EFFECT OF MONTHLY VARIATION IN WATER TEMPERATURE ON ARTIFICIAL BREEDING OF COMMON CARP (*CYPRINUS CARPIO* L.)

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DEPARTMENT OF BIOLOGICAL SCIENCES,

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OCTOBER, 2015

DECLARATION

I declare that the work in this dissertation entitled "**EFFECT OF MONTHLY VARIATION IN WATER TEMPERATURE ON ARTIFICIAL BREEDING OF COMMON CARP (*CYPRINUS CARPIO* L.)**" has been carried out by me in the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. The information derived from the literature has been duly acknowledged in the text and in a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any Institution.

Isa USMAN	______________________	______________________
	Signature	Date

CERTIFICATION

This dissertation entitled **"EFFECT OF MONTHLY VARIATION IN WATER TEMPERATURE ON ARTIFICIAL BREEDING OF COMMON CARP (*CYPRINUS CARPIO* L.)"** by ISA USMAN meets the regulations governing the award of the degree of Master of Zoology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this work to God Almighty for seeing me all through my studies and Mr. Abel Usman for sponsoring me.

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ABSTRACT

The effect of monthly variation in water temperature on artificial breeding of common carp, *Cyprinus carpio* using ovaprim as an ovulation stimulator was studied. Two years old common carp broodstocks weighing 800-2500grams were obtained from Panyam fish farm in Jos, Nigeria. They were transported to the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria and maintained on commercial feed at 3% body weight for a period of two months before the breeding exercise. A total eighteen (18) broodstock were divided into three (3) temperature groups of 23±1, 26±1 and 29±1°C. Each group had three (3) female and three (3) male brooders. Effect of female body weight on fecundity of common carp was also evaluated. Ovaprim hormone at dosage of 0.5ml/kg and 0.2ml/kg was administered intramuscularly to female and male brooders respectively and simultaneously. Temperature has significant effect ($p \leq 0.05$) on latency period of *C. carpio*; latency period decreased with increase in temperature. At 23±1°C, latency was found to be 16.55±0.13 hours, at 26±1 and 29±1°C latency was 13.00±0.13 and 11.32±0.07 hours respectively. While temperature had no significant effect on the weight of eggs, fecundity and viability of the eggs of *C. carpio*, increase in female body weight yielded a significant increase ($r = 0.97$) in the weight of eggs and fecundity of *C. carpio*. The present results showed that temperature has a significant effect ($p \leq 0.05$) on fertilization and hatchability. *C. carpio* performed best at 26±1°C in terms of fertilization (89.00±3.00%) and hatchability (67.50±2.50%) followed by the temperature of 23±1°C with fertilization of 72.67±1.76% and hatchability of 46.83±0.84%, while the temperature of 29±1°C gave fertilization of 52.30±10.49% and hatchability of 28.98±0.96%. There was also a high significant difference ($p \leq 0.05$) in hatching duration of *C. carpio* eggs incubated at different water temperatures, increase in temperature leads to decrease in the hatching duration of *C.*

carpio eggs. Hatching duration was found to be 41.96±0:42 hours at the temperature of 23±1°C, at 26±1°C hatching duration was 37.18±0:04 hours and 28.98±0.96 hours at 29±1°C. There was an increase in survival of larvae with increase in temperature (r = 0.67) though there was no significant difference (p≤0.05) in the survival of larvae between the three temperature groups. At 23±1°C survival was 15.27±0.52% while at 26±1 and 29±1°C survival was 33.20±1.60 and 30.13±6.29% respectively. It is concluded that *C. carpio* can be successful reproduced artificially with ovaprim within the temperature range of 23±1 to 29±1°C, and optimally at 26±1°C. It is therefore recommended that the temperature of 26±1°C be used during artificial breeding of *C. carpio* in Nigeria.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Breeding performance is an important parameter to evaluate the success of artificial reproduction of fish species in captive, which also depend on the environmental conditions, type of hormone used and its potency, dose of hormone and maturity status of the fish (Sahoo *et al.*, 2007). The success of induced breeding also depends on the latency period (Bogdan *et al.,* 2010); appropriate combinations of inducing agents and stripping time always yield maximum egg output during induced breeding while improper coordination between these two will lead to breeding failure (Kiran *et al.,* 2013).

Fish reproduction is regulated by a number of environmental factors which stimulates internal mechanisms into action; the final product of the reproductive cycle is the release of eggs and sperm, which can be controlled by either placing fish in an appropriate environment or by stimulating the fish's internal regulating factors with hormones or other stimulants (Amer *et al.,* 2009).

The precise combination of environmental factors and hormonal treatments required for maturation, ovulation and spawning when available, brings about an accelerated release of gonadotropin from the pituitary into the blood stream. As a result, gonadotropin, which was hitherto being secreted only at a tonic level for the maintenance of yolky oocytes, suddenly appears as a surge, and then the elevated titres of gonadotropin bring about maturational changes culminating in the act of spawning (Pao *et al.*, 2003).

Temperature is the main environmental factor governing the development of eggs in fish embryonic development (Nwosu and Holzlohnev, 2000). Temperature is known to influence the efficiency of yolk utilization although it has been reported that growth rate increase with increasing water temperature but when it becomes super-optimal this negate the fish's growth (Yang and Chen, 2005). In addition temperature determines the development of certain morphological features, hatching rate and larval behaviour (El-Gamal Abd, 2009).

An essential step in successful breeding of any fish species is to understand the optimal temperature for broodstock conditioning and egg incubation. Deformity in fry can be a result of wrong timing of stripping, especially when ova are either premature or overripe (Legendre and Oteme, 1995); in other to overcome such challenge it becomes imperative to determine the latency period of common carp under Nigerian environmental conditions.

The 'fecundity' of a fish is defined as the number of eggs that are likely to be laid by the fish during reproduction. The fecundity and its relation to female size makes it possible to estimate the potential of eggs output and potential number of offsprings in a season which determine the reproductive capacity of fish stock. This reproductive potential, is an important biological parameter that plays a significant role in evaluating the commercial potentials of fish stocks (Gomez-Marquez *et al.,* 2003). Successful fisheries management including practical aquaculture relies on having an accurate assessment of fecundity to understand the recovery ability of fish populations (Tracey *et al.*, 2007).

The common carp (*Cyprinus carpio*) originated in European rivers around the Black sea and the Aegean basin (Freyhof and Kottelat, 2008). It was introduced to Nigeria in the year 1952 by the “father” of Panyam fish farm Jos K.K Zwilling (Barker, 1994). Since the introduction of common carp to Nigeria many research works have been done both on private projects especially at the Rock water fish farm in Jos and governmental projects at the Panyam fish farm, National Institute for Freshwater Fisheries Research (NIFFR) New-Bussa but their breeding history has gone largely unrecorded and carp breeding has not attracted much interest from individuals and research institutes in the country (Barker, 1994).

According to the Food and Agriculture (FAO) statistics of 2013, production of farmed *C. carpio* was 6% (4,080,045 tonnes) of the total global freshwater aquaculture production amounting to 5.7 billion United State Dollars (FAO, 2015). Common carp production increased by an average of 6.6% per year between 2005 and 2013. It is the third most cultured fish species in the world with Asia being the major producing continent of the species.

For many years fish farmers and scientists have been using hormonal preparations for the artificial and semi-natural propagation of carps and other fish species (Pao *et al*., 2003). In practice, acetone-dried common carp (*Cyprinus carpio*) pituitary is the most commonly used agent to induce ovulation. This small organ, which contains the active hormone (gonadotropin), is collected from mixed populations of matured carp usually 3 year old fish (Charula, 2008).

A relatively new development in the technology of induced breeding is the stimulation of endogenous gonadotropin released from the pituitary of the treated fish by the use of inducing agents such as the synthetic analogue of gonadotropin releasing hormone (GnRH) (Mylonas *et al.*, 2010). However, the injection of GnRH analogues alone is generally ineffective in inducing ovulation in cyprinids fish species where there is a strong dopamine inhibitory tone on gonadotropin secretion, which facilitate the gonadotropin releasing activity of the GnRH analogue that is combined with a dopamine receptor antagonist (Peter *et al*., 1988).

The usual practice for the breeding of carp fish is to administer the hormone in two consecutive injection dose, that is the priming and resolving dose (Amin, 2006), others still make use of pituitary extracts as the priming dose in combination with synthetic hormone as the resolving dose. The work of Nandeesha *et al*. (1990a) on the breeding of Indian major carp through the single application of ovaprim has made a major breakthrough in the breeding of carp and farmers have adopted the single application of ovaprim to cause ovulation in carps (Haniffa *et al*., 2007). Hence the single application of ovaprim to induce ovulation in common carp is adopted for this research.

1.2 Statement of the Research Problems

Latency period being one of the major determinants of artificial breeding success is affected greatly by temperature. Thus is one of the most decisive environmental variables affecting the maturation and embryonic development of fish eggs (Haniffa *et al*., 2007). Hence, determination of the optimal temperature for common carp broodstock conditioning after inducement and egg incubation is necessary to maximize the fish seed production.

Obtaining high quality gametes and larvae is one of the most important challenges in modern aquaculture hence the development of a sustainable aquaculture system requires intensive year-round production of fish. Common carp is not easily available in Nigeria although can survive in our aquaculture system and attract more profit to the farmers.

1.3 Justification

Many fish populations worldwide have experienced a drastic reduction, largely due to the effects of the industrialisation and habitat loss (Rahdari *et al*., 2014). One of the useful ways to replace declining natural stocks is through captive breeding or hatchery programs.

Correct timing of females stripping in relation to hormonal inducement at different temperature is of prime importance to obtaining good sexual products which can help in avoiding the risk of getting premature or overripe eggs. Besides obtaining a good sexual product, the quantity of ova is also likely to vary according to the latency and body weight of broodfish (Bogdan *et al*., 2010).

The percentage of fertilized eggs, live embryo and the number of larvae produced per female or per kilogram of fish is a major important response for any induced spawning of fish in an environment (Amer *et al*., 2009), hence determining the effect of temperature as a major environmental determinant on breeding becomes imperative.

1.4 Aim of the Research

The aim of this research is to determine the optimum temperature at which *C. carpio* induced with Ovaprim will have the best breeding performance.

1.5 Objectives of the Research

The objectives of this study are to;

I. Determine the effect of different temperatures on the latency period of *C. carpio* induced with Ovaprim.

II. Evaluate the effect of different temperatures and female body weight on the fecundity and viability of *C. carpio* induced with ovaprim.

III. Determine the effect of different temperatures on the fertilization rate, hatching time and rate of *C. carpio* eggs.

IV. Determine the survival of *C. carpio* larvae hatched at different temperatures.

1.5 Hypotheses

I. There is no significant difference (P>0.05) in latency period of *C. carpio* when induced with ovaprim at different temperatures.

II. There is no significant difference (P>0.05) in fecundity and viability of *C. carpio* induced with ovaprim at different temperatures and having different female body weight.

III. There is no significant difference (P>0.05) in the fertilization rate, hatching time and rate of *C. carpio* eggs treated at different temperatures.

IV. There is no significant difference (P>0.05) in the survival of *C. carpio* larvae hatched at different temperatures.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History of Induced Breeding

The concept of pituitary injection application for successful spawning in fish was developed by Houssay (1930) of Argentina. The present day concept of the role of pituitary in vertebrate's reproduction, according to Atz and Pick-ford (1959) may be said to have originated from the experiments of Aschheim and Zondek in 1927.

Brazil was the first country to develop a technique for hypophysation in fish, Von Ihering and his co-workers, Martins, Cardoso, de Azevedo and others, conducted experiments with various hormone injections on the lines of Houssay (1930) and achieved success in 1934 (Rath, 2000). Since then, Brazilian fish culturists have been employing this technique to obtain seed from indigenous fishes as a part of their routine piscicultural programme.

The Russians are considered the next to introduce hormone treatment in fish culture after the Brazilians. It was not until 1937 that Gerbilskii (1938) could succeed, and since then this method is being utilized with advantage in the Soviet Union for production of sturgeon eggs in the farms situated along the lower Volga, Ural, Kuban and other rivers (Gerbilskii, 1965).

In India, Chaudhuri and Alikunhi (1957) successfully induced spawning, for the first time, in an Indian major carp species using pituitary gland extract. They also bred *Pseudotropius atherinoides* by administering pituitary extract from *Cirrhinus reba*.

2.2 Hypophysation Technique

According to Pao *et al.* (2003) the pituitary gland (PG), or the hypophysis, lies beneath the diencephalon, connecting with the hypothalamus, when the fish brain is lifted upside down, the PG is easily separated from the hypothalamus. It is buried in the sphenoid. The hypophysis secretes two gonadotrophic hormones: Luteinizing Hormone (LH) which stimulates ovulation, and Follicle Stimulating Hormone (FSH), which promotes the development and maturity of the eggs and stimulates the development and maturity of the follicles (Fabio *et al.,* 2011).

Hypophysis can be collected from mature male or female common carp weighing more than 0.5 kg. It can also be collected from dead, unspoiled fish. The hypophysis of a common carp that has never spawned is the best (Patil and Wisniewski 2011). To remove the hypophysis, first, cut off the head of the fish, set the head on its cut surface with the snout facing upward, and use a knife to cut from the nostrils to the upper edge of the eyes and remove the front parietal bone. Turn the whole brain upside down with a pair of forceps and the hypophysis can be removed. If fresh hypophysis is used, it should be ground before use or dehydrated and defatted with pure acetone or absolute alcohol (use a volume about 15-20 times that of the hypophysis). The hypophysis can be preserved for future use after the acetone or alcohol has been renewed twice immersed for 1-2 h each time (Woynarovich and Horvath, 1980). There are methods of preservation. First, the dehydrated hypophysis is dried on a piece of filter paper for 15-20 min and then stored in a tightly sealed, labelled small brown bottle. Second, the hypophysis is left in the second immersion liquid and stored in a tightly sealed, labelled small brown bottle. Both methods give good results. Generally, the hypophysis is still effective after 2 years of preservation.

In usual practice, the female alone is injected with a stimulating dose of 2.0 to 3.0 mg kg^{-1} body weight followed by a second dose of 5.0 to 8.0 mg kg^{-1} weight after a lapse of 6 hours, if required. Two males per female by number, are given a single dose, each of 2.0-3.0 mg kg^{-1} bw at the time of second injection to the female. Both the injected males and the females are kept together in a breeding 'hapa' for spawning. Slight alterations in doses may be made depending on the stage of maturity of the breeders as well as on environmental factors (Charula, 2008).

Mahmood (2006) successfully bred Climbing perch (*Anabas testudinues*) with Pituitary gland dose of 1.2 mg/100g administered in both single and double injection Pituitary gland. It was found that administration of PG in split doses (double injection) had no additional advantages (ovulation 100%, fertilization 81.56% and hatching 72.68%) over the single injection (ovulation 100%, fertilization 83.03% and hatching 75.48%) treatment. Similarly, the ovulation time of the fish did not vary significantly ($P>0.05$) in either single or double injection of PG (single injection 7.12 hr; double injection 7.20 hr). The results demonstrated PG can used successfully to induced ovulation in *A. testudinues* by splitting of pituitary gland dose (double injection) has no additional advantages on breeding performance of *A. testudineus*.

Hussan *et al.* (2007) compared the induced breeding technique and its success rate between ovaprim and use of pituitary extract from African catfish, *Clarias gariepinus* in *Pangasius sutchi*. The broodstock was injected pituitary extract intraperitonially at doses of 2.0-4.0 mg kg^{-1} and 8.0-14.0 mg kg^{-1} for females and 2.0 mg kg^{-1} for males, while the control (ovaprim) received 0.25 ml kg^{-1} and 0.5 ml kg-l for females and for males 0.2 ml kg^{-1}. The results were almost similar to the control using the ovaprim. The fertilization and hatching rate of eggs from pituitary extract ranged from 43-90 percent and 50-90 percent respectively. The pituitary extract induced breeding technique can be considered much less cost effective when compared to that of ovaprim.

Amer (2009) compared two spawning induction agents, ovaprim and carp pituitary (single injection and double injection) extract in the common carp (*Cyprinus carpio).* For observation of spawning parameters (latency time, spawning index, fertilization rate and hatching rate) in three experimental groups of female common carps that were intraperitonially injected with one of the following treatments: a single dose (3.0 mg/kg) of Carp Pituitary Extract (CPE); two successive doses of 0.3 mg/kg (as a preliminary dose) and 2.7 mg/kg of CPE; one single dose 0.5 ml Ovaprim. Males of equivalent female numbers were simultaneously induced, although they received one single dose only (2.0 mg CPE/male) as stimulating dose at the time of the females' first injection. Significantly ($p<0.05$) lower reproductive performance (Latency 15hrs, Fertilization 71.00±1.87%, Hatchability 50.00±2.34 and fecundity of $11.05 \pm 1.32 x 10^3$) was observed in fish group which received one single CPE injection when compared to those received two CPE doses as indicated by the experimental spawning parameters. No significant differences ($P>0.05$) in fertilization and hatching rates between Ovaprim and CPE (double dose) treated fish. However, the hypophysation technique (double CPE doses) was remarkably efficient than Ovaprim. Furthermore, the results revealed that the hypophysation technique is more economical and productive than that of Ovaprim. CPE is an important management/production tool in the propagation of cyprinid as well as many commercial aquaculture species.

Gomina (2011) reported on the effect of pituitary extracts of carp, Clarias and ovaprim hormone on the fecundity and fertility of the common carp in Nigeria. The pituitary of matured Common carp and Clarias were extracted and used for the inducement of *C. carpio*. Extracts were administered at different dosages; 6.0 mg/kg, 5.0 mg/kg and 4.0 mg/kg while ovaprim dosages of 0.6 ml/kg, 0.5 ml/kg and 0.4 ml/kg. The carp pituitary induced *C. carpio* gave a fertilization rate of 82.1, 74.3 and 66.5% when given dosage of 6.0, 5.0 and 4.0 mg/kg respectively, the Clariid pituitary induced *C. carpio* did not ovulate, on the other hand the ovaprim induced *C. carpio* gave fertilization rate of 80.8, 93.5 and 72.8% at the dosage of 0.6, 0.5 and 0.4ml/kg respectively.

2.3 Alternative Inducing Agents

Nandeesha et al., (1990a) highlighted problems associated with the process of hypophysation during induction of fish species as;

a. Varying potency of the pituitary gland results in unsuccessful spawning and this problem cannot be easily rectified since the farmer cannot measure the potency of the available gland.

b. Rightful collection of pituitaries and proper cryopreservation.

c. There is large gap between the supply and demand of pituitary. As a result, several fish breeders either use poor quality glands yielding unsatisfactory results or do undertake only limited breeding operations in accordance to the glands availability.

d. For preparation of pituitary extract, basic equipments like chemical balance and centrifuge are required which are not available in several farms.

e. For achieving successful spawning, pituitary extract has to be administered necessarily in two split doses to female fish. This not only results in increased handling of brood Fish, often leading to spawning failure, but also consumes considerable amount of labour and time.

f. Pituitary glands need to be stored in a refrigerator to avoid spoilage.

Alternatives to hypophysation were tried by different researchers in different parts of the world.

2.3.1 Mammalian gonadotropins (human chorionic gonadotropin)

Human Chorionic Gonadotropin (HCG) has been found as an alternative for pituitary gland. HCG was discovered in beginning of 1927 by Aschheim and Zondek. They extracted good quality hormone with luteinizing gonadotrophic activity from the urine of pregnant women. Russian workers first used chorionic gonadotropin in 1964 with a trade name as Choriogohin and got good results on Cypriniformes. A perusal of literature indicates that HCG is effective either alone or in combination with Pituitary Gland (PG) extract in inducing various fishes all over the world (Murthy *et al*., 2013).

Legendre and Oteme (1995) studied the effect of varying latency period on quantity and quality of ova of *Heterobranchus longifilis*. Females were induced by single injection of HCG at a dose of 1.5 IU g^{-1} body weight. The ova quality was estimated by hatching percentage and proportion of deformed larvae obtained after artificial fertilization. Their results showed that, in an individual female, ovulation of postvitellogenic oocytes is not synchronous and takes 3 to 4 hours to be completed, between 7-8 hours and 11 hours after HCG injection at 30°C. The quantity of ova that could be collected by hand-stripping increased with the latency period, from 18000±12000 to 72000±29000 ova per female at 8 hours and 1l hours after injection, respectively. However, the first ova obtained (8 hours after injection) were of good quality (94% hatching) and showed no signs of incomplete final maturation. Eleven hours after injection, the percentages of ovulated oocytes within the whole ovarian population of postvitellogenic oocytes were high in all the females, being generally around 90%. After ovulation, aging of ova occurred rapidly: the proportion of deformed larvae increased significantly (from 4 to 20%) 2 hours after completion of ovulation and, 4 hours post-ovulation, hatching percentage dropped from 92 to 36%. This latency period was found to be 11 hours following HCG injection at 30°C, and corresponds approximately to the completion of ovulation

Zakes and Szczepkowski (2004) induced pike perch *(Sander lucioperca)* to spawn 3 months prior to the natural spawning period through photothermal and hormonal stimulations. Female (five specimen in each group) were stimulated with injection of HCG once (200 IU kg^{-1}), twice (200 IU kg^{-1}, second dose after 48 hours - 400 IU kg^{-1}) or three times (200 IU kg^{-1}, after 24 hours -200 IU kg^{-1} and after another 24 hours - 200 IU kg^{-1}). The control group was injected once with 0.9% NaCl. The males were stimulated with a single hormone dose of 200 IU kg^{-1}. Eggs were obtained from all treatments, none of the control group ovulated any egg. The time of ovulation was 66-71 hours following the first injection and the egg viability from 71.5 to 77.5% that were not depended on hormone doses.

Selvaraj and Francis (2007) observed gonadal development, Gonadosomatic Index (GSI), histology and level of serum steroid hormones in captive striped murrel *(Channa striatus* Bloch) implanted with HCG capsules and cholesterol capsules as control for a period of five months after implantation, HCG implants induced a significant increase in the GSI of male and female fish. The GSI values were highest in the fourth month and lowest in first month. Ova diameter was higher in HCG as compared to control. Similarly, fecundity was highest in December (15,415 eggs) in HCG implanted fish, whereas, only 2,245 eggs were observed in the control fish. Serum testosterone level was highest (18 µg ml^{-1}) during the December in HCG implanted male fish whereas the serum estradiol-17β level was highest (61.5 PG ml^{-1}) in third month in HCG planted female fish.

2.3.2 Purified fish gonadotropin (GnRH and LH-RH)

Attempts have been made in various countries to use the analogues of luteinizing hormones-releasing hormones (LH-RH) for induced breeding of fishes with varying degrees of success. One of such alternatives is the use of ovaprim to induce ovulation in different species of fish.

The effects of LH-RH on Gonadotropin Releasing Hormone (GnRH) receptor capacity correlated very closely with changes in serum gonadotropin concentration. These investigations showed the blocking action of dopamine with dopamine receptor antagonists, potentiates the action of LH-RH-a in gonadotropin release. Such investigations led to the development of "Linpe method" (Peter *et al.*, 1988), where in the LH-RH-a is combined with dopamine antagonist for successful spawning.

The usual dopamine antagonists like pimozide or domperidone are used in combination with Luteinising Releasing Hormone antagonist (LRH-a) for spawning carps. As domperidone or pimozide are not dissolved in water, its application encountered certain problems in fish breeding. 'Ovaprim', a ready to use drug in solution form, manufactured by Syndel laboratories in Canada has overcome such problems. With reference to the chemical constituents of ovaprim, it contains calibrated amount of S-GnRH (Salmon Gonadotropin Releasing Hormone) and domperidone, a dopamine antagonist dissolved in an organic solvent. ***It contains an analogue of 20 µg of Salmon gonadotropin releasing hormone (sGnRHa) and a dopamine antagonist, domperidone at 10 mg/ml (Hill et al., 2009). The dopamine***

antagonist, domperidone used in ovaprim is also reported to be better than another commonly used antogonist, pimozide. Ovaprim being a ready to use product and one which does not require refrigerated storage, appears to be the most convenient and effective ovulating agent (Brzuska, 2006).

This drug is administered to both female and male brood fish simultaneously in a single dose. This reduces not only the handling of brood fish but also helps in saving considerable amount of time and labour which will add on to the cost of seed production. The efficiency of ovaprim for induced breeding of carps have given highly encouraging results in common carp, Catla, Rohu, Mrigal, Silver carp, Grass carp, Big head, etc. The effective dose required for various species of carps is found to vary considerably (Naeem et al., 2005a).

Leu and Chou (1996) experimented induced spawning and larval rearing of captive yellow porgy, *Acanthopagrus latus* (Houttuyn) by single injection of ovaprim (S-GnRH-a in combination with domperidone) 1000 *IU* per kg or a combination of HCG (500 IU per kg) and ovaprim (0.25 ml per kg). Ovaprim at a dose of 0.5 ml per kg was found most effective. Fertilization rates ranged between 72 to 84% and hatching rates between 60 and 88%. Larval rearing trials of 50-56 days for growth and survival produced juveniles of 20mm average total length and survival ranging from 0.7 to 27.5%.

Nandeesha *et al.* (1990b) tested Ovaprim C for its induced breeding efficacy in three species of Indian major carps, viz. Catla (*Catla catla*), Rohu (*Labeo rohita*) and Mrigal (*Cirrhinus mrigala*). Breeding response of these carps were evaluated vis-a-vis that obtained in the controls, injected split doses of carp pituitary extract or a single dose of carp pituitary extract or a single dose of ovaprim solvent. They found out that all the three species could be bred with a single intramuscular injection of Ovaprim at 0.5 ml/kg body weight. Mrigal responded positively to even lower dosages of 0.3 and 0.4 ml/kg, but 0.4 ml/kg was found to be the minimum dosage required for Rohu. In all these trials, males were injected with carp pituitary extract at 3-4 mg/kg, 6 hr after injecting ovaprim to females. In another trial, wherein female and male Rohu were injected simultaneously with ovaprim at 0.4 and 0.15 ml/kg, respectively, the spawning response was excellent. As against this, the control fish receiving pituitary extract in a single dose failed to respond. The percentage of fertilization in most cases ranged from 70 to 99%. The results of their investigation clearly indicate the suitability of Ovaprim-C for inducing breeding of the Indian major carps.

Naeem *et al*. (2005a) also carried out an induced spawning of Major Carp *Catla catla* by a single intramuscular injection of ovaprim. Fish were spawned successfully following the single injection of ovaprim at a dosage of 0.7ml/kg for female and 0.2ml/kg of male brooders at a temperature of 26°C, the percentage fertilization and hatching was 91.01 and 67.50% respectively.

Naeem *et al*. (2005b) obtained fertilization of 72.56% and hatchability of 71.09% when they induced silver carp (*Hypophthalmichthys molitrix*) to spawn with ovaprim through single application at dosage of 0.6ml/kg and 0.2ml/kg for female and male respectively with temperature ranging 24.8-25.8°C. Based on their findings on experiments with ovaprim, it is therefore concluded that ovaprim usage may have the following advantages over that of commercial pituitary.

i. Reduced handlings of brood fish due to the single injection given to both the sexes simultaneously. This not only well decreased/avoid post spawning mortality of fish but also increase spawning response

ii. Additional studies may provide valuable information on the growth of hatchlings.

iii. Additional experiments are essential to confirm the impact of ovaprim on the growth and survival of hatchling.

Froud *et al.* (2010) experimented on the effect of ovaprim, ovatide and other inducing agent in Benni (*Barbus sharpeyi*). 56 fish were divided into 7 treatments and injected intramuscularly with 4mg kg^{-1} body weight of Carp Pituitary Extract (CPE) as positive control, Propylene glycol as negative control, 0.5 mg kg^{-1} bw of Ovaprim, 0.5 mg kg^{-1} bw of Ovatide, 1000 IU kg^{-1} bw of HCG, 10µg kg^{-1} bw of LHRH-A2, 10µg+2 mg kg^{-1} bw of LRHa+CPE in double injection of 10 hours apart. Results showed that LRHa+CPE combination yielded 87.5% spawning success in comparison with HCG, Ovaprim, Ovatide, LRHa and CPE, none of fish were ovulated in the groups of negative control, HCG and LRHa, while 3/8 fish were ovulated in the group of Ovaprim and Ovatide (37.5%). 6/8 fish were ovulated in the group of CPE (75%). Spawning success of 37.5%, latency period of 25.2±0.1, fertilization success of 85.26±2.54 and hatching rate of 77±1.36 was found when the fish was induced with ovaprim through single application of Ovaprim at water temperature of 15-17°C.

Ghosh *et at*. (2012) researched on Induced breeding, embryonic and larval development of Koi carp (*Cyprinus carpio*) in Khulna, Bangadesh. The aim of the experiment was to perform induced breeding and observe embryonic and larval development stages in different seasons for mature male and female *Cyprinus carpio*, which was achieved by administering the synthetic hormone Ovaprim, in a single dose for males and divided dose for females at 0.4-1.0 ml/kg body weight via intramuscular injection. Spawning occurred between 5-6 hrs after the 2nd injection in summer (April) at a temperature of 26-29°C and 17-20°C in winter (December). Using doses of 0.4ml/kg, 0.7ml/kg and 1.0 ml/kg of ovaprim, the fertilization rate of eggs were obtained at 42.31%, 54.55% and 61.82 % in summer and 26.92%, 38.00% and 39.23 % in winter respectively. Hatching rates were 42.78%, 44.60% and 55.00 % in the summer season and 30.22%, 31.01% and 31.67 % in the winter season with the same doses of ovaprim. The fertilized eggs were adhesive, transparent, and spherical with diameters ranging between 0.8mm and 1.0 mm and yellowish white in colour. Due to the positive response of Koi carp to Ovaprim, considerable fertilization and hatching rate, short embryonic period and larval development, it is possible to conduct a successful breeding programme of this *Cyprinus carpio* commercially.

Malik *et al.* (2014) studied the effect of ovaprim hormone on the spawning of Koi carp at a fish hatchery in Pakistan. Females having average weight of 536.7±6g were injected single dose of ovaprim intraperitonially, they obtained an average number of eggs/kg, average number of fertilized eggs/kg and average number of hatchlings of 9930, 74534 and 622 respectively. Fertilization and hatching percentage was 75.2 and 83.3% respectively, at a temperature range of 20-24°C, water pH range of 7.2-7.6mg/L and dissolved oxygen range of 5.4-5.8ml/L. Hence from their findings it was concluded that successful use of ovaprim hormone in artificial ovulation in common carp fishes is well accepted (Sharma and Singh, 2002; Szabó, 2003). Generally, fish response with Ovaprim was well found by considering the ratio of successful breeding, fecundity, fertilization rate and hatching rate. In carp fishes ovaprim hormone solve many problems in carp seed production.

2.4 The Effect of Temperature on Breeding Performance

Many aspects of fish life are controlled by different levels of environmental and biological factors, such as temperature, food availability, pollution, predation or a combination of these factors (Kupren *et al.*, 2011). Temperature is one factor that affects greatly the breeding performance of fish species, temperature do not just affect the duration of time between inducement and ovulation of fish species but affects to a great extent the interval between the start of embryonic development (Fertilization) and hatching (Haniffa *et al.*, 2007). Temperature affects virtually all biochemical and physiological activities of an animal, it should therefore be viewed as an environmental factor which evokes multiple effects on an animal’s reproduction.

Arabaci *et al.* (2001) in their research on the induction of spawning in common carp using Luteinising Hormone Releasing Hormone antagonist combined with Haloperidol (LHRHa+HAL): Effect of different treatment time and determination of Latency Period dependence on temperature. Time between injection and initial egg release in fish treated with LHRHa combined with domperidone was 28-30 hour. However, the administration of LHRHa+HAL in their study, found shorter latency of 14-16 hour at water temperature of 24°C, latency was negatively correlated with water temperature in LHRHa+HAL injected fish.

When eggs of common carp were naturally fertilized and incubated at different temperatures 20, 24, 27, 35 and 38°C for a period of 70 hour of fertilization by El-Gamal Abd (2009), the results of his findings indicated that the optimum percent of healthy eggs was 77% at temperature of 27°C, followed by 59% at temperature of 30°C. However, no eggs were survived to hatch at neither 20°C nor 38°C. The hatching rate at 27°C and 30°C increased than the other treated groups. The lengths of newly hatching larvae were 3.3±0.015 mm at 24°C and yolk volume ranged from 0.421±0.005 to 0.676±0.005µl. It likely seems that, the yolk volume of newly hatching larvae varied inversely with increasing of water temperature. The growth of larvae increased at the optimum temperature from 27-30°C.

Yang and Chen (2005) found out that the rate of embryonic development to hatching was accelerated with increase in incubation temperature, the time taken for 50% of the embryos to hatch at 15, 19, 23, and 27°C were 11.3, 6.6, 5.0, and 4.2 days, respectively. There were significant differences in time to 50% hatch among all temperatures of this experiment Lower survival of obscure puffer eggs during early development at temperature of 15°C suggested that egg incubation at or below this temperature are not suitable for this species. Although there was no significant difference in total hatch rate and total mortality rate among the three higher temperatures, trend towards lower total hatch rate and higher total mortality rate existed at the warmer temperature of 27°C. Therefore, the optimal temperature for incubating obscure puffer eggs is between 19 and 23°C according to the results of total hatch rate, viability of newly hatched larvae 24 hours post hatching and total mortality rate of eggs incubated at 15, 19, 23, and 27°C. Within a viable range, incubation temperature strongly affects the rate of embryonic development of fish. Generally, lower temperature retards the rate of embryonic development and higher temperature accelerates it, the time from fertilization to 50% hatch of obscure puffer eggs decreased from 11.3 days at 15°C to 4.2 days at 27°C and was consistent with the widely observed phenomena in many other fishes.

Oyelese (2006), researched on the effect of water temperature on fertilization and hatchability rates of artificially induced *Clarias gariepinus* in Nigeria. In the research sixty six (66) broodstock with average weight of 398g were conditioned for two weeks after which some were sacrificed for fresh pituitary and milt collection. Female spawners were injected at different hours of the day (8.00am, 4.30, 8.00 and 12.00pm) at average temperature of 26.00, 24.30, 27.66 and 25.00°C respectively and stripped after 10 hours latency for eggs collection. Significant variations exist between water temperatures at the time of injection and fertilization/hatchability rate. It was further confirmed by a positive correlation coefficient of $r=0.95$ ($p<0.05$) in both fertilization and hatchability rates. The highest hatchability rate of 77.84% and highest fertilization rate of 66.65% was reported at 8.00pm with water temperature of 27.66°C. This was followed by 75.51% at 26.00°C hatchability rate while 51.23% fertilization rate was obtained for fish induced at 8.00am. Increase in temperature leads to increase in the fertilization and hatchability rate of *Clarias gariepinus.*

Fahad *et al.* (2010) experimented on the effect of varying holding temperatures on hatching success, occurrence of deformities and mortality rates were investigated for goldlined seabream eggs. Wild broodstock (600 g) were stocked at a 2:1 male-female ratio in a $2m^3$ fiberglass tank supplied with filtered seawater (37 g L^{-1} salinity, temperature range 24±0.5°C [day] and 22±1°C [night], DO_2 in excess of $5.0mgL^{-1}$). Females were injected with 200 IU kg^{-1} HCG between 08.00 and 10.00 hours and returned to tanks to spawn after which, eggs were collected by hand using a 100µm net. Fertilized eggs at the gastrulation stage (120 L^{-1}) were randomly placed into one of 12 experimental 6 L aerated (DO_2 5 mg L^{-1}) plastic containers with water temperatures maintained at 24±0.5°C (ambient), 26±0.5°C, 28± 0.5°C and 30±0.5°C using thermostats. No significant differences ($p<0.05$) were recorded between eggs reared at 24 and 26°C with respect to viability, deformity, mortality or unhatched egg rates. Increasing temperature reduced the number of viable eggs with those at 30°C returning poorest performance ($p<0.05$). Mortality levels were lowest for eggs incubated at 24 and 26°C. The greatest level of deformities recorded was that of eggs reared at 28°C.

Valeta *et al.* (2013) attempted to improve hatching success and fry production by assessing the effect of temperature on egg development in *Oreochromis karongae.* Temperature-dependent development rates and hatching periods for fertilized eggs stripped from *O. karongae,* a mouth brooder, were determined in a recirculating system set up in a hatchery. Three treatments namely; 25, 27 and 29°C, were replicated thrice in 2.0-L McDonald type incubation jars stocked with 265 fertilized stage 1 eggs of *O. karongae*, at water flow rate of 0.15 L/s. There was a curvilinear relationship between temperature and egg development, which was best described by a logarithm regression function. Hatching period decreased with increase in incubation temperature. The shortest hatching period was 7.3 days, which was observed at the highest incubation temperature (29°C) while the longest hatching period was 14.7 days, observed at the lowest temperature (25°C), hence, increase in temperature leads to significant decrease in the hatching period. Hatchability and fry survival were higher at higher temperatures.

Nowosad *et al.* (2014a) determined the effect of water temperature on the effectiveness of artificial reproduction of dace brooders under laboratory and field conditions. Three temperatures of 9.5, 12 and 14.5°C (±0.1°C) were tested in the laboratory. The water temperature under field conditions were 11.0±0.3 °C (CzarciJar Fish Farm) and 13.2±1.4 °C (Janowo Fish Farm). The study showed that artificial reproduction of dace is possible in all the temperature ranges under study and an embryo survival rate of over 87% was achieved. Dace has also been found to be very sensitive to rapid temperature changes, even within the optimal temperature ranges for the species. Such changes have an adverse effect on the outcome of the reproduction process, such as decrease in the percentage of reproducing females, pseudo-gonado-somatic index (PGSI) and embryo survival rate.

Orina *et al.* (2014) experimented on the optimal breeding environment for the cyprinid *Labeo victorianus* induced with ovaprim at temperatures of 22±1°C, 26±1°C and the control group where temperature was not controlled. They found out that latency period significantly varies among treatments. Temperature had a great effect on the spawning time, brooders under 26±1°C had a latency of 6-8 hours those under 22±1°C had 12-18 hours latency while the control group had latency of 16-18 hours. Fecundity significantly dependent on the length and weight of broodstock. They also found out that mean fertilization and hatching rate were both dependent on temperature, with temperature of 26±1°C being the best performer for all population having above 93% fertilization and 83% hatchability. Larval survival was directly dependent on temperature with 26±1°C having the highest larval survival (89%), while the control had the least survival (67%) but not significantly different from the 22±1°C temperature (71%).

2.5 Effect of female body weight on the fecundity of fish species

The body weight of female fish species has been reported by different researchers and fish farmers to have a great effect on the fecundity of the fish species.

Ataguba *et al.* (2012) researched on the effect of broodstock size combination in artificial spawning of cultured *Clarias gariepinus.* The broodstock used for the study was obtained from a homogenous population of same age (one year and three months), four body weight range groups were used A (200-300g), B (400-500g), C (600-700g) and D (800-900g). They found out that broodstock combination involving D females had the highest fecundity of 61700.0-74100.0, fertilization rate 77.3-83.7%

and hatchability rate of 74.4-88.7%. On the other hand, the broodstock combination A had the least fecundity of 20500.0-30400.0, fertilization rate of 53.5-69.5% and hatchability rate of 69.4-77.4%. Generally, from the study it was concluded that fecundity, fertilization and hatchability increased significantly with increase in broodstock body weight.

Hossain et al. (2012) worked on the relationships between body size, weight and fecundity of a threatened species Puntius ticto from the Ganges River, northwestern Bangladesh. A total of 24 mature female specimens were collected using the traditional fishing gears from March to August 2006. The results showed that Total Length of P. ticto varied from 9.10 to 10.80 cm, with calculated mean ± SD as 9.77±0.57 cm. Body weights extended from 14.00 to 24.00 g, with calculated mean ± SD as 17.83±3.39 g. The mean total fecundity was 2586±700 and ranged from 1611 to 4130. Body weight was more significantly correlated with total fecundity ($r=0.633$; $p<0.001$) than other body meristics.

2.6 The Biology of Carp

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 fish species that have a global distribution (Froese and Pauly, 2002).

2.6.1 Habitat

Carp exploit large and small manmade or natural reservoirs, and pools in slow or fast moving streams. They prefer larger, slower-moving bodies of water with soft sediments but they are tolerant and hardy fish that thrive in a wide variety of aquatic habitats. Best growth is obtained at water temperatures of 23-30°C. The fish can survive cold winter periods, salinity up to about 5‰ is tolerated, optimal pH is 6.5-9.0, low oxygen concentration (0.3-0.5 $mg.l^{-1}$) as well as super-saturation (Flajshans and Hulata, 2007).

2.6.2 Physical description

Carp often grow up to 30-60cm in length and 0.5-4 kg in weight, males are usually distinguished from females by the larger ventral fin. Carps are characterized by their deep body and serrated dorsal spine. The mouth is terminal on the adult and sub terminal on the young. Colour and proportions are extremely variable, but scales are always large and thick. Three sub-species with slightly different scale patterns are recognized; *C. carpio communis* (scale carp) has concentric scales, *C. carpio specularis* (mirror carp) with large scales running along the side of the body in several rows with the rest of the body naked, and *C. carpio coiaceus* (leather carp) with few or no scales on the back and a thick skin (Flajshans and Hulata, 2007).

2.6.3 Reproduction

Carp generally spawn in the spring and early summer depending upon the climate but in the tropics the fish can spawn year round. Males externally fertilize eggs, which the female scatter, incubation is related to water temperature and has been documented at three days at temperature of 25 to 32°C. Fry usually ranges from 5 to 5.5 mm in total length. Temperature, stocking density, and availability of food influence individual growth. By the time the fish reach 8mm the yolk has disappeared and they begin to actively feed (Chumchal, 2002).

2.6.4 Food habits

Carp are primarily selective benthic omnivores that specialize on invertebrates that live in the sediments, newly hatched carp initially feed on zooplankton, specifically rotifers, daphnia, copepods, and algae. Carp feed by sucking up mud from the bottom ejecting it and then selectively consuming items while they are suspended (Freyhof and Kottelat, 2008).

2.7 History of Common Carp (*Cyprinus carpio*) Culture in Nigeria

The introduction of common carp (*Cyprinus carpio*) to Nigeria in 1952 coincided with one of the first attempt to culture fish commercially in the country at the newly constructed government fish farm at Panyam, in the central Jos Plateau region (Baker, 1994). The original common carp broodstock were brought to Panyam from central Europe, as 100g fingerlings, by the fathers of the Panyam fish farm, an Austrian, K. K Zwilling. They consisted of 50% fully scaled and 50% mirror varieties, derived principally from the Dinkel Bula strain produced in Bavaria, Germany.

Breeding of this fish was very successful since its introduction to the country and their progenies were soon to be found throughout Nigeria not until 1980 during an FAO/UNDP project that carp bred for commercial production had seriously deteriorated genetically, due to lack of any programme of selective breeding from 1952. Other introduction of the fish species into Nigeria were achieved by the Rockwater fish farm (Raynage Fish farm at present) from United Kingdom and from Israel. At present the Panyam fish farm established by the government and the Raynage fish farm depends on natural reproduction of Common carp hence their inability to make the fish species commercially available to both farmers and consumers.

The above findings of collected literature suggest that the application of synthetic hormones and hypophysis for inducing ovulation and successful breeding and achieving quality egg and larval production of fish species. Many species of fish will not readily reproduce under certain culture conditions. Others will, but not necessarily when the farmer desires. In these cases, induction of spawning can be of great value (Murthy *et al.,* 2013). Methods vary from species to species and situation to situation. However, at least two generalizations can be drawn. First, brooders are very vulnerable to rough handling. Care should always be used to avoid damaging these valuable animals. Second, a fish that does not have mature gametes will not produce viable eggs or sperm no matter how many times it is injected with hormones. Ripeness is the result of environmental factors working over a period of time, leading to maturation of the gonads and production of viable eggs (Rath *et al.,* 2007). Many procedures have been developed for inducing fish to undergo the last steps of spawning. Farmers should thoroughly research the procedures that have been developed for their species of fish through experimentation, and select those that best suit the circumstances. In addition, once the fish have spawned, there are many techniques involved in incubating and caring for the eggs, and caring for the hatched fry. These too must be thoroughly researched.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Fish Collection

Two years old broodstocks of *Cyprinus carpio* weighing 800g to 2500g were obtained from Panyam Fish farm in Jos and transported in oxygenated polythene bags to the Department of Biological Sciences, Ahmadu Bello University, Zaria, where they were kept in the fish pond to acclimatize for the period of three weeks.

During this period, the broodstocks were fed on commercial feed (Coppens) containing about 56% crude protein. The fish were fed two times daily at the rate of 3% body weight and starved 24 hours prior to the breeding exercise.

3.2 Identification of Gravid Brooders

At the time of breeding the male and female brood fishes were identified on the basis of their secondary sexual characters. The ripe males were identified by roughness of the dorsal surface on the pectoral fin, which on the contrary was very smooth in the female. The roughness in pectoral fins was felt by touching the surface of fin close to the body. The matured male and female fishes were also distinguished from the shape of their body, condition of the vent and secretion of milt in males (Charula, 2008). The detailed key characters adopted for distinguishing and identification of the mature male and female are explained in Table 3.1.

3.3 Experimental Design

A total of Eighteen (18) broodstock (9 female and 9 male) having body weight ranging from 800grams to 2500grams were used for this experiment.

Table 3.1: Details of the Characteristics Features of Sexually Matured Female and Male Common Carp used for the Induced Breeding Trials

S/No	Characters	Female	Male
1.	Scales	Scales smooth and silky	Rough with sandy texture
2.	Operculum	Operculum smooth	Rough with sandy tubers
3.	Pectoral fin	Pectoral fins very smooth and slippery, slightly smaller in length	Rough with sandy touch, particularly on the dorsal side, slightly stouter and longer
4.	Abdomen	Bulging out on both sides, soft and palpable. A distinct cleavage is formed midventrally along the abdomen when the fish is placed on its back.	Round and firm. Not very soft to the touch
5.	Vent	Round slit extrovert (convex) fleshy and pinkish in colour, papillae prominent.	Elongated slit, introvert (concave), white in colour
6.	Putting pressure on abdomen	Yellowish discharge or a few ova may come out through the vent. In very mature condition, ova in a chain would slip out on slight pressure	On slight pressure above the vent on the abdomen milky white fluid (Milt) runs out through the vent.

Source: Charula (2008)

The brooders were divided into three groups (I, II and III) of 3 females and 3 males, each of the group of brooders were bred in three different months with different water temperatures. Group I were bred in January with water temperature of 23±1°C, February with water temperature of 26±1°C for group II and March with water temperature of 29±1°C for group III (Table 3.2).

To determine the effect of body weight of female brooders on their fecundity, fishes were divided into four body weight groups A (800-1199g), B (1200-1599g), C (1600-1999g) and D (2000-2400g). Each body weight group had two female brooders with similar body weight.

3.4 Inducing Agent (OVAPRIM)

Ovaprim a synthetic hormone produced by Syndell Laboratories, Canada, was used for induction of reproduction in *C. carpio*. This product is a liquid synthetic formulation containing D-Arg^6, Pro^9, NEt S-GnRH and domperidone. It contains 20 mg of salmon gonadotropin and 10 mg of domperidone per ml of injectable solution dissolved in an organic solution.

Single application of ovaprim was used as described by Nandeesha *et al.* (1990b), both female and male *C. carpio* were administered single dose of the inducing hormone intramuscularly at the base of the last ray of the dorsal fin with a hypodermic syringe after cleaning the area with a clean dry towel. Female brooders received a dosage of 0.5ml/kg body weight while males received 0.2ml/kg body weight (Naeem *et al.*, 2011).

Table 3.2: Experimental design

Group	Ratio of Female to Males breeders	Temperature (°C)	Period
I	3/3	23±1	January
II	3/3	26±1	February
III	3/3	29±1	March

3.5 Determination of Latency Period of *C. carpio*

The broodstock were administered with ovaprim and returned to the circular plastic tank containing 100 litres of water under aeration. The fish were left undisturbed for about 10 hrs after which period they were checked at 30 minutes interval for ovulation indicated by the release of eggs in the experimental tank (Arabaci *et al.,* 2001). When eggs are observed at the bottom of tanks the fish are immediately brought out and stripped. The duration of time from inducement with ovaprim to ovulation was recorded as the latency period of the fish.

3.6 Stripping and Determination of Fecundity of *C. carpio*

Eggs were stripped into a clean dry plastic bowl by a gentle massage of the abdomen with slight pressure on the lower sides of the females from anterior to posterior region of the fish until the ovaries are empty or when a drop of blood appeared (Plate I).

To determine the fecundity of spawners, the eggs obtained were immediately weighed using sensitive weighing balance (Sartorius CP8201), the weighing balance was kept in air tired environment to avoid interference. The weighing balance was initially at zero before placing the plastic bowl containing the eggs on the weighing balance. One gram of the eggs was then weighed in triplicates and the number of eggs contained in the one gram egg was counted to obtain the estimate of the total number of eggs spawned by the female fish (Naeem *et al.,* 2013).

3.7 Fertilization and Stickiness Elimination

Fertilization was accomplished using the ‘dry’ method, i.e. the milt was added directly into the plastic bowl by stripping the male brooder. The egg with the milt was stirred gently with a chicken feather and clean fresh water was added to activate the eggs before eliminating the stickiness.

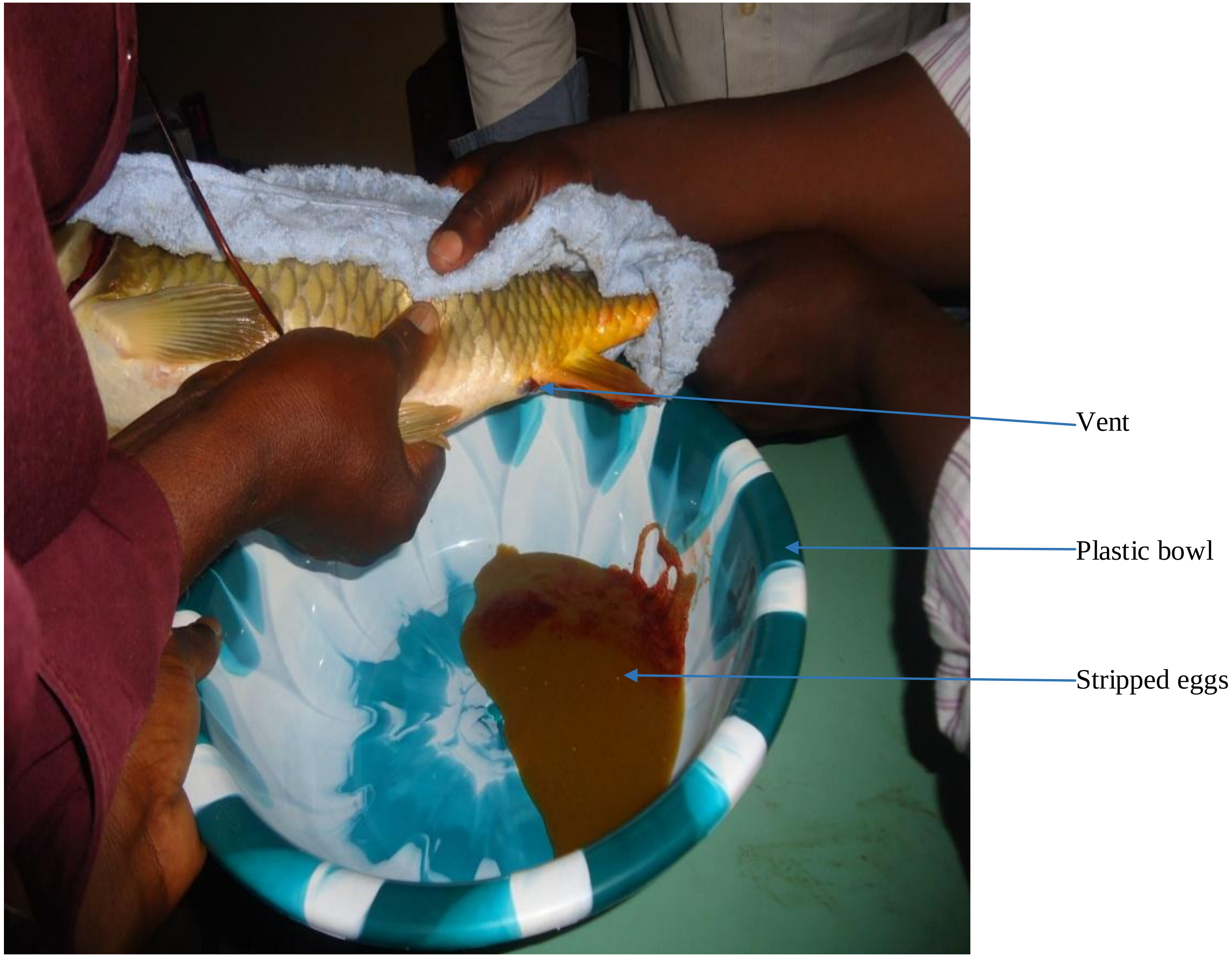

Plate I: Stripping of *Cyprinus carpio* eggs

Eliminating egg stickiness is one of the most critical treatments in artificial reproduction of common carp when incubating jars are to be used, the treatments reduces the adhesive effect of the cortical envelope to avoid mass egg adhesion. Stickiness elimination enables the use of incubators, enhances embryonic development, raises hatching, and facilitates controlling and effecting of ambient conditions (Hussein and Al Hazzaa, 2003). In other to remove stickiness, 6.0 grams of NaCl dissolved in one litre of water was then added to the mixture of milt and egg then thoroughly stirred for two minutes. A composition of 10.0 grams full cream powdered milk (Nido) and 1.2 grams of NaCl per litre of water added to cover fertilized eggs in the tray. The mixture were stirred softly and continuously for 40 minutes (Hussein and Al Hazzaa, 2003).

3.8 Percentage Fertilization

For estimation of the fertilization rate, three sub samples' of water hardened eggs were taken from each set and the number of fertilized egg out of total eggs produced in each sub sample was counted (Garg *et al.,* 2002). The fertilized egg percentage was then calculated by the following formula:

$$\text{Percentage Fertilization} = \frac{\text{Total number of fertilized eggs}}{\text{Total number of eggs produced}} \times 100$$

3.9 Incubation and Hatching of eggs

Milk treated eggs were rinsed several times with fresh water and transferred into the McDonald incubator jars. Incubators were receiving fresh borehole water, which was circulated using the water recirculatory system; water flow rate (6 Litre per minute) in each incubator was adjusted so that the fertilized eggs rotate gently in such a way that the eggs are not carried out of the incubators by the flowing water gravity.

The incubators keeps the eggs oxygenated, suspended and in constant motion from the water inflow (Plate II). Dead eggs were siphoned off several times a day to keep all jars clean in other to prevent infection of eggs by fungi. In some cases where fungal infections were observed the eggs were treated with potassium permanganate (Akankali *et al.*, 2011).

The embryos develop into larva and hatch by breaking out of the egg. Hatching is by mechanical process, aided by enzymes. Immediately after hatching the larvae remains in the jar kept in motion by the water current for some time, after which the swim bladder is develops. The swim bladder enables the larvae to swim vertically and are carried by water current from the hatching jars into the aquaria tanks

3.10 Hatching Duration

The hatching duration is the average time required from initiation of incubation process to complete incubation of eggs in each set of experimental trial. The duration of time from the fertilization to the hatching of the eggs of *C. carpio* was observed and expressed in hours as the hatching time in each of the temperature groups.

3.11 Determination of Percentage Hatchability

The numbers of hatchlings were estimated using the volumetric method. The hatchling after swimming into the plastic aquaria containing a known volume of water, a sample of 1 litre was collected in triplicate and the number of hatchlings was estimated from the samples. The percentage hatchability was calculated from the number of fry counted as described by Oyelese (2006).

$$\text{Percentage hatchability} = \frac{\text{Number of hatched larvae}}{\text{total no. of fertlized eggs}} x100$$

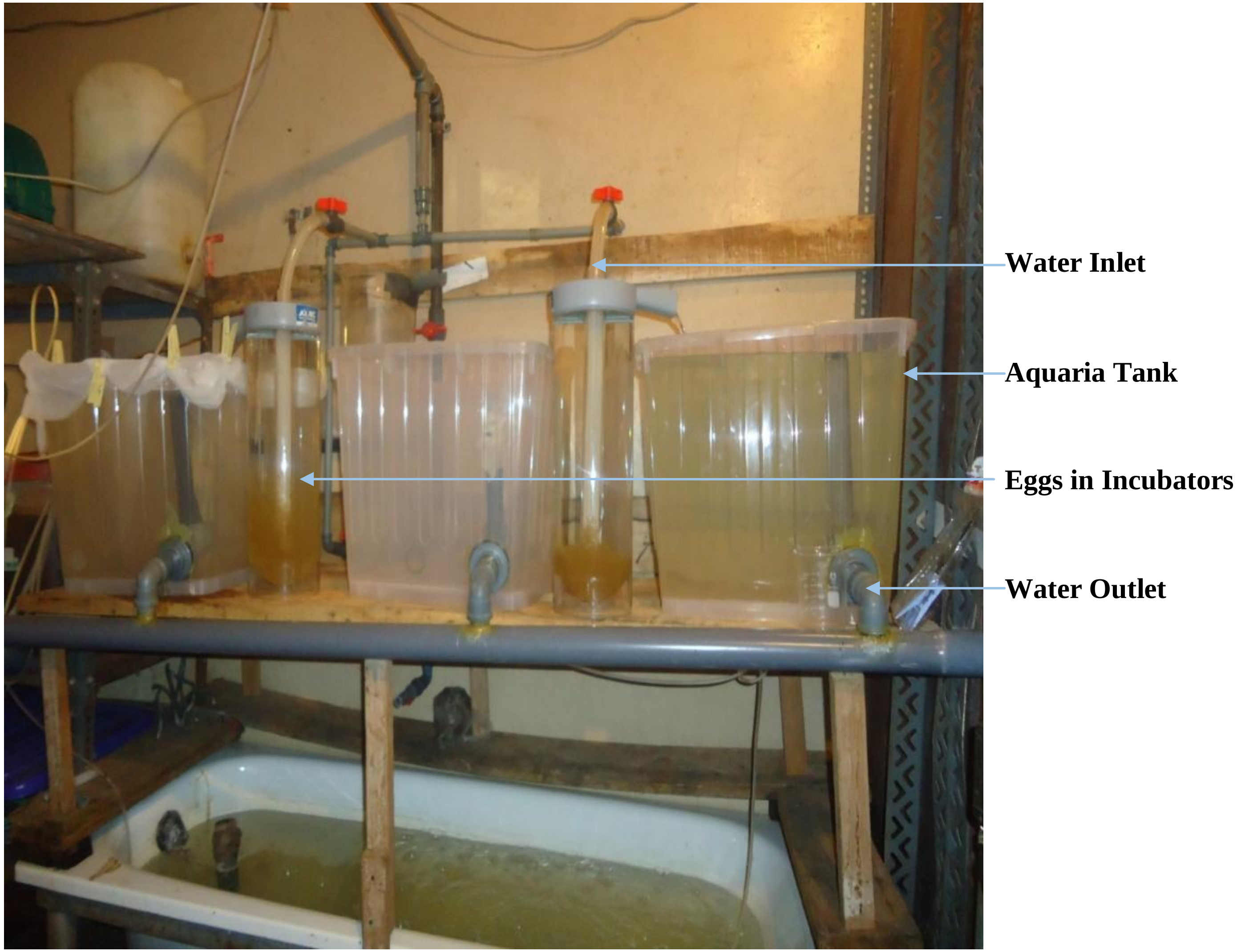

Plate II: ***Cyprinus carpio*** **eggs incubating in McDonald jar**

3.12 Percentage Survival

Five hundred (500) larvae in triplicates were removed from the total spawn produced by each set of brooders and were reared for four weeks (28 days). During this period of rearing the hatchlings were fed with zooplankton twice a day (Charula, 2008). Zooplanktons were harvested from concrete ponds and cultured in a large plastic bowl; the culture medium was fertilized with organic manure. Larval survival was observed (Plate III) and calculated as follows:

$$\text{Percentage Survival} = \frac{\text{Number of survived larvae}}{\text{total number of hatched larvae}} x100$$

3.13 Water Quality Analysis

Selected water quality parameters of water used in breeding process, for conditioning of broodstocks after inducement and for incubation was monitored every three (3) hour interval. Water parameters for larval rearing were monitored weekly. The water during incubation and seed rearing was aerated at least for 10 hours a day. The following physico-chemical parameters of water were analysed as follows;

3.13.1 Temperature

The temperature of water was noted directly by immersing the sensor of the mercury in glass thermometer in the water of the experimental tanks, after stabilizing the reading, the temperature was noted up to single decimal point in °C.

3.13.2 pH, Electrical Conductivity (EC) and Total Dissolved Solids (TDS)

The pH, Electrical conductivity and total dissolved solids of water in the experimental tanks were measured with the help of a multiparameter HANNA instrument (Model: HI98129). The measurement was done by selecting the desired parameter to be measured on the instrument then immersing the sensor into the water for one to two minutes before taking the reading.

Plate III: Some survived *Cyprinus carpio* fry

3.13.3 Dissolved Oxygen (DO)

The dissolved oxygen of sample waters was determined using the Winkler's titrimetric methods as described by Chiya and Nakai (1995);

Water sample was poured into a 300ml BOD bottle and 2ml $MnSO_4$ solution and 2ml alkali-iodide azide reagent was added then stoppered with care to exude air bubbles, it was the mixed gently by inverting the bottle a number of times until a clear supernatant was obtained. The solution was then allowed to settle for two minutes after which 2ml conc. H_2SO_4 was added by allowing the acid run down the neck of the bottle, it was stoppered again and mixed by gentle inversion until dissolution was complete. 100ml of the prepared solution was transferred into a conical flask and titrated with 0.012N of $Na_2S_2O_3.5H_2O$ solution to a pale straw/yellow colour. 1ml of freshly prepared starch solution was added and the colour becomes blue, titration was continued by adding the thiosulphate drop wise until the blue colour disappeared.

3.14 Data Analysis

The statistical analysis of the data was done using the analysis of variance (ANOVA) to test for significance difference at $p \leq 0.05$. ANOVA was also carried out to test for the significance difference ($p \leq 0.05$) between the different body weight of the female *C. carpio* and weight of eggs produced as well as the fecundity. Where there was significant difference Duncan's Multiple Range Test (DMRT) was carried out to separate means. Correlation analysis (r) was carried out to determine the relationship between temperature and the various breeding parameters and the relationship between body weight of female fish and its fecundity. Principal Component Analysis (PCA) was also carried out to determine the temperature at which *C. carpio* performs best during the breeding process.

CHAPTER FOUR

4.0 RESULTS

The results regarding the various aspects of breeding performance that is latency, fecundity, viability, fertilization, hatching duration, hatchability, and survival percentage of *C. carpio* induced ovaprim as affected by different temperatures from the experiment are presented and compared here under.

4.1 Effect of Temperature on the Latency Period of *C. carpio*

The results in Table 4.1 shows that latency period reduces with increased in temperature, at 23±1°C the latency was 16.55±0.13 hrs, at 26±1 and 29±1°C, latency was 13.00±0.13 and 11.32±0.07 hrs respectively. From the same table, the statistical analysis of result (ANOVA) indicates that there was a high significant difference ($p \leq 0.05$) between latency periods at different temperature regimes. From the result of the correlation analysis (Table 4.2) there is a strong negative relationship between temperature and latency ($r = -0.98$, $p \leq 0.05$).

4.2 Effect of Temperature on the Weight of Eggs, Fecundity and the Viability of Eggs of *C. carpio*

The mean weight of female fish and weight of eggs produced by the three groups (I, II and III) of fish are 1717.67±462.82, 1639.50±199.50 and 1632.33±373.90g and 76.67±23.15, 85.70±7.10 and 82.87±14.47g respectively (Table 4.1). Statistically, there was no significant different ($p > 0.05$) between the weight of eggs produced by *C. carpio* at different temperatures even though there was an increase in the weight of eggs produced by fish induced at temperature of 23±1 to 26±1°C and a slight decrease from 26±1 to 29±1°C.

Table 4.1: Breeding indices of *Cyprinus carpio* induced with ovaprim at different temperatures

Parameters	**Temperature (°C)**			**LSD Mean±SE**	**P value**
	23±1	**26±1**	**29±1**		
Weight of Female Fish (g)	1717.67±462.82[a]	1639.50±199.50[a]	1632.33±373.90[a]	**1666.13±198.95**	0.986ns
Latency (Hrs.)	16.55±0.13[a]	13.00±0.13[b]	11.32±0.07[c]	**13.70±0.87**	0.000**
Weight of Eggs (g)	76.67±23.15[a]	85.70±7.10[a]	82.87±14.47[a]	**81.25±9.15**	0.942ns
Fecundity	53666.67±16207.85[a]	59990.00±4970.00[a]	58006.67±10126.67[a]	**56875.00±6401.72**	0.942ns
Number of Viable Eggs	52356.50±15744.86[a]	58281.00±4471.00[a]	55742.87±9806.31[a]	**55107.51±6193.93**	0.950ns
Percentage (%) Fertilization	72.67±1.76[ab]	89.00±3.00[a]	52.30±10.49[b]	**69.11±6.51**	0.047*
Hatching Duration (Hrs.)	41.96±0.42[a]	37.18±0.04[b]	28.98±0.96[c]	**35.87±2.15**	0.000**
Percentage (%) Hatchability	46.83±0.84[b]	67.50±2.50[a]	28.58±10.08[c]	**45.16±6.62**	0.033*
Percentage (%) Survival	15.27±0.52[b]	33.20±1.60[a]	30.13±6.29[a]	**25.33±3.64**	0.062ns

Means along the same row with different superscript alphabet(s) were significantly different ($p \leq 0.05$), ns-no significant difference ($p > 0.05$)

Table 4.2: Relationship between temperature and various breeding indices of *Cyprinus carpio*

	Temperature	Wt. of female fish	Latency	Wt. of egg	Fecundity	No. of viable eggs	% fertlization	% hatchability	Hatching time
Wt. of female fish	-0.07								
Latency	-0.98*	0.06							
Wt. of egg	0.11	0.97*	-0.14						
Fecundity	0.11	0.97*	-0.14	1.00					
No. of viable eggs	0.09	0.97*	-0.12	1.00	1.00				
% fertilization	-0.51*	0.23	0.37	0.18	0.18	0.20			
% hatchability	-0.45*	0.06	0.30	0.08	0.08	0.09	0.94*		
hatching time	-0.98*	-0.02	0.94*	-0.18	-0.18	-0.15	0.62*	0.59*	
% Survival	0.67*	0.18	-0.76*	0.34	0.34	0.33	0.26	0.30	-0.56*

*** Shows significant (p≤0.05) relationship between the two parameters which intercepted**

Similarly there was an increase in the fecundity of fish from 53666.67±16207.85 in 23±1°C to 59990.00±4970 in 26±1°C then a decrease to 58006.67±10126.67 in 29±1°C temperature group. Statistically there was no significant difference ($p>0.05$) in the fecundity of *C. carpio* in the three temperature groups as depicted in (Table 4.1).

There was also an increase in the viability from 52356.50±15744.86 in fish induced at temperature of 23±1°C to 58281.00±4471.00 in 26±1°C then a decrease to 55742.87±9806.31 in 29±1°C temperature group fishes. Statistically there was no significant difference in the viability of fishes in the different temperature groups ($p>0.5$).

From the correlation results in Table 4.2 temperature had low relationship with the weight of eggs produced and the fecundity of *C. carpio* ($r = 0.11$) as well as the viability of eggs ($r = 0.09$).

4.3 Effect of Female Body Weight on the Fecundity of *C. carpio*

The result of the effect of female body weight on the fecundity of *C. carpio* is presented in Table 4.3. The result shows that increase in female body weight leads to significant increase in the weight of eggs and the fecundity of *C. carpio*. Fish in group A with the mean body weight of 899±39g spawn eggs of 45.5±10.5g with fecundity of 31850±7350, group B fish with mean body weight of 1589.5±149.5g produced eggs weighing 82.8±4.2g with fecundity of 57960±2940 eggs. On the other hand group C and D fish with average body weight of 1842±3.0 and 2334±114 g respectively had 86.4±6.4 and 110.3±4.7g weight of eggs resulting into corresponding fecundity of 60480±4480 and 77210±3290 respectively. Statistically, there was significant difference in the fecundity of *C. carpio* in the different female body weight groups ($P \leq 0.05$).

Regarding the relationship between female body weight of *C. carpio* and the weight of eggs, fecundity and the viability of eggs, there is a strong positive correlation ($r = 0.97$) between the body weight of female *C. carpio* and the weight of eggs produced and also fecundity. Also from the same correlation Table 4.4 there was a very low relationship between female body weight of *C. carpio* and number of viable eggs produced by the same fish ($r = 0.03$, $p > 0.05$).

4.4 Effect of Temperature on the Fertilization, Hatching Duration and Hatchability of *C. carpio* Egg

4.4.1 Fertilization

The result in Table 4.1 shows that fertilization rate increase from 72.67±1.76% in group I fish induced at temperature of 23±1°C to 89.00±3.00 at 26±1°C then decreases drastically to 52.30±10.49% at 29±1°C. The statistical analysis of results of fertilization rate shows a significant difference ($p \leq 0.05$) in the fertilization rate between fishes induced in the month of February (26±1°C) and March (29±1°C) while there is no significant difference between fishes induced in January (23±1°C) and February (26±1°C), there is also no significant difference in fertilization rates of fishes induced in January (23±1°C) and March (29±1°C). The fish induced in the month of February had the highest percentage fertilization while those induced at 29±1°C temperature had the least percentage fertilization. The result of relationship between temperature and fertilization rate (Table 4.2) showed temperature was negatively correlated ($r = -0.51$ $p \leq 0.05$) with fertilization rate of *C. carpio*.

Table 4.3: Effect of female body weight on the weight of eggs and fecundity of *Cyprinus carpio*

Group	Mean weight of female fish (g)	Mean weight of eggs (g)	Fecundity
A (800-1199g)	899.0±39.0[c]	45.5±10.5[d]	31850±7350[d]
B (1200-1599g)	1589.5±149.5[b]	82.8±4.2[c]	57960±2940[c]
C (1600-1999g)	1842.0±3.0[b]	86.4±6.4[b]	60480±4480[b]
D (2000-2400g)	2334.0±114[a]	110.3±4.7[a]	77210±3290[a]
P value		**0.012**	**0.012**

Means across the same column with different alphabet(s) were significantly different (P≤0.05)

Table 4.4: Relationship between female body weight, weight of eggs and fecundity of *Cyprinus carpio*

	Body weight of fish	Weight of egg	Fecundity
Weight of egg	0.97*		
Fecundity	0.97*	1.00	
% Viable eggs	0.03	-0.11	-0.11

*** Indicate a strong relationship between the two parameters which intercepted**

4.4.2 Hatching duration

The results of hatching duration in Table 4.1 showed that increase in temperature leads to decrease in the hatching duration of *C. carpio* eggs. The eggs in group I with 23±1°C incubating water temperature had hatching duration of 41.90±0.42 hours, at 26±1°C the hatching duration was 37.18±0.04 hours while 29±1°C the hatching duration was 28.97±0.96 hours. Statistically there was a high significant difference ($p \leq 0.05$) in the hatching duration of *C. carpio* eggs incubated at different water temperatures. The result of relationship between temperature and hatching duration (Table 4.2) shows a strong negative correlation ($r = -0.98$).

4.4.3 Hatchability

The result in Table 4.1 shows that temperature also affects hatchability rate of *C. carpio*. Group I fishes had a hatchability rate of 46.83±0.84%, group II had the maximum percentage hatchability of 67.50±2.50% while the group III had the minimum percentage hatchability of 28.58±10.08%. The statistical analysis of results of percentage hatchability indicates a significant difference ($p \leq 0.05$) between fish incubated in the three temperature groups. Result of the relationship between temperature and percentage hatchability shows a significant negative correlation ($r=-0.45$, $p \leq 0.05$).

4.5 The Effect of Temperature on the Survival of *C. carpio* Larvae

The results of the percentage survival of the larvae of *C. carpio* hatched in different months as affected by temperature is also presented on Table 4.1 which indicates that the group I fish had the least survival rate of 15.27±0.52% followed by the group III larvae which had 30.13±6.29% while the group II larvae had the highest survival rate of 33.20±1.60%. Statistically there was no significant difference ($p>0.05$) in the survival rate of *C. carpio* hatched at different temperatures even though there was means separation in the survival rates between the groups. There was no significant difference in the survival of larvae reared in the month of January and March, there was also no significant difference in survival of larvae reared in February and March but there was significant different between the larvae reared in January and February. The result of the relationship between percentage survival of *C. carpio* larvae and temperature shows a significant positive relationship ($r = 0.67$, $p \leq 0.05$).

4.6 Principal Component Analysis of Breeding Indices

From the principal component analysis (Figure 1), the temperature group II (26±1°C) had positive effect on most of the breeding parameters including weight of eggs, number of viable eggs, percentage fertilization, percentage hatchability and the percentage survival of the *C. carpio* larvae because of the clustering and closeness of these parameters to the temperature of 26±1°C. The two other temperature groups had positive effect on some of the parameters as well as negative effect on some of the breeding parameters of *C. carpio* because of the axis on which these groups are placed (Positive-Negative Axis).

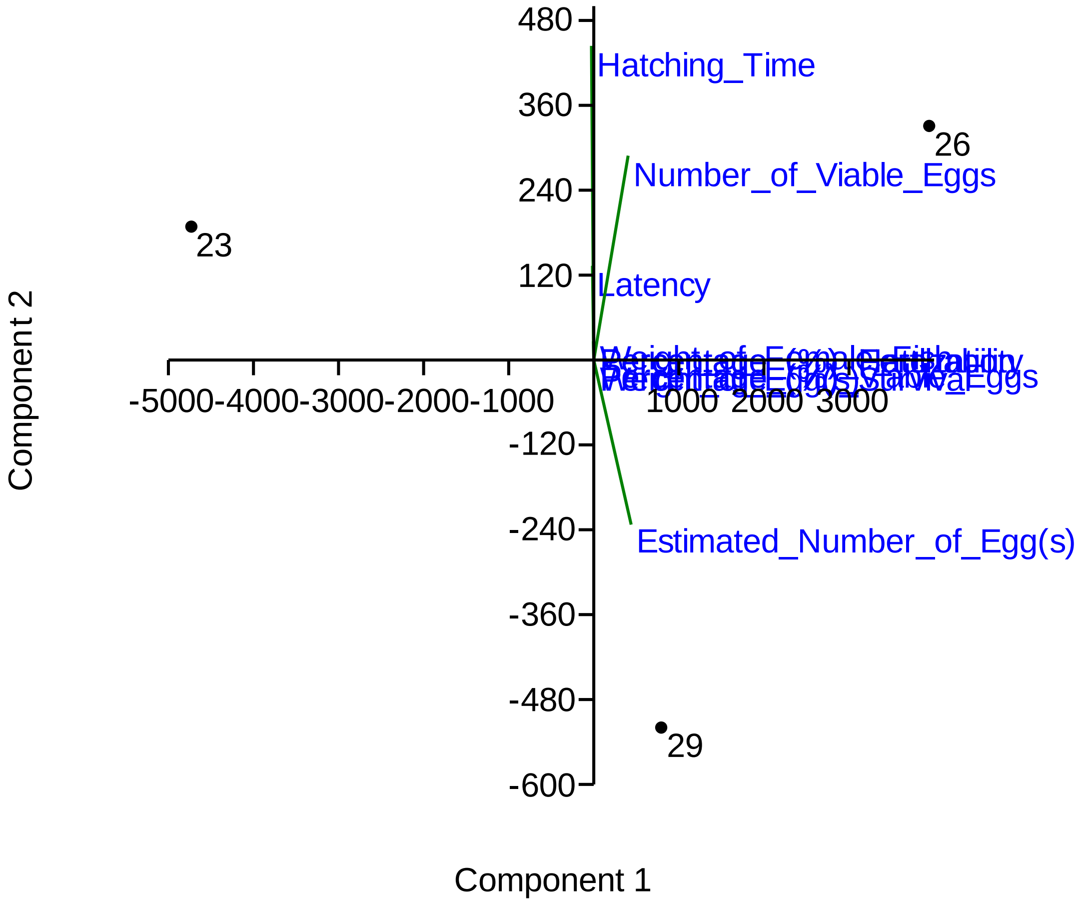

Figure 4.1 Principal Component Analysis (PCA) of the effect of temperature on artificial breeding of *Cyprinus carpio* with ovaprim

Temperature groups	PCA Scores	
	Axis 1	Axis 2
I (23±1°C)	-4731.8	188.56
II (26±1°C)	3939.8	330.87
III (29±1°C)	791.98	-519.43

4.7 Water Quality Parameters

The result of the water quality parameters is presented on Table 4.5 for the conditioning of broodfish after inducement with ovaprim, the average air temperature for group I fish was 25.04±0.06°C while the average water temperature was 22.78±0.05°C, the mean pH and Dissolved oxygen was 8.09±0.05 and 6.13±0.06 ml L^{-1} respectively, the group II fish had average air temperature, water temperature, pH and DO of 28.67±0.09°C, 25.95±0.06°C, 7.97±0.11 and 5.62±0.24 mg L^{-1} respectively, while the group III fish had average air temperature, water temperature, pH and DO of 29.73±0.09°C, 28.62±0.05°C, 7.97±0.07 and 4.87±0.14 mg L^{-1} respectively, the other two water quality parameter for conditioning of *C. carpio* broodstocks are presented on Table 4.5.

Table 4.6 shows the water quality parameter for the incubation of *C. carpio* eggs, the average water temperature for group I, II and III eggs are 23.19±0.13, 26.14±0.03 and 28.96±0.03°C respectively, for average water temperature for same groups are 25.89±0.03, 28.85±0.01 and 29.97±0.09 °C respectively, mean values of pH and DO was found to be 7.99±0.03 and 6.09±0.03 ml L^{-1}, 7.90±0.06 and 5.84±0.12 ml L^{-1}, and 8.00±0.03 and 5.27±0.03 ml L^{-1} for group I, II and III incubating eggs respectively. Electrical conductively and Total dissolved solids values are also shown on Table 4.6.

Table 4.5: Water quality parameters for conditioning of *Cyprinus carpio* broodstock induced with ovaprim

Group	Water Temperature (°C)	Room/Air Temperature (°C)	pH	Dissolved Oxygen (ml/L)	Electrical Conductivity (µS/cm)	Total Dissolved Solids (ppm)
I	22.78±0.05	25.04±0.06	8.09±0.05	6.13±0.06	626.56±7.31	311.83±3.27
II	25.95±0.06	28.67±0.09	7.97±0.11	5.62±0.24	588.07±28.19	294.73±15.99
III	28.62±0.05	29.73±0.09	7.97±0.07	4.87±0.14	574.40±7.37	285.40±1.79

Mean values (±SE)

Table 4.6: Water quality parameters for incubation of *Cyprinus carpio* eggs

Group	Water Temperature (°C)	Room/Air Temperature (°C)	pH	Dissolved Oxygen (ml/L)	Electrical Conductivity (µS/cm)	Total Dissolved Solids (ppm)
I	23.19±0.13	25.89±0.03	7.99±0.03	6.09±0.03	671.18±3.40	336.09±2.08
II	26.14±0.03	28.85±0.01	7.90±0.06	5.84±0.12	599.39±0.28	298.37±0.07
III	28.96±0.03	29.97±0.09	8.00±0.03	5.27±0.03	585.85±0.3	293.81±0.30

Mean values (±SE)

CHAPTER FIVE

5.0 DISCUSSION

5.1 Latency Period

During this breeding experiment, the longest latency period was observed in the fish group which were induced with ovaprim at the temperature of 23±1°C while the fish which were induced at 29±1°C had the minimal latency period of 11.32±0.07 hours. The higher the temperature the lower the latency of period as indicated by the linear negative correlation coefficient (r=-0.98). This findings agrees with the work of Arabaci *et al.* (2001) when they induced Common Carp (*Cyprinus carpio)* with LHRHa combined with Haloperidol, at 20°C the latency was about 20hours, at 22°C latency was found to be 14 hours and at 26°C the latency period was at 12 hours. The findings from this research on the effect of temperature on latency period of *C. carpio* also correspond with the result of Drori *et al*. (1994) who obtained a latency of 14-16 hours when the fish was induced at 23°C.

The reason for this linear negative correlation could be because in poikilothermic organisms at higher temperature metabolic activities is accelerated and their metabolism is dependent on the ambient temperature (Arabaci *et al.*, 2001). Therefore increase in metabolism leads to an increase in the secretion of the pituitary gland which in turn release gonadotropin into the blood stream in shorter time, this release of the gonadotropin into the blood stream is what brought about the luteinisation and the release (ovulation) of the eggs by the female broodfish. At lower temperature the case is different because metabolic activities are slow, hence the release of gonadotropin by the pituitary gland into the blood stream is slower thereby retarding the duration between inducement and ovulation of the female fish.

5.2 Fecundity of *Cyprinus carpio*

There was no significant difference in the fecundity of *C. carpio* induced at the three temperatures groups; the overall mean number of eggs produced by *C. carpio* with average weight of 1666.13±198.95g was 56875.00±6401. Charula (2008) found fecundity of 19750±510 eggs, when *C. carpio* (average weight 475g) was induced with ovaprim at dose of 0.4ml kg^{-1}, this result tends to correspond with the findings in this research. However a higher number of eggs were produced (188100 eggs) when *C. carpio* (mean weight 2955g) was induced with ovaprim by Jhajhria (2002).

The relationship between female body weight of *C. carpio* and the weight and number of eggs produced indicates a strong positive correlation between the parameters. Hence, body weight of female *C. carpio* had an effect on the weight of eggs produced and fecundity, increase in body weight leads to an increase in the fecundity of the fish.

These increase in weight of eggs along with that of the body weight of female brooders increases was also reported by Naeem *et al.* (2005a), Naeem *et al*. (2011) who reported strong positive correlation (r) value of 0.983 and 0.901 between body weight and total number eggs produced by *Catla catla* and Grass carp (*Ctenopharyngodon idella*) in the two publications respectively. Ataguba *et al.* (2012) also reported a similar increase in the fecundity of fish with increase in weight of female brooders result in *C. gariepinus*, females with weight of 297g produced egg number of 30,400 while the females with weight of 824g produced egg number of 72,000. The correlation between Silver carp female body weights and weight of eggs presented in grams by Amin (2006) was characterized by a positive significant correlation coefficient of 0.60 which also correspond with the findings in this study.

5.3 Fertilization Rate, Hatching Duration and Hatching Rate

Fertilization of *C. carpio* was highest (89.00±3.00%) in the fish induced at 26±1°C and the lowest fertilization rate (53.30±10.08%) was found in the fish group III (29±1°C). This results corresponds with the findings of Orina *et al*. (2014) when *Labeo victorianus* was induced with ovaprim (0.5mg Kg^{-1}) at different temperatures. The fish induced at 26±1°C had fertilization rate of 93% while those induced at 22±1°C had fertilization rate of 73.8±1.1%. It was therefore concluded that temperature had effect on the fertilization rate of *Labeo victorianus.*

Malik *et al*. (2014) in Pakistan found fertilization rate of 75.2% by Koi carp when induced with ovaprim at temperature range of 22-24°C, similar results was obtained in the present study on *C. carpio* which were induced at 23±1°C temperature with fertilization rate of 76.67±23.15%. Nandeesha *et al*. (1990b) reported fertilization percentage of 85.43% in the Indian major carp (*Cirrhinus mrigala*) with the use of ovaprim when induced breeding trials were conducted in various states of India during May to July (Temperature of 25-30°C). These results are comparable with the fertilization rate of *C. carpio* in group II (26±1°C) in the present study.

The significant decrease in fertilization rate at higher temperature of 29±1°C could be that the temperature was closer to thermal tolerance limit of *C. carpio* eggs. Temperature affects the quality and size of eggs produced (Kucharczyk *et al*., 2014; Nowosad *et al*., 2014b). Moreover, temperature in fish is responsible for the period of final gamete maturation and can even inhibit the reproduction process or reduce the larvae survival rate to 0% (Bromage *et al*., 2001; Kupren *et al*., 2011). Fertilization involves the action of the gamete (egg) from the female fish as well as the gamete (Milt) from the male fish and it is a known fact that sperm motility is a key prerequisite for determining the quality and fertilizing ability of semen (Cosson and Alavi (2004). It has been confirmed that spermatozoa are motile for longer period at lower temperature of 20°C than at elevated temperature of 26 or 30°C in common carp or 30°C in grass carp (Jezierska and Witeska, 1999).

Increase in temperature leads to significant decrease in the duration of hatching of *C. carpio* egg. The hatching duration in this research tend to be lesser when compared with the findings of Haniffa *et al.* (2007) who reported hatching of *C. carpio* eggs after 72hours of fertilization at water temperature of 26-28°C, the fishes were induced with ovaprim at a dose of 0.3 ml kg^{-1}. In comparison with the present study Charula (2008) also found a higher hatching duration of 58.54±0.347 hours when *C. carpio* was induced with ovaprim (dose 0.4ml kg^{-1}) at temperature range of 23-26.5°C. Ghosh *et al.* (2012) found a hatching duration of 36-48 hours at temperature of 28-29°C when Koi carp was induced with ovaprim, this hatching duration was also higher than the hatching duration of the group III eggs (28.98±0.96 hours) found in the present study.

The hatching duration of *C. carpio* eggs as affected by temperature in the present study was less than all the other hatching duration of *C. carpio* reported in related researches on the breeding of *C. carpio* reviewed by the researcher; this could be due to other unknown factors (environmental, water quality, maturation of the breeders etc) in Nigeria which may favour the hatching process of *C. carpio*.

The results of the effect of temperature on the percentage hatchability of *C. carpio* eggs in this present study was found to be 46.83±0.84, 67.50±2.5 and 28.98±10.08% for the temperature group I, II and III respectively. The group II fishes with temperature of 26±1°C had the highest percentage hatchability followed by the group

II fishes while the group III fishes with incubation temperature of 29±1°C had the least hatchability.

El-Gamal Abd (2009) documented hatching rate of 48.0±0.118% for eggs of *C. carpio* incubated at 24°C temperature, 74.53±0.232 and 54.20±0.273% hatchability of *C. carpio* eggs was also found for the incubation temperature of 27 and 30°C respectively, the findings in this current study had a lesser hatchability rate when compared with the results obtained by El-Gamal Abd (2009) but hatchability increases from the eggs incubated at temperature of 24°C to 27°C and decreases at 30°C. There was also similarity between the hatchability rate of *C. carpio* in the present study with the result of Ghosh *et al.* (2012) who found hatchability rate of 42.78 and 30.22% when *C. carpio* was induced with ovaprim in divided dose.

Oyelese (2006) researched on water temperature as a determinant of fertilization and hatchability in *Clarias gariepinus* and found hatchability of 72.35, 75.51 and 77.84% at temperature of 24.3, 26 and 27°C respectively, these findings tends to disagree with the findings in the current study. Temperature tends not have great effect on the hatchability rate of *C. gariepinus* while on the other hand temperature tends to have significant effect on the hatchability rate of *C. carpio*. Though from the correlation, there was a strong positive relationship between fertilization rate of *C. carpio* and the hatchability rate which denotes that increase in fertilization rate increases hatchability rate.

Orina *et al*. (2014) in comparison with the present study found a higher percentage hatchability of the cyprinids fish (*Labeo victorianus*), when the fish in Yala River was induced with ovaprim (0.5ml Kg^{-1}) at temperature of 22±1 and 26±1°C the hatchability was found to be 62.7±0.9 and 83.0±1.0%

The reason for the reduced hatchability rate at elevated temperature of 29±1°C °C could be due to the lethal effect of higher temperature on the developing embryos; since the developing embryos are fragile their resistance to environmental stress is minimal (Golovanor and Smirnor, 2007). Every organism have tolerance limit to every environmental condition which it survive and when these conditions are exceeded the resultant effect is the deformity and subsequently mortality of the organism.

5.4 Percentage Survival

The group I hatchlings had the least survival of 15.27±0.52%, followed by the group III hatchlings with survival rate of 30.13±6.29% while the group II hatchlings had the highest survival rate of 33.20±1.60%. Hence, increase in temperature leads to an increase in the survival rate of *C. carpio* larvae induced with ovaprim at different temperature. El-Gamal (2009) found a higher survival rate when compared to the present study in his work on the effect of temperature on hatchling and larval development and mucin secretion in common carp, at 24°C the survival rate was 38.0±0.281%, at 27°C the survival rate was 72.00±0.556% while at 30°C survival rate was 52.33±0.191%.

The high mortality observed during the rearing period could not be link directly to the effect of temperature. The mortality observed could be due to feeding, the larvae was fed with zooplanktons which was not sufficient to the large number of hatchlings which resulted in the first set of mortality, when this problem was encountered the larvae after one week was fed with decapsulated Artemia which again lead to considerable number of mortalities. Feeding with decapsulated artemia was discontinued immediately and zooplanktons were used to feed the surviving larvae. It is therefore, important to note that before the breeding of *C. carpio* sufficient and good quality feed be kept in place to avoid the problem encountered in this research.

From the principal Component Analysis, *C. carpio* performs optimally at 26±1°C. Orina *et al*., (2014) reported a similar finding on *Labeo victorianus.* The water temperature 26±1°C provides the optimal breeding conditions for breeding of *L. victorianus* in captivity.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

Monthly variation in water temperature showed significant effect on artificial breeding of *Cyprinus carpio*. Latency period was longest (16.55±0.13 hrs) at water temperature of 23±1°C and shortest (11.32±0.07 hrs) at 29±1°C. Latency period was negatively correlated (-0.98) with temperature. There was no significant difference ($p>0.05$) between water temperature and weight of eggs, fecundity and number of viable eggs. There was negative correlation (-0.51) between fertilization and temperature. Fertilization rate was maximal (89.00±3.00%) at 26±1°C and minimal (52.30±10.49%) at 29±1°C. Hatching duration was significantly affected by temperature, at 23±1°C hatching duration was 41.96±0.42 hrs while at 26±1°C and 29±1°C hatching duration was 37.18±0.04 and 28.98±0.96 hrs respectively. Hatching rate was highest (67.50±2.50%) at 26±1°C followed by 46.83±0.84% at 23±1°C and least (28.58±10.08) at 29±1°C. Hatching *C. carpio* eggs was negatively correlated with water temperature. Survival of larvae was highest (33.20±1.60%) at temperature of 26±1°C and least (15.27±0.52%) at 23±1°C

6.2 Conclusions

Temperature has significant effect on latency period. Increase in water temperature leads to significant decrease in the latency period of *C. carpio*.

Temperature has no effect on the weight of eggs, fecundity of *C. carpio,* and number of viable eggs rather weight of eggs and fecundity of *C. carpio* is significantly affected by female body, increase in female body weight leads to significant increase in the weight of eggs and fecundity (r = 0.97). Female body weight has no effect on the number of viable eggs.

Fertilization rate, hatching duration and hatchability rate were affected significantly by temperature. Increase in water temperature leads to decrease in fertilization rate (r = -0.51), hatching duration (r = -0.98) and hatchability rate (r = -0.45).

No significant difference was observed in the survival of *C. carpio* larvae. Survival was positively correlated with temperature (r = -0.67), it increases with increasing water temperature.

6.3 Recommendations

Considering the fact that *C. carpio* can be successfully bred artificial in hatchery under Nigerian environmental conditions, it is therefore recommended that;

i. The water temperature range of 23±1-29±1°C be used by farmers during breeding of *C. carpio* in Nigeria, if possible water temperature of 26±1°C be selected because *C. carpio* performs best at this temperature in the present study.

ii. Fish farmers in Nigeria should be encouraged to diversify into the culture of Common carp.

REFERENCES

Akankali, J.A., Seiyaboh, E.I. and Abowei, J.F.N. (2011). Fish breeding in Nigeria. International Journal of Animal and Veterinary Advances, **3**(3): 144-155.

Amer, M. A., Akar, A.M., Saleh, O. A., and Eissa, A. E. (2009). A comparative study on two spawning induction agents, ovaprim and carp pituitary extract in the common carp (*Cyprinus carpio*). *Egyptian Society of Animal Reproductive Fertility Twenty First Annual Congress*, Cairo / Hurgada, February 7 - 11, **171**

Amin, A.E. (2006). Timing of hypophysation processes in the silver carp *Hypophthalmichthys molitrix* (Valenciennes) and an attempt to employ the concept of latency time. *Egyptian Journal of Aquatic Biology and Fisheries,* **10**(3): 33-54.

Arabaci, M., Çağırgan, H. and Sari, M. (2001). Induction of spawning in common carp (*Cyprinus carpio*) using LHRHa ([D-Ser (tBu)6, Pro9-NEt]-LHRH) combined with Haloperidol: Effects of different treatment time and determination of latency period dependence on temperature. *Turkish Journal of Fisheries and Aquatic Science*, **1**: 1–5.

Ataguba, G.A., Solomon, S.G. and Onwuka, M.C. (2012). Broodstock size combination in artificial spawning of cultured *Clarias gariepinus*. *Livestock Research for Rural Development*, **24**(12): 1-3

Atz, J. W. and Pick-ford, G.B. (1959). The use of pituitary hormones in fish culture. *Endeavour,* **18**: 125-129.

Barker, J. (1994) *Common carp (Cyprinus carpio) breeding in Nigeria. Naga, the ICLARM Quarterly*, **17**(3): 28-29.

Bogdan, K., Adrian, G., Ada, C., Ioan, B. and Nicolae, P. (2010). Spawning latency period in hormonal induced reproduction of Pikeperch (*Sander lucioperca*). *Animal Science and Biotechnologies,* **43**(2): 32-35

Bromage, N., Porter, M. and Randall, C. (2001). The environmental regulation of maturation in farmed fin fish with special reference to the role of photo period and melatonin. *Aquaculture,* **197**: 63–98.

Brzuska, E. (2006). Artificial propagation of female Hungarian strains 7 carp (*Cyprinus carpio*) after treatment with carp pituitary homogenate, Ovopel or Dagin. *Czech Journal of Animal Science*, **51**: 132-141.

Charula, S. (2008). Assessment of synergistic efficacy of pituitary extract with ovaprim and ovatide on breeding performance and survival of spawn of

Cirrhinus mrigala (Ham). (Doctoral thesis of Limnology and Fisheries, Maharana Pratap University of Agriculture and Technology, Udaipur). Retrieved from http://www.mpuat.ac.in/index.php

Chaudhuri, H. and Alikunhi, K.B. (1957). Observations on the spawning of Indian major carps by hormone injection. *Current Science*, **26**: 381-382.

Chiya, N. and Nakai, I. (1995). "XAFS studies of some precipitation and coloration reaction used in analytical chemistry". *Physica B: Condensed Matter*, pp. 208–209, 387–388. doi:10.1016/0921-4526(94)00706-2

Chumchal, M. (2002). "*Cyprinus carpio*" (on-line). Retrieved from Animal diversity web; http://animaldiversity.ummz.umich.edu/site/accounts/information/Cyprinus_carpio.html.

Cosson, J. and Alavi, S.M.H. (2004). Sperm motility in fishes. I. Effects of temperature and pH: a review. *Cell Biology International*, **29**: 101-110.

Drori, S., Offer, M., Sivan, B.L. and Yaron, Z. (1994). Spawning induction in common carp (*Cyprinus carpio*) using pituitary extract or GnRH superactive analogue combined with Metoclopramide: analysis of hormone profile, progress of oocyte maturation and dependence of temperature. *Aquaculture*, **119:** 393–407.

El-Gamal Abd. E.E. (2009). Effect of temperature on hatching and larval development and mucin secretion in common carp, *Cyprinus carpio* (Linnaeus, 1758). *Global veterinaria,* **3**(2): 80-90.

Fabio P. A., Helio B. S., Elizete R., Yoshimi S. and Nilo B. (2011). Influence of water temperature on induced reproduction by hypophysation, sex steroids concentrations and final oocyte maturation of the *Prochilodus argenteus* (Pisces: Prochilodontidae). *General and Comparative Endocrinology***,** **172**(3): 400–408

Fahad, S.I., Ewen, M., Mohammad, M.A., Ghazi, A.A. and Adel, H.A. (2010). The Effect of Variable Incubation Temperatures on Hatchability and Survival of Goldlined Seabream, Rhabdosargus sarba (Forsskål, 1775) Larvae. International Scholarly and Scientific Research and Innovation, 4(5): 275-278.

FAO, (2015). Yearbook of Fishery Statistics Summary tables. Retrieved from ftp://ftp.fao.org/FI/STAT/summary/a-6.pdf

Flajshans, M. and Hulata, G. (2007). Common carp – *Cyprinus carpio*. In: "Genetic effects of domestication, culture and breeding of fish and shellfish, and their impacts on wild populations." D. Crosetti, S. Lapègue, I. Olesen, T. Svaasand (eds). GENIMPACT project: Evaluation of genetic impact of aquaculture activities on native populations. A European network. WP1 workshop "Genetics of domestication, breeding and enhancement of performance of fish and shellfish", *Viterbo, Italy,* 12-17th June, 7. http://genimpact.imr.no/

Freyhof, J. and Kottelat, M. (2008). *Cyprinus carpio*. The IUCN Red List of Threatened Species. Version, 2014. 3. www.iucnredlist.org

Froese, R. and Pauly, D. (2002). "*Fishbase: Species summary for Cyprinus carpio*" (On-line). http://www.fishbase.org.

Froud, B.K., Mohammad, Y.F., Farokh, A. and Khuzestan, I. (2010). Effect of ovaprim, HCG, LHRH-A2, LHRHA2+CPE and carp pituitary in Benni (*Barbus sharpeyi*) artificial breeding. *Global Veterinaria,* **5**(4): 209-2012.

Garg, S.K., Bhatnagar, A., Kalla, A. and Johal, M.S. (2002). Experimental Ichthyology, CBS Publishers and Distributors 4596/1-4, Darya Ganj, New Delhi – 110002 (India) pp. 129.

Gerbilskii, N.I. (1938). Expedition for the study of the physiology of spawning. *Rybnoe Khozyajstvo,* **18**: 33-36.

Gerbilskii, N.L. (1965). The present approach to the problem of neurohormonal control of the fish sexual cycle and techniques of hormonal influence applied in fish culture. Lecture-Seminar on fish culture in the inland waters of the USSR for the FAO group fellowship study, *Leningard,* pp. 23.

Ghosh, A.K., Biswas, S., Sarder, I., Sabir, W. and Rahaman, M.B. (2012). Induced breeding, embryonic and larval development of koi carp (*Cyprinus carpio*) in Khulna, Bangladesh. *Mesopotamia Journal of Marine Science,* **27**(1): 1-14.

Golovanov V.K. and Smirnov A.K. (2007). Influence of the Water Heating Rate Upon Thermal Tolerance in Common Carp (*Cyprinus carpio* L.) During Different Seasons. *Journal of Ichthyology,* **47**(7): 538–543.

Gomez-Marquez, J.L., Pena-Mendoza, B., Salgado-Ugarte, I.H. & Guzman-Arroyo, M. (2003). Reproductive aspects of Oreochromis niloticus (Perciformes: Cichlidae) at Coatetelco lake, Morelos, Mexico. Revista de Biologia Tropical, **51**(1): 221-228.

Gomina, O.R. (2011). Effect of pituitary extracts of Carp, *Clarias* and ovaprim hormone on the fecundity and fertility of the common carp, (*Cyprinus carpio*) (Unpublished master's thesis). Ahmadu Bello University, Zaria, Nigeria.

Haniffa, M.A., Benziger, P.S.A., Arockiaraj, A.J., Nagarajan, M. and Siby, P. (2007). Breeding behaviour and embryonic development of koi carp (*Cyprinus carpio*). *Taiwania*, **52**(1): 93-99.

Hill, J.E., Kilgore, K.H. and Pouder B.D. (2009). Survey of Ovaprim use as a spawning aid in ornamental fishes in the United States as administered through the University of Florida Tropical Aquaculture Laboratory. *North American Journal of Aquaculture*, **71**: 206-209.

Hossain, M.Y., Rahman, M.M. and Elgorban M.A. (2012). Relationships between Body Size, Weight, Condition and Fecundity of the Threatened Fish *Puntius ticto* (Hamilton, 1822) in the Ganges River, Northwestern Bangladesh. *Sains Malaysiana*, **41**(7): 803–814

Houssay, B.A. (1930). Accion sexual de la hipofisis en los perces y reptiles. *Revista de la Sociedad Argentina de Biology,* **6**: 686-688.

Hussan, A.B., Nurhidayah, B.M.R. and Sukumaran, M. (2007). Studies on the induced breeding of patin bunga *(Pangasius sutchi)* using pituitary extract replacing ovaprim. *Abstracts of 8th Asian Fisheries Forum,* November 20-23, Kochi, India, pp. 330-331.

Hussein, A. and Al Hazzaa, R. (2003). Stickiness elimination of himri barbell (*Barbus lutes*, Heckel) eggs. *Turkish Journal of Fisheries and Aquatic Sciences,* **3**: 47-50

Indira, T., Damodaran, R. and Priyadarshini, R. (2012). Comparative study of synthetic hormones ovaprim and carp pituitary extract used in induced breeding in Indian major carp. *Advanced Biotechnology,* **12**(07): 49-52.

Jezierska, B. and Witeska, M. (1999). The effect of time and temperature on motility of spermatozoa of common and grass carp. *Electronic Journal of Polish Agriculture,* **2**(2): 04

Jhajhria, A. (2002). Comparative efficacy of ovaprim and ovatide in induced spawning of carps at Jodhpur. *Aquaculture,* **3:** 61-68.

Kiran, B.R., Shankar M.K. and Venkateshwarlu, M. (2013). A review on induced breeding of cat fishes, murrels and climbing perches in India. *Advances in Applied Science Research*, **4**(4): 310-323.

Kucharczyk, D., Żarski, D., Targońska, K., Łuczyński, M.J., Szczerbowski, A., Nowosad, J., Kujawa, R. and Mamcarz A. (2014). Induced artificial androgenesis in common tench, *Tinca tinca* (L.), using common carp and common bream eggs. *Italian Journal of Animal Science,* **13**(2890): 196-200.

Kupren, K., Mamcarz, A. and Kucharczyk, D. (2011). Effect of variable and constant thermal conditions on embryonic and early larval development of fish from the genus Leuciscus (Cyprinidae, Teleostei). *Czech Journal of Animal Science,* **56**: 70–80.

Legendre M. and Otémé Z. (1995). Effect of varying latency period on the quantity and quality of ova after hCG induced ovulation in the African catfish, *Heterobranchus longifilis* (Teleostei, Clariidae). *Aquatic Living Resources,* **8**(4): 309-316.

Leu, M.Y. and Chou, Y.H. (1996). Induced spawning and larval rearing of captive yellow fin porgy, *Acanthopagrus latus* (Houttuyn). *Aquaculture,* **143**: 155-166.

Mahmood, S.U. (2006). Comparason between single and double injection of pituitary gland (PG) on the breeding performance of Climbing perch, *Anabas testudineus* (Bloch). *Journal of Biological Science,* **14**: 57-60.

Malik, A., Abbasi, A.R., Kalhoro, I.B., Shan, S.A., Narejo, N.T. and Kalhoro, H. (2014). Effect of Ovaprim Hormone (Syndel Laboratory, Canada) on Spawning of Koi Carp at Fish Hatchery Chilya Thatta, Sindh, Pakistan. *Sindh University Research Journal (Science Series)*, **46**(3): 273-276.

Murthy, K.S., Arlu, M.V. and Kiran, B.R. (2013). Induced breeding of Indian Major, Minor and Cyprinids Carps in India: An overview. *Journal of International Academic Research for Multidisciplinary*, **1**(6): 165-193.

Mylonas, C.C, Fostier, A., Zanuy, S. (2010). Broodstock management and hormonal manipulation of fish reproduction. *General and Comparative Endocrinology,* **165**: 516-534.

Naeem, M., Salam, A. and Jafar, A. (2005a). Induced spawning of major carp *Catla catla* by a single interamuscular injection of Ovaprim-C and facunditity at fish hatchery Islamabad, Pakistan. *Journal of Biological Science*s, **5**(6): 776-780.

Naeem, M. A., Salam, F.,Diba, A. and Saghir, S. (2005b). Fecundity and Induced Spawning of Silver carp, *Hypophthalmichthys molitrix* by using a Single Intramuscular Injection of ovaprim-C at Fish Hatchery Islamabad, Pakistan. *Journal of Biological Sciences*, **8**(8): 26-30.

Naeem, M., Zuberi, A., Salam, A., Ashraf, M., Elahi, N., Ali, M., …Ahmad, B. (2011). Induced spawning, fecundity, fertilization rate and hatching rate of Grass carp (*Ctenopharyngodon idella*) by using single intramuscular injection of ovaprim-C at a fish hatchery Faisalabad, Pakistan. *African Journal of Biotechnology*, **10**(53): 11048-11053. DOI: 10.5897/AJB10.1481

Naeem, M., Zuberi, A., Ashraf, M., Ahmad, W., Ishtiaq, A. and Najam, H. (2013). Induced breeding of *Labeo rohita* through single application of ovaprim-C at Faisalabad Hatchery, Pakistan. *African Journal of Biotechnology*, **12**(19): 2722-2726. DOI: 10.5897/AJB12.1037

Nandeesha, M.C., Rao, K.G., Jayanna, R., Parker, N.C., Varghese, T.J., Keshavanath, P. and Shetty, H.P.C. (1990a). Induced spawning of Indian major carps through single application of ovaprim. In : *"Proceedings of the Second Asian Fisheries Forum"*. Hirano, R. and Hanyu, 1. (Eds.) *Asian Fisheries Society*, Manilla, Philliphines, pp. 581-585.

Nandeesha, M.C., Das, S.K., Nanthaniel, D.E. and Varghese, T.R. (1990b). Breeding of carps with ovaprim in India. Special publication number-4, *Asian Fisheries Society*, Indian Branch, COF Mangalore pp. 1-41.

Nowosad, J., Targońska, K., Chwaluczyk, R., Kaszubowski, R. and Kucharczyk, D. (2014a). Effect of temperature on the effectiveness of artificial reproduction of dace Cyprinidae (*Leuciscus leuciscus* L.) under laboratory and field conditions. *Journal of Thermal Biology*, **45**: 62–68

Nowosad, J., Kucharczyk, D., Liszewski, T., Targońska, K., Kujawa, R., (2014b). Comparison of temperature shock timing to induced artificial mitotic gynogenesis and androgenesis in common tench. *Aquaculture International*, **23**(1): 45-53. http://dx.doi.org/10.1007/s10499-014-9796-0.

Nwosu, F.M. and Holzlohnev, S. (2000). Influence of temperature on eggs hatching, growth and survival of larvae of *Heterobranchus longifilis*. (Teleostei: Clariidae). *Journal of Applied Ichthyology*, **16**: 20-23.

Orina, P.S., Rasowo, J., Gichana, E., Maranga, B. and Charo-Karisa H. (2014). Artificial breeding protocol and optimal breeding environment for *Labeo victorianus* (Boulenger, 1901). *International Journal of Fisheries and Aquatic Studies*, **1**(6): 138-143.

Oyelese, O.A. (2006). Water temperature a determinant of fertilization and hatchability rates in artificially induced breeding of *Clarias gariepinus* (Teleostei: Clariidae). *Research Journal of Biological Sciences*, **1**(1-4): 83-87.

Pao, X., Kuanhong, M., Jian, Z., Jianxin, W. and Yongseng G. (2003). Comparative studies on spawning inducing using Ovaprim and other hormones. Retrieved from http://www.syndel.com/spawning/ovaprim-ffrc-reporthtml.

Patil, J.G. and Wisniewski, C. (2011). Hypophysation: A technique for deployment of odour donor fish for control of the common carp *Cyprinus carpio*. Revision of Patil J.G. (2006) Technical Report No 5, *Inland Fisheries Service*, *Hobart*. pp 8.

Peter, R.E., Lin, H.R. and Van-der-Kraak, G. (1988). Induced ovulation and spawning of cultured freshwater fish in China: Advances in application of GnRH analogues and dopamine antagonists. In: *"Proceedings on Induced Spawning of Asian Fishes, a Fish Breeding Workshop"* Singapore, 7-10 April, 1987.

Rahdari, A., Gharaei, A. and Ghaffari M., (2014). Spawning latency period in hormonal induced reproduction of Snow trout (*Schizothorax zarudnyi* Nikolskii 1897). *Iran Journal of Biotechnology,* **12**(1): e10268. DOI: 10.5812/ijb.10268

Rath, R.K. (2000). Freshwater Aquaculture 2nd Edition Scientific Publishers (India), *Jodhpur*, pp. 103-172.

Rath, S.C., Sarkar S.K., Gupta, S.D. and Mondal, B. (2007). Gonadal development and induced spawning in spontaneously bred *Labeo rohita* (Ham). *Indian Journal of Fisheries* **54**(2): 163-170.

Sahoo, S., Giri, S., Chandra, S. and Sahu, A. (2007). Effect of Ovaprim doses and latency period on induced spawning of *Clarias batrachus*: Observation on larval deformity. *Indian Journal Experiment Biology,* **45**(10): 20-29.

Selvaraj, S. and Francis, T. (2007). Influence of human chorionic gonadotropin on maturation in striped murrel, *Channa striatus. Asian Fisheries Science,* **20**: 1-124.

Sharma, A.P. and Singh, V.K (2002). Induced breeding response of Indian major carps, viz. *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* using ovaprim and carp pituitary extract. *Indian Journal of Animal Science*, **72**: 35-354.

Szabo, T. (2003). Ovulation induction in northern pike (*Esox lucius* L). Using different GnRH analogues, Ovaprim, Dagin and carp pituitary. *Aquaculture Research,* **34**: 479-486.

Tracey, S.R., Lyle, J. and Haddon, M. (2007). Reproductive biology and per-recruit analyses of striped trumpeter (Latris lineata) from Tasmania, Australia: Implications for management. Fisheries Research, **84**: 358-368.

Valeta, J.S., Likongwe, J.S., Kassam, D. and Maluwa, A.O. (2013). Temperature-dependent egg development rates, hatchability and fry survival rate of Lake Malawi Tilapia (Chambo), *Oreochromis karongae* (Pisces: *Cichlidae*). *International Journal of Fisheries and Aquaculture*, **5**(4): 55-59. DOI: 10.5897/IJFA12.056

Woynarovich, E. and Horvath, L. (1980). The artificial propagation of warm water finfishes: a manual for extension. *FAO Fish Technical Paper*, **201**: 183

Yang, Z. and Chen, Y. (2005). Effect of temperature on incubation period and hatching success of obscure puffer (*Takifugu obscurus* Abe) eggs. *Aquaculture*, **246**: 173–179. www.elsevier.com/locate/aqua-online

Zakes, Z. and Szczepkowski, M. (2004). Induction of out of season spawning of pikeperch, *Sander lucioperca* (L.). *Aquaculture International*, **12**: 11-18.

Appendix I: Descriptive statistics of the breeding indices of *Cyprinus carpio*

		N	Mean	Std. Deviation	Std. Error	95% CI
						Lower Bound
Weight of Female Fish	23	3	1717.6667	801.62107	462.81614	-273.6705
	26	2	1639.5000	282.13561	199.50000	-895.3878
	29	3	1632.3333	647.62206	373.90477	23.5509
	Total	8	1666.1250	562.70201	198.94520	1195.6943
Latency	23	3	993.6667	13.65040	7.88106	959.7572
	26	2	780.5000	12.02082	8.50000	672.4973
	29	3	679.3333	8.62168	4.97773	657.9159
	Total	8	822.5000	148.12061	52.36854	698.6681
Weight of Eggs	23	3	76.6667	40.10403	23.15407	-22.9573
	26	2	85.7000	10.04092	7.10000	-4.5141
	29	3	82.8667	25.05700	14.46667	20.6216
	Total	8	81.2500	25.86686	9.14531	59.6248
Estimated No. of Eggs	23	3	53666.6667	28072.82197	16207.85132	-16070.0891
	26	2	59990.0000	7028.64140	4970.00000	-3159.8375
	29	3	58006.6667	17539.90118	10126.66667	14435.1367
	Total	8	56875.0000	18106.79983	6401.72047	41737.3365
No. of Viable Eggs	23	3	52356.5000	27270.90016	15744.86155	-15388.1715
	26	2	58281.0000	6322.94884	4471.00000	1471.5586
	29	3	55742.8667	16985.03231	9806.31298	13549.7074
	Total	8	55107.5125	17519.06553	6193.92502	40461.2072
Percentage Fertilization	23	3	72.6667	3.05505	1.76383	65.0775
	26	2	89.0000	4.24264	3.00000	50.8814
	29	3	52.3000	18.16618	10.48825	7.1727
	Total	8	69.1125	18.41424	6.51042	53.7178
Percentage Hatchability	23	3	46.8333	1.45717	.84130	43.2135
	26	2	67.5000	3.53553	2.50000	35.7345
	29	3	28.5833	17.45769	10.07920	-14.7840
	Total	8	45.1563	18.73575	6.62409	29.4928
Hatching Time	23	3	2514.6667	43.82161	25.30042	2405.8078
	26	2	2231.0000	5.65685	4.00000	2180.1752
	29	3	1738.0000	93.95212	54.24328	1504.6100
	Total	8	2152.5000	366.99085	129.75086	1845.6880
Percentage Survival	23	3	15.2667	.90185	.52068	13.0263
	26	2	33.2000	2.26274	1.60000	12.8701
	29	3	30.1333	10.89097	6.28791	3.0787
	Total	8	25.3250	10.28782	3.63729	16.7242

Appendix II: Descriptive statistics of the breeding indices of *Cyprinus carpio* continued

		95% CI	Minimum	Maximum
		Upper Bound		
Weight of Female Fish	23	3709.0038	860.00	2448.00
	26	4174.3878	1440.00	1839.00
	29	3241.1157	938.00	2220.00
	Total	2136.5557	860.00	2448.00
Latency	23	1027.5761	978.00	1003.00
	26	888.5027	772.00	789.00
	29	700.7508	670.00	687.00
	Total	946.3319	670.00	1003.00
Weight of Eggs	23	176.2906	35.00	115.00
	26	175.9141	78.60	92.80
	29	145.1117	56.00	105.60
	Total	102.8752	35.00	115.00
Estimated No. of Eggs	23	123403.4224	24500.00	80500.00
	26	123139.8375	55020.00	64960.00
	29	101578.1966	39200.00	73920.00
	Total	72012.6635	24500.00	80500.00
No. of Viable Eggs	23	120101.1715	23887.50	78246.00
	26	115090.4414	53810.00	62752.00
	29	97936.0260	37553.60	71190.00
	Total	69753.8178	23887.50	78246.00
Percentage Fertilization	23	80.2558	70.00	76.00
	26	127.1186	86.00	92.00
	29	97.4273	38.20	72.80
	Total	84.5072	38.20	92.00
Percentage Hatchability	23	50.4531	45.30	48.20
	26	99.2655	65.00	70.00
	29	71.9506	9.50	43.75
	Total	60.8197	9.50	70.00
Hatching Time	23	2623.5256	2485.00	2565.00
	26	2281.8248	2227.00	2235.00
	29	1971.3900	1635.00	1819.00
	Total	2459.3120	1635.00	2565.00
Percentage Survival	23	17.5070	14.40	16.20
	26	53.5299	31.60	34.80
	29	57.1880	21.60	42.40
	Total	33.9258	14.40	42.40

Appendix III: ANOVA Table of the breeding indices of *Cyprinus carpio*

		Sum of Squares	df	Mean Square	F	Sig.
Weight of Female Fish	Between Groups	12813.042	2	6406.521	.015	.986
	Within Groups	2203621.833	5	440724.367		
	Total	2216434.875	7			
Latency	Between Groups	152912.167	2	76456.083	574.138	.000
	Within Groups	665.833	5	133.167		
	Total	153578.000	7			
Weight of Eggs	Between Groups	110.467	2	55.233	.060	.942
	Within Groups	4573.193	5	914.639		
	Total	4683.660	7			
Estimated No. of Eggs	Between Groups	54128666.667	2	27064333.333	.060	.942
	Within Groups	2240864733.333	5	448172946.667		
	Total	2294993400.000	7			
No. of Viable Eggs	Between Groups	44057279.902	2	22028639.951	.052	.950
	Within Groups	2104366318.807	5	420873263.761		
	Total	2148423598.709	7			
Percentage Fertilization	Between Groups	1676.902	2	838.451	6.017	.047
	Within Groups	696.687	5	139.337		
	Total	2373.589	7			
Percentage Hatchability	Between Groups	1830.909	2	915.454	7.309	.033
	Within Groups	626.288	5	125.258		
	Total	2457.197	7			
Hatching Time	Between Groups	921249.333	2	460624.667	106.989	.000
	Within Groups	21526.667	5	4305.333		
	Total	942776.000	7			
Percentage Survival	Between Groups	496.902	2	248.451	5.092	.062
	Within Groups	243.973	5	48.795		
	Total	740.875	7			

Appendix IV: Raw data of the breeding indices of *Cyprinus carpio* as affected by temperature

Temperature (°C)	Wt. of female fish (g)	Latency(min.)	Wt. of egg (g)	Estimated no. of egg	No. of viable eggs	Percentage fertilization	Percentage hatchability	Hatching time (min.)	Percentage survival
23	860	1003	35	24500	23887.5	72	47	2565	14.4
23	1845	978	80	56000	54936	76	45.3	2494	15.2
23	2448	1000	115	80500	78246	70	48.2	2485	16.2
26	1440	772	78.6	55020	53810	86	70	2227	34.8
26	1839	789	92.8	64960	62752	92	65	2235	31.6
29	938	681	56	39200	37553.6	45.9	32.5	1819	26.4
29	1739	687	87	60900	58485	38.2	9.5	1635	21.6
29	2220	670	105.6	73920	71190	72.8	43.75	1760	42.4