dose was eight hours as described by Ufodike and Amadi, (1991). The first dose was given at 10:00am while the second dose was given at 6.00pm. A total of eight hours latency period was observed using both Carp pituitary Extract and Ovaprim. The injected brooders were placed separately in plastic basins each containing 15 litres of water to complete the latency period.

Plate x: Intraperitonal injection

3.7 Stripping and Artificial Fertilization

Stripping is the term given to the method of actually forcing eggs or milt out of fish. The females were stripped by gently pressing the abdomen in anterior-posterior direction. The eggs were stripped into a Petri dish. Subsequently, a male of the common carp was also stripped and the milt mixed with the stripped eggs.

The eggs and milt was carefully mixed for 30 seconds using a sterilized feather.

A solution containing 0.3% urea and 0.4% sodium chloride in distilled water was added to the mixture. This solution temporarily reduced the stickiness of the eggs and prolonged the fertilizing capacity of the milt.

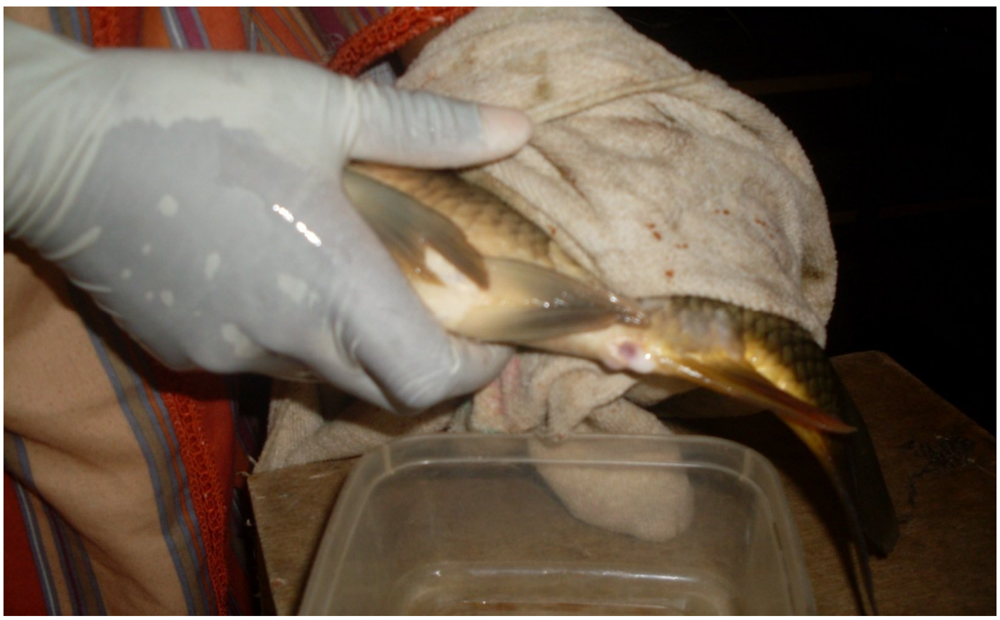

Plate xi: Stripping of Milt

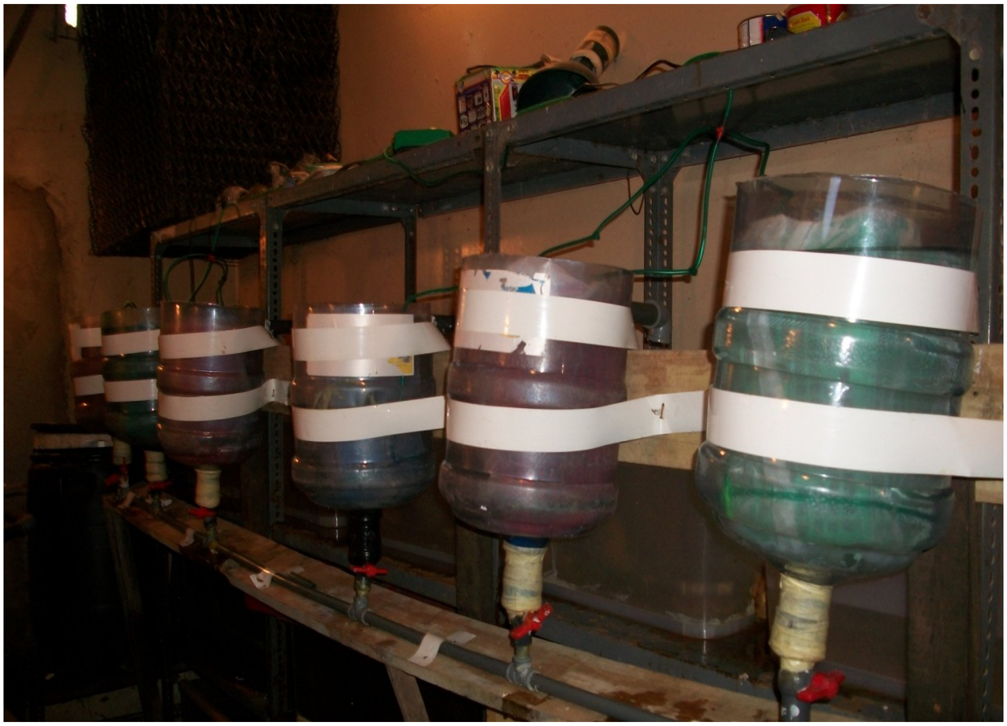

Plate xii: Incubating Jars

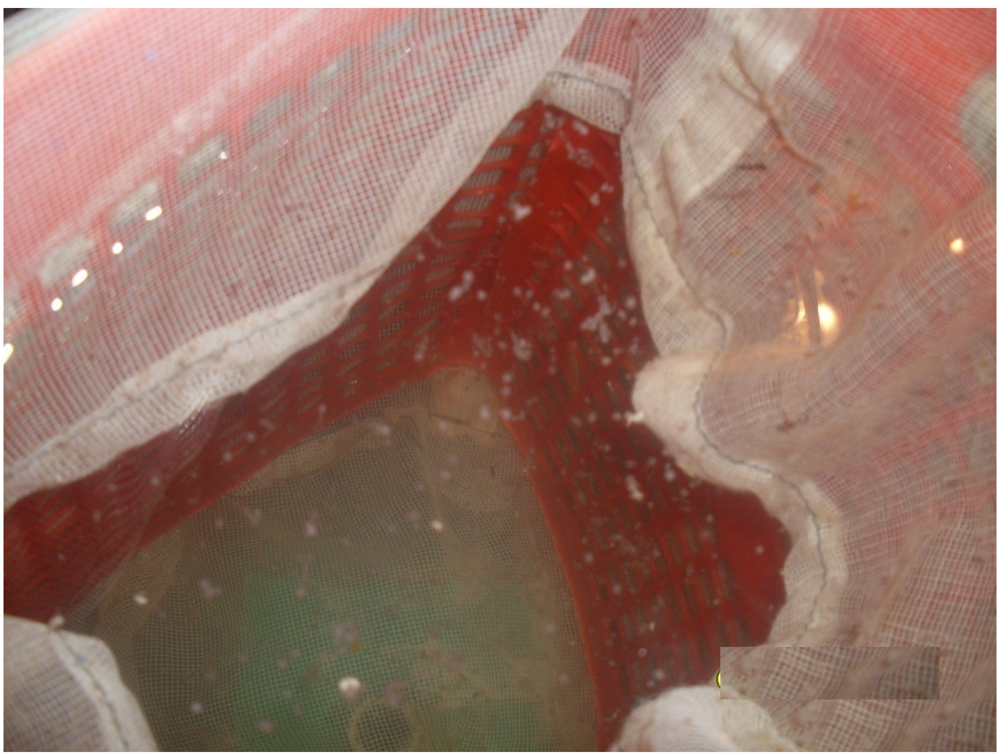

Plate xiii: Incubating Eggs

3.8 Hatching of the Eggs

The fertilized eggs were placed in funnel-type incubators which were previously washed and treated with malachite green in (plate xii) for hatching. A continuous water flow through the inlets of the incubators was maintained. The thermostatic heater in the hatchery was set at 26^0C which ensured the hatching of the eggs.

Plate xiv: Hatchlings (Two Days Old)

Plate xv: Fingerlings (Eight Weeks Old)

3.9 Estimation of Number of Eggs Stripped and Percentage Fertilization

The volumetric method was used to estimate the number of eggs spawned. The stripped eggs were put in known volume of water. 10ml was taken out of it and the water displaced by this sample was noted in finely graduated cylinder. The weights of the eggs were also used to calculate the number of eggs spawned. 1gram of weight of eggs = 800 eggs.

The fertilized eggs were differentiated from the unfertilized ones by the presence of “eye spot” and the swelling of the fertilized ones. The unfertilized eggs were white and opaque while the fertilized eggs were transparent. The percentage fertilization was estimated from the number of unfertilized eggs by the equation.

$$\text{Percentage fertilization} = \frac{N-n}{N} \times \frac{100}{1}$$

Where N = Total number of eggs stripped

n = Number of unfertilized eggs.

The hatchlings emerged after a period of 48 hours. The unhatched eggs were counted and this was used to calculate hatchability.

Percentage hatchability =

$$\frac{\text{Total No. of eggs incubated} - \text{No. of unhatched eggs} \times 100}{\text{Total No. of egg incubated}}$$

The Hanna instrument was used to record physicochemical parameters including temperature, pH, and dissolved oxygen.

3.9.1 Statistical Analysis

One way analysis of variance was used to determine the significant difference in the mean number of eggs stripped, fertilized and hatched in the various treatments.

CHAPTER FOUR

4.0 RESULTS

4.1 Effect of Carp Pituitary Extracts on Fecundity and Fertility of the Common Carp

All the six fish administered the common carp pituitary extract responded positively. (Table 2). Table 2 indicates that the fish administered 6.00mg/kg body weight of carp pituitary extract had the highest number of eggs stripped (fecundity) which was 51,116. The mean number of eggs fertilized was 42,010 eggs, while percentage fertilization was 82.1%. The mean number of eggs hatched was 35,451, while percentage hatchability was 84.38%.

The mean number of eggs stripped from the fish administered 5mg/kg body weight was 42,125 and the percentage fertilization was 74.35%. The mean number of eggs hatched was 29,116 while percentage hatchability was 69.1%.

The mean number of eggs stripped from the fish administered 4mg/kg body weight was 36,000, while percentage fertilization was 66.5%. The mean number of eggs hatched was 15,621 eggs while percentage hatchability was 60.3%.

4.2 Effect of *Clarias* Pituitary Extracts on Fecundity and Fertility of the Common Carp

Table 3 indicates that all the six fishes administered *Clarias* pituitary, none of them spawned. This could be due to the phylogenetic difference between the donor and the recipient fish which renders the hormone ineffective. (Table 3).

4.3 Effect of Ovaprim on Fecundity and Fertility of the Common Carp

All the six fish administered ovaprim responded positively (table 4). The mean number of eggs stripped from the fish administered 0.5ml/kg body had the highest fecundity which was 61,400 eggs; the mean number of eggs fertilized was 57,080 while percentage fertilization was 93.5%. The mean number of eggs hatched was 53,617 while percentage hatchability was 90.1%.

The fish administered 0.6ml/kg body weight of ovaprim had a mean number of stripped eggs as 51,681 and the mean number of eggs fertilized was 41,760 while percentage fertilization was 80.8% and percentage hatchability was 76.75%.

The fish administered 0.4ml/kg body weight of ovaprim had the lowest number of stripped eggs which was 47,720, the mean number of eggs fertilized was 34,708 while percentage fertilization was 72.8%. The mean number of eggs hatched was 23,885 while percentage hatchability was 68.8%.

The results obtained here are much higher than the values gotten from the control experiment (Carp pituitary extract). The analysis of variance (ANOVA) shows that common carp pituitary extracts and ovaprim are statistically significant ($P<0.05$) on their effects on fecundity and fertility of the common carp.

Table 2: Effect of Carp Pituitary Extract on Fecundity and Fertility of the Common Carp (Control).

Sex	Mean weight of fish (g)	Dosage mg/kg body weight	Weight of eggs (g)	Mean number of eggs stripped	Mean No of eggs fertilized	Percentage fertilization%	Mean No of eggs hatched	Percentage hatchability
Female	1.37	6	63.9	51,116	42,010	82.1%	35,451	84.38%
Male	1.32	3						
Female	1.2	5	52.6	42,125	31,304	74.3%	29,116	69.1%
Male	1.4	2.5						
Female	1.32	4	45	36,000	23,916	66.5%	15,621	60.3%
Male	1.37	2						

Table 3: Effect of *Clarias* Pituitary Extract on Fecundity and Fertility of the Common Carp (*Cyprinus carpio*).

Sex	Mean weight of fish (g)	Dosage mg/kg body weight	Weight of eggs (g)	Mean number of eggs stripped	Mean No of eggs fertilized	Percentage fertilization%	Mean No of eggs hatched	Percentage hatchability
Female	1.4	6	-	-	-	-	-	-
Female	1.5							
Male	1.3	5	-	-	-	-	-	-
Female	1.4	2.5						
Male	1.32	4	-	-	-	-	-	-
	1.4	2						

Table 4: Effect of Ovaprim on Fecundity and Fertility of the Common Carp (*Cyprinus carpio*)

Sex	Mean weight of fish (g)	Dosage mg/kg body weight	Weight of eggs (g)	Mean number of eggs stripped	Mean No of eggs fertilized	Percentage fertilization%	Mean No of eggs hatched	Percentage hatchability
Female	1.38	0.6	64.6	51,681	41,760	80.8%	34,151	76.75%
Male	1.35	0.3						
Female	1.32	0.5	76.7	61,400	57,080	93.5%	53,617	90.1%
Male	1.4	0.25						
Female	1.35	0.4	59	47,720	34,708	72.8%	23,885	68.8%
Male	1.5	0.2						


Mean No. of eggs stripped, fertilized and hatched (X10^3)


Mean No. of eggs stripped, fertilized and hatched (X10^3)

CHAPTER FIVE

5.0 DISCUSSION

In fish as in other animals, natural final maturation and release of eggs (ovulation) is regulated and brought about by the release of gonadotrophic hormones that are produced and subsequently released from the pituitary gland. Such gonadotrophic hormones are the luteinizing hormone (L.H) and follicle stimulating hormone (F.S.H). Gonadotropin thus released, reach the ovary and trigger off final maturation of eggs and ovulation.

Direct injection of gonadotropins into a suitable brood fish by passes the fish's own gonadotropin producing system, the hypophysis. However, ovulation may not follow inducement and may even be inhibited by the suppression of gonadotropin release via negative feedback mechanism.

Fertilization and quality of hatchlings are influenced by the quality of the parent stock (brooders)` and the chances of milt penetrating the eggs spawned. Outstanding among environmental factors which affect development of spawns are temperature, dissolved oxygen and pH. Extreme or sudden changes in these qualities may stress the spawners, thus affecting the response of the fish to treatments.

In the course of this investigation, after hypophysation of various brood stocks with different dosages of carp pituitary extracts, *Clarias* pituitary extract and ovaprim, all the fish hypophysized with carp pituitary extract and ovaprim in all the dosages spawned except those hypophysized with the *Clarias* pituitary extract. This result is in agreement with the work of Ofojekwu *et al*; (2001) on common carp *(Cyprinus carpio)* where all the nine brood fish injected with carp pituitary at 2.5, 3.0 and 4.0mg/kg body weight spawned. This result also agrees with the work of Obi and Popoola (1994) and Omoregie *et al*; (1998) that all the fish injected with CPE spawned satisfactorily. This result also showed that fecundity increased with increase in dosage of the hormone as earlier reported by Ufodike and Amadi (1991), Salami *et al*; (1994); Ofojekwu *et al*; (2001) and Haniffa and Sridhar (2002), that the numbers of eggs stripped, fertilized and hatched are dependent on the hormone levels of the spawners.

The analysis of variance (ANOVA) (appendix I) also showed that there was significant difference ($P < 0.05$) in the mean number of eggs stripped, fertilized and hatched in *Cyprinus carpio.*

Clarias pituitary extract did not induce ovulation as well as spawning in the Common carp *(Cyprinus carpio).* All the six female fish injected with *Clarias* pituitary extract, none was found to have ovulated. These results are similar to the work of Ufodike and Amadi (1991) as well as Jauro (1993). They discovered that there was no fecundity and fertility in the common carp which received the *Clarias* pituitary injection. They found out that ovogenesis progressed in carp pituitary extracts hypophysized fish as opposed to the dormant eggs in the heteroplastic hypophysized fish (*Clarias* pituitary extracts). They then concluded that *Clarias* pituitary extract is ineffective or may require higher doses to be effective in carp ovogenesis and fertility.

Ovaprim induced ovulation and fertility in all the six female fishes administered, however, 0.5ml/kg weight of ovaprim had the highest number of stripped eggs as well as the highest percentage fertility 93.5% and hatchability 90.1%. This result is similar to the work of Brzuska and Adamek (1999), that ovaprim produced the best result in fecundity in the closely related species, *Silurus glanis*.

However, there was limitation to the extent of increase in dosage of the ovaprim in this work. For example fecundity increased from 51,681 in 0.4ml/kg body weight to 61,400 in 0.5ml/kg body weight, but decreased to 47,720 at 0.6ml/kg body weight of ovaprim. This result corroborates with the recent work of

Audu (2009) who compared the effect of CPE and ovaprim on *Clarias gariepinus* and found out that at 0.5ml / kg weight of ovaprim gave the highest number of stripped eggs; but at 0.6ml/kg weight of the dosage, fecundity reduced.

Whereas the report of Ofojekwu *et al*; (2001) who used 2.5, 3.0, and 4.0mg/kg body weight of carp pituitary extract on nine brood fishes and his result showed a steady increase in the number of eggs spawned with increase in dosage of the hormones.

This result also tallies with the work of Nwokoye *et al*; (2007), Nandeesha *et al*; (1991), Francis, (1996) and Haniffa *et al*; (1996) that the dosage of ovaprim for induced spawning in Carp and Murrel ranged between 0.3ml/kg and 0.6ml/kg body weight.

In comparing the effects of the three dosages each of CPE (4, 5 and 6mg/kg body weight) and Ovaprim (0.4, 0.5 and 0.6 ml/kg body weight) on fecundity, it could be seen from the result that Ovaprim at a dose of 0.5ml/kg body weight performed significantly better (P<0.05) (appendix IV) than all the dosage of CPE. This confirmed the report of Okoro *et al*; (2007) who effectively induced oocyte maturation with 0.5ml/kg of Ovaprim in *Clarias gariepinus* and found it worthy of recommendation for its use. It also confirmed the result of Cheah and Lee (2000) that successfully induced ovulation in *I. punctatus* with 0.5mg/kg body weight of Ovaprim as the best induction output.

Percentage fertilization also showed that Ovaprim at 0.5ml/kg body weight was significantly better ($P<0.05$) from the other doses of CPE. The highest percentage fertilization of CPE was 82.1% and recorded in 6mg/kg body weight dose which is much less than that of Ovaprim. This proves the superiority of Ovaprim over CPE. This result agrees with that of Nandeesha *et al*; (1991) who found out that nearly 40 percent more fry can be obtained by using Ovaprim in place of commercial Carp pituitary. He then concluded that in economic terms, the use of Ovaprim is advantageous as spawning success, quantity of eggs obtained, the fertilization rate and hatching percentage remained consistently higher with Ovaprim than that of CPE in almost all instances. They then recommended the use of Ovaprim for breeding Carps as its exceptional potency can be compared to none.

Hatching rate showed a significant difference ($P < 0.05$) between CPE and Ovaprim. The data showed increase hatching rate in all the dosages of Ovaprim than the CPE. The highest percentage hatching rate of 90.1% was recorded in Ovaprim at a dose of 0.5ml/kg body weight and lowest at 0.6ml/kg body weight.

CPE recorded 84.3% as its best hatching effect at 6mg/kg body weight dosage and lowest 60.3% at 4mg/kg body weight dosage. This result showed Ovaprim to be better than CPE in hatchability of eggs in *Cyprinus Carpio*.

5.I Conclusion

Though inference could be drawn from the obtained data that CPE and Ovaprim could be used in the induction of ovulation, fertilization and hatchability in *Cyprinus Carpio* at any of the three dosages of CPE (4mg, 5mg and 6mg/kg body weight) or Ovaprim (0.4ml, 0.5ml and 0.6ml/kg body weight), it could be concluded that ovaprim at the dosage of 0.5ml/kg body weight gave the best result in all the three tested parameters in *Cyprinus* c*arpio* while the dosage with the lowest output was obtained in CPE at 4mg/kg body weight.

5.2 Recommendations

Whenever a fish breeder or farmer embarks on induced breeding; his main aim is to have a successful spawn and eventual production of large number of larvae. This aim cannot be achieved unless the breeder knows the most effective hormone and the right dosage to use in the induction of *Cyprinus* carpio.

In the induction of *Cyprinus Carpio*, the use of ovaprim and particularly, at 0.5ml/kg body weight dosage is recommended for farmers or fish breeders in Nigeria for inducing ovulation (spawning), fertilization and hatching in *Cyprinus Carpio*.

REFERENCES

Adebayo, O.T. and Popoola O.M (2008). Storage period; its effect on efficacy of non-piscine (Frog) Hormone used in inducing ovulation in African Catfish. (*Clarias gariepinus*) Burchell, 18822). *International Journal of Zoology*. 4:124-128.

Adigun, N.O. (1982). *Induced Spawning of African Catfish Clarias lazera. Using Acetone dried carp pituitary extract, Deoxycorticosterone acetate and Human Chorionic Gonadotopin* M.Sc. Thesis, University of Jos, Nigeria. Pp. 32 – 48.

Adigun, N.O. Anthony F.A and Parker, J.C. (1983*). Induced spawning of the African Catfish, Clarian Lazera using acetone dried carp pituitary extract, Deoy Corticosterone acetate (DOCA) and Human Chronic gonadotropin.* Proceeding of the 3rd Annual Conference of FISON. 22nd – 15th September 1983: 108 – 112.

Audu I.W and Ofojekwu P.C (2010). *Nigerian Journal of Experimental and Applied Biology,* 11:107-113.

Audu, Idi Wakama (2009). *Comparative Effect of Carp Pituitary Extract (CPE) and Ovaprim on Induced Breeding of African Catfish* (*Clarias gariepinus*, Burchell 1823) M.Sc Thesis; University of Jos. Nigeria pp. 12 – 15.

Billard, E. and Marcel .T. (1996). *Rhythms of reproduction in telost fish*. Academic Press London. pp 31 – 32.

Brian, H and Carosfeld J. (1993). *Induced breeding in tropical fish culture.* International Development Research Centre Canada. Pp 36-98.

Brzuska .E. and Adamek .J. (1999). Artificial Spawning of European Catfish, Silurus Glanis L. Stimulation of Ovulation using LHRH – A, Ovaprim and Carp Pituitary Extract, *Aquaculture Research,* 1:59-64.

Brzuska E. (2001). Artificial spawning of European Catfish, *Silurus glanis L;* Differences between Propagation Results after stimulation of ovulation with carp pituitary and Ovopel, *Aquaculture Research,* 32:11-19.

Chang, J.P. and Peter, R.E (1983). Effect of dopamine on gonadotropin release in female goldfish, Carassives auuratus. *Neuroendocrinology,* 36:351-357.

Chattopadhyay, N.R., Mazumder B. and Mazumder B. (2002). Induced Spawining of *Pangasisu sutchi* with pituitary extract. *Aquaculture Asia,* 2:43 – 44.

Cheah, S.H. and Yeo, M.E. (1994) *Induced spawning of the catfish, Clarias batrachus (Linnaeus) with ovaprim* In: Chou, L.M. Munro, A.D., Lam, T.J., Chen, T.W, Cheeng, L.K.K., Ding, J. K. et al. (eds). The third Asian Fisheries Forum pp. 826 – 829.

Cheah, S.H., Lee, C.L. (2000). Induced Ovulation of the Australian Eel Tailed Catfish Neosilurus ater (Penegia) with Ovaprim. *Asian Fisheries Science,* 13:87-96.

Cheah, S.H., Lee, C.L. and Parry, D.L (1993). *A case for the development of Aquaculture Technology for the Australian Eel – Tailed Catfish, Neosilurus ater.* Seminar on Aquaculture in the Kimberley Region. 23-24 October, 1993. Broome, Western Australia, 8pp.

De Silva, S.S., Ingram, B., Sungan, S., Tinggi, D., Gooley, G. and Sim, S.Y. (2004). Atificial propagation of the indigenous Tor species, emparau (T. Tambroides) and semah (T. douronensis), Sarawak, East Malaysia. *Aquaculture Asia*. 9(4): 15-20.

Eyo, J.E. (1997) Effect of In-vivo Human Chorionic Gonadotropin on Ovulation and Spawning of the African Catfish, *Clarias gariepinus* (Burchell, 1822). *Journal of Applied Ichthyology* 13:45-46.

Fasasi A.T. (2003) Keynote address of the programme Manger of the Ogun State Agriculture Development Programme (AGADEP), Abeokuta at the 1st Annual Fish Farmers Seminar and Training Workshop Organized by FISON (Ogun State Chapter) Abeokuta on July 31st 2003. App.

Francis, T. (1996) Studies on the effect of pituitary Hormone and Feeds on the Reproduction of *Heteropneustes fosilis* (Bloch). Ph.D Thesis. Tamil Nadu Veterinary and Animal Sciences University, Madras, India. Pp 41-53.

Goudie A.C, Simco B.A., Davis KB and Parker NC (1992). Reproductive performance of pigmented and albino female channel catfish induced to spawn with HCG or ovaprim, *Journal of World Aquaculture Society,* 23:136-139.

Gracia – Alanso, J. and Vizziano, D. (2004) Induction of Oocyte Maturation in the White Croaker, *Micropogonias furnieri* (Pisces: Ciaenidae) by Human Chronic Gonadotropin. *Brazil Journal of Biology,* 64:73-80.

Habibi, H.R., T.A. Marchant, C.S. Nahomiak, H. VanDerloo, R.E Peter, J.E Rivier and W.W Vale, (1989*) Functional relationship between receptor binding and biological activity for analogue of mammalian and salmon gonadotropin –*

releasing hormones in the pituitary of goldfish (carassius auratus). *Biological Report*, 40:1152 – 1161.

Haniffa, A.M., Merlin, T. and Shaik – Mohammed, J. (2000) Induced Spawning of the stripped Murrel, *Channa striatus* using pituitary extracts, Human Chorionic Gonadotopin Leutenizing Hormone Releasing Hormone Analogue and Ovaprim. *Acta Ictyological et Piscatorial,* 1:53-60.

Hannifa, M.A., Shaik Mohammed, J., Merlinrose, T. (1996). Induction of Ovulation in Channa Striatus (Bloch) by SnGnRh fishing *Chines,* 1:23-24.

Haniffa, A.M. and Sridhar, S. (2002*).* Induced Spawning of Spotted Murrel (Channa punctatus) and Catfish (Heteropneustes fossils) using HCG and Synthetic Hormone (Ovaprim) *Veterinarski Archieve,* 72:51-56.

Hill, J.E., Baldwin, J.D., Graves, J.S., Leonard, R., Powell, J.F.F and Watson, C.A. (2005*) Preliminary observations of topical gill application of reproductive hormones for induced spawning of a tropical ornamental fish.* North American Journal of Aquaculture, 67:7-9.

Ita E.O. (1989). Principles and practice of integrated commercial fish farming in Nigeria. National Institute for Freshwater Fisheries Research (NIFFR) Technical Report Series No. 19, ISS No. 331 – 6744, 14-24.

Jauro, Ilyasu Abdu (1993*). Effect of priming crude carp pituitary extracts with crude pituitary extracts of Tilapia zilli and Clarias gariepinus*. M.Sc thesis University of Jos, pp.25-26.

Khan, M.N., M.Y Janjua and M. Naeen, (1992*). Breeding of carps with ovaprim (LH-RH Analogue) at Fish Hatchery Islamabad. Proceeding of Pakistan Congress of Zoology*, 12:545 – 548.

Lam, T.J. (1982) Applications of endocrinology to fish culture. *Canadian Journal of Aquatic Science,* 39:111-137.

Madu C.T. (1989). *The Hatchery Management of the Mudfish Clarias gariepinus* Ph.D thesis, University of Jos, 1-20pp.

Madu C.T. (2008) *Fish Seed Propagation.* A paper presented at the 23rd Annual International Conference / Workshop of the Association for Aquatic Sciences of Nigeria on 21st – 25th April 2008.

Manob, T., Khamchai, L. and Sujan, N. (1988) Induced Spawning by HCG Produced from the Urine of Pregnant Women. FAO Corporate Document Repository. Retrieved on the 10th December 2010 from www.FAO.org/cocrep/fied/0003/AC2758/oohtm.

Middaugh, D.P and Yoakum, R.L. (1974). The use of chorionic gonadotropin to Induce Laboratory spawing of the Atlantic Croaker, Micropogonias undulates with notes on subsequent Embryonic Development. *Chesapeake Science,* 15:110-123.

Mohammad – Zaini, S., Saaadon, K. and Omar, A.B. (1994) *OVAPRIM* – Satu teknologi baru pembiakan ikan akuarium. Prog. Fish Res. Conf. Dept Fish. Malaysia. 4:257 – 260.

Nandeesha, M.C, K.G Rao, R. Javanna, R. Parker, N.S. Parker, J.J. Varokese, P. Keshvanath and H.P.C. Shety, (1990). *Induced spawning of Indian major carps through single application of ovaprim* C. in The Second Asian Fisheries Forum (Hirano, R, and I. Hanvu Eds.), Asian Fisheries Society, Manila, Philippines, pp. 581 – 585.

Nandeesha, M.C., Bhadraswamy, G., Patiil, J.G., Varghese, T.J., Kamal S. and Keshavanath, P. (1993) Preliminary Results on Induced Spawning of Pond-Raised Maseer. Tor. Khudru. *Journal of Tropical Aquaculture,* 8:55-60.

Nikolski (1969*).* A method for induced fish spawn prog. *Fish culture* 34: 15 – 16.

Nwokoye, C.N., Nwuba, L.A. and Eyo, J.E. (2007). Induced propagation of African Clariid catfish, Heterobranchus bidorsalis using synthetic and Homoplastic hormones. *African Journal of Biotechnology,* 6:2687 – 2693.

Obi, A. and Popoola, O.T. (1994*).* Effects of Pituitary extracts of *Oreochromis niloticus, Cyprinus carpio* and *Clarias gariepinus*. *Journal of Aquatic Sciences*, 9:19-22.

Ofojekwu, P.C., Umaru, M.S. and Abutu S. (2001) Induced spawning of common carp (*cyprinus carpio L.)* using carp pituitary extracts. *Nigerian Journal of Experimental and Applied Biology,* 2:169 – 173.

Okoro, C.B., Nwadukwe, F.O and Ibimere I. (2007*).* The use of in oocyte maturation and ovulation in ovaprim *Clarias gariepinus (*Burchell, 1822). *Journal of Applied Zoology and Environmental Biology*. 9:83-84.

Okpanachi Martin. A. (1999) *A Comparative Study of Induced Breeding of Cyprinus carpio and Tilapia zilli and Fry Nutrition.* M.Sc. Thesis University of Jos Nigeria pp. 40 – 47.

Omoregie, E., Ufodike, E.B.C. and Jauro, A. (1998*). Variations in Fecundiy and fertility of common carp primed with pituitary* *extracts of carp, Tilapia and Clarias. Journal of Aquatic sciences*, 13:15-18.

Orji R.C.A., Nnadi, C.N., Onyike, G and Ogbona, J. (1997) *Effect of various doses of acetone-dried CPE and Season of Propagation of Heterobranchus bidorsalis,* Pp. 68 – 74.

Peter, R.E., Chang, J.P., Nahorniak, C.S., Omeljanuik, R.J., Sokolowska, M., Shih, S.H. and Billard, R. (1986). *Interaction of Catecholamines and GnRH in regulation of gonadotropin secretion in teleost fish,* 42:513-548.

Rottmann, R.W., Shiveman, J.V. and Chapman, F.A. (1991) *Introduction to Hormone – Induced Spawning of Fish. Southern Regional* Aquaculture Centre (SRAC). SRAC Publication No. 421 Florida, USA.

Sahoo, S.K., Giri, S.S and Sahu, A.K 2005. Induced spawning of Asian catfish, *Clarias batrachus* (Linn): Effect of various latency periods and sGnRha and domperidon doses on spawning performance and egg quality. *Aquaculture Research,* 36:1273-1278.

Salami, A.A., Fagbenro O.A., Edibite L and Fagbermiro, S.O (1994*).* Induced spawning of the African catfish Clarias gariepinus using non-piscine pituitary extracts. *Journal of the world Aquaculture society,* 25:166-160.

Salvaraj, T.F. (2007) Influence of Human Chorionic Gonadotrophin on Maturation in Stripped Murrel, C*hanna striatus. Asian Fisheries Society*, 20:23-39.

Sarkar, U.K., Deepak, P.K, Negi, R.S., Singh, S and Kapoor, D. 2006. Captive breeding of endangered fish Chitala chitala for species conservation and sustainable utilization. *Biodiversity and conservation.* 15.3579 – 3589.

Shehadeh, Z.H. (1975*): Induced Breeding Technique. A Review of Problems and Progress.* Workshop on Controlled Reproduction of Cultivated Fishes EIFAC/FAO Tech. Paper No.25: 72-89.

Sower, S.A. and A. Gorbman (1991). Agnatha, Pp 83-90*. In Encyclopedia of reproduction*, Vol. 1 E. Knobil and J.D Neill (Eds) Academic Press, San Diego.

Thomas, P., Arnold, C.R. and Holt G.J. (1995) Redrum and Other Sciaenids, In: Bromage, N.R. and Robert, R.J. (eds) *Broodstock Management and Egg and Larva Quality.* Blackwell Science, pp 118 – 1374.

Ufodike, E. B. C., Edu, E.A.B. and Anthony A.D. (1986). Effects of intramuscular dose levels of deoxycorticosterone acetate and crude pituitary hormone on fertility and fecundity of African catfish *Clarias lazera. Nigerian Journal of Applied Fisheries and Hydrobiology,* 1:7-20.

Ufodike, E. and Amadi, O. (1991). Comparative Assessment of the Effects of Homoplastic and Heteroplastic Hypophysation on Ovogenesis, fecundity and fertility of the mirror carp. *Journal of Aquatic Sciences,* 6:35-38.

Woynarovich, E. and Horvath, L. (1980) Artificial Propagation of warm water Fin Fishes *(A manual for extension). Technical paper No. 201*.183pp.

APPENDIX I

Summary of mean Physico-chemical parameters in the various tanks

Parameter	Incubating tanks
Temperature	25.9 $\pm$ 0.8
pH	6.64 $\pm$ 0.07
Dissolved oxygen	5.94 $\pm$ 0.21
Alkalinity (mg/l^{-1})	56.57$\pm$5.82

APPENDIX II

Analysis of Variance between parameters

Source of Variation	Df	Fish Wt.	Egg Wt.	Fecundity	Fertility	Hatchability
Treatment	5	0.0052*	238.4495**	156404079.2**	258822764.0**	327407628**
Doses	2	0.0064*	103.1875**	70010647.0**	128894209.8**	389811422.6**
Type	1	0.0102*	518,7675**	339416033.3**	439738347.0**	233447765.3**
Dose x Type	2	0.0015*	233.5525**	151291534.3**	298293525.8**	311983764.1**
Error	11	0.0018	5.4558	3311683.7**	6122871	990575

* Significance
** Highly significance

APPENDIX III

Table of means (hormones)

Types of hormones	Fecundity	Fertility	Hatchability
Ovaprim	53600[a]	44516[a]	37218[a]
Carp Pituitary	42964[b]	32409[b]	28396[b]
Mean	48282	38462.5	32807
S.E	742.77	1009.98	1284.63

APPENDIX IV

Analysis of variance between doses of Ovaprim

Doses	Fecundity	Fertility	Hatchability
0.4	43641[b]	32838[c]	24886[b]
0.5	51763[a]	44190[a]	43867[a]
0.6	49443[a]	38359[b]	29668[b]
Mean	48282.33	38462.33	32807

Values with the same letters within columns are not significant.