University of Natural Resources and Life Sciences, Vienna

UNESCO-IHE Institute for Water Education

EGERTON UNIVERSITY

COMPARATIVE STUDY OF SYNTHETIC HORMONE OVAPRIM AND CARP PITUITARY GLAND USED IN INDUCED BREEDING OF AFRICAN CATFISH (*Clarias gariepinus*)

Master of Science Thesis

by

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This thesis is submitted in partial fulfilment of the requirements for the joint academic degree of Master of Science in Limnology and Wetland Management jointly awarded by the University of Natural Resources and Life Science (BOKU), Vienna, Austria. UNESCO-IHE Institute for Water Education, Delft, the Netherlands, Egerton University, Njoro, Kenya

MSc research host institution

University of Natural Resources and Life Science (BOKU)

Vienna, April 2015

Acknowledgement

First and foremost I would like to express my deepest gratitude to my supervisor Ao.Univ.Prof. Dr.Phil. Herwig Waidbacher, University of Natural Resources and Life Sciences Vienna, Department of Water, Atmosphere and Environment for his consistent advice and support from the beginning up to the completion of this work. Really this work would not have been completed without his kindly support and follow up. I highly appreciate his social interaction and respect for the people during his short visit to Ethiopia. I am indeed grateful for all support and advice.

Many thanks to my local supervisor Dr. Getinet G.tsadike from the National Fisheries Research Centre for their immense help during my research work and gave me scientific papers, important comments during my proposal writing and data collection.

Many thanks to Mr. Paul Meulenbroek who helped me on data arrangement, analysis and editing and also advising me in each paper work and spent his time on checking my progress and gave me commonts which greatly improved my thesis quality.

I sincerely thank IPGL-course coordinator Mag. Gerold Winkler and Dipl.-Ing.(FH) Hanna Michael Hentschel, Nina Haslinger and Elaine Liu for their valuable and immense support during my stay in Europe. It is your support that brought me to this success.

I extend my gratitude to my home institute (EIAR) too for allowing me to study abroad keeping my position until I finish my study. My specially thanks goes to Mr Esayas Alemayehu who worked with me in every activities and hosting me in his room untill I finished my data collection. Thanks to all fishermens (Seyifu Hayle, Tadesse Mulugeta and Alemu Chala,) and all sebeta researchers staffs arranging all facilities during my hatchery work.

Finally, I would like to thank you to all my family, specially my father and step mother being sent me in school and encouraged me not drop out school when I had number of obstacles to drop out my school.

Abstract

The study was done in a hatchery for 14 days. It was compared the effectiveness of Carp pituitary gland (PG), synthetic hormone Ovaprim (OV) and control (CR) on spawning success of *C.gariepinus* in a hatchery. In total 21 females and 9 males matured catfish were used for the study. The pituitary gland was administered at dose rate of 3 mg kg^{-1} female body weight and synthetic hormone Ovaprim was administered at dose rate of 0.5 ml kg^{-1} body weight for females while control has no any treatment. The study was examined the mean fecundity, fertility rate, egg hatching and survival of *C.gariepinus* using different hormones. The mean number fecundity was calculated from the total weight of eggs released. The percentage of fertilization was determined from the surviving embryos after 45 second fertilization. The hatching percentage was calculated from the total number of fertilized eggs, while the percentage of survival rate was determined by physical counting after 14 days feeding with *Artemia nuplii*. The mean number of eggs ovulated was higher in Pituitary gland injected catfish compared to Ovaprim treated catfish. However, the control group one brood fish was ovulated poor quality eggs out of three female fish but was not hatched at all. The highest percentage of fertilization rate of 73.2 % was in Ovaprim treated catfish compared to 72 % in Carp pituitary treated catfish. On the other hand, the highest hatching rate was 89% in Ovaprim treated fish compared to 69 % Pituitary gland injected catfish. Mean survival rate of fry was higher in Ovaprim 85 % while Carp pititary gland treated fish survival rate was 79 %. However, Ovaprim treated fish showed slightly higher hatching and survival success as compared to Carp pituitary gland but has no significan differences in stimulating effect ($P>0.05$) in all the parameters investigated. In terms of accessibility, Ovaprim is imported products and more expensive while Carp Pituitary gland extract is relatively affordable and avilable locally in order to use it. Hence, Carp pituitary gland is highely recommended for benefit of small scale aquaculture farmers and commercial hatchery users.

Key Words: Aquaculture, Induced spawning, Fecundity, Fertility, Hatching rate, *Clarias gariepinus*, Pituitary gland, Ovaprim hormones.

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1 Introduction

Aquaculture in Africa is predominantly rural, and orientated principally to the immediate needs of the farmers and their families. In many instances the fish are consumed directly within the farmers' family as much as 50% of the harvest in Kenya, Ivory Coast and Rwanda (FAO 1994). Fish farming is still at its infant stage in Ethiopia despite favorable physical conditions support its development (figure 1; FAO 2013). The present agricultural policy encourages professional production developments, further on sustainable utilization of water resources (Ministry of agriculture annual report 2008). However, country mainly depends on capture fisheries from natural water bodies. The annual fish demand is increased rapidly with population growth. The maximum exploitation fish resources from aforementioned natural water bodies indicate that there is a strong need to develop the remaining available aquaculture resources. Commercial aquaculture for export also demonstrates promising opportunities based on current demand. The African sharp tooth catfish *Clarias gariepinus* is considered a commercial success amongst the candidate species in accelerating Ethiopian aquaculture developments. The biological, ecological attributes and life history of *Clarias gariepinus* are well documented scientifically. Since 1996, the *Clariids* and *bagrids* attracting vast interest within African aquaculture sectors. In result, *Clarias* species rearing has recently undergone major developments throughout Africa. The *C.gariepinus* can sustain on a varied natural diet features detritus, zooplankton, and larger animal prey, but also achieve satisfactory growth on an artificial diet (Babiker 1984; Habibi et al. 1986). They respond efficiently to induce breeding and artificial propagation. The growth is directly related to temperature, as they can attain a length of 22-32 cm after one year. They can adjust to fluctuating temperatures, while tolerating hypoxia with their ability to respire atmospheric oxygen under necessary conditions (Babiker 1984). The rearing is carried out typically in lakes and ponds; both mono cultural and poly cultural environments are considered suitable. In some cases the specie also functions as predators in tilapia fingerling control in poly culture (Babiker 1984). African catfish production levels range from 0.1-4 kg /m^3/year in extensive and semi-intensive pond systems. In high density, intensive systems, yield that exceeds 800 kg/m^3/ year is possible to obtain. Food conversion ratios vary between 1:12 using agricultural waste to 1:1.1 using formulated, least-cost feeds. The marketable size could be achieved within six months in small-scale rural fish farmers under poly cultural conditions. In rural poly cultural ponds

production varies from 0.2-0.35kg /m^3/year. Marketable sizes for both species would normally arrive at the same time (120-150 g for tilapia and 250-450 g for catfish) after 10-12 months (FAO Fisheries Statistics 1994). However, several possible drawbacks during production period could occur. The cases of low reproduction success; aggressive behavior and cannibalism may cause injuries that lead to infection and creates obstacles for transportation (Babiker 1984).

The research and development efforts have not yet met the ever-rising demand of improved seeds. In natural habitat *C. gariepinus* do not spawn all-year round and hence fry production is required for regular supply to the farmers. The study output will be significant on improving the rural area supply of *Clarias garipinus* seed through integration with irrigation and livestock farming practices. Particularly in areas of the country where supplies of fish are most limited. The results we draw from this study also indirectly contribute to a more nutritious diet for the local rural population through the affordable supplies of animal protein.

This case study is structured to address the existing problem, propose a set of attainable goals with the aim to establish cohesive production cycle. It is initiated to generate and evaluate catfish seed production techniques by using synthetic (Ovaprim) and Carp pituitary hormones. Such methods could be adopted by a wide range of development agents from governmental sectors and most importantly for the local farmers.

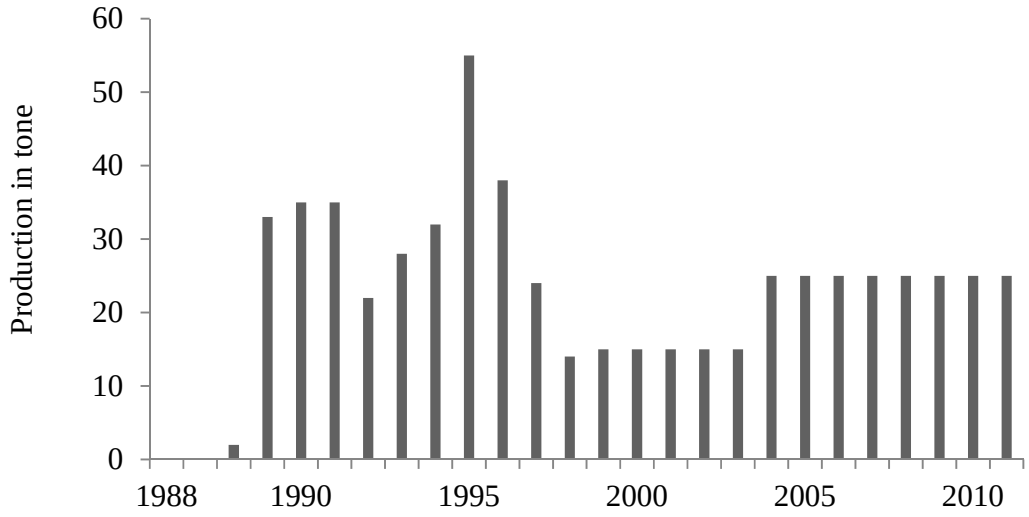


Figure 1: Trends of aquaculture production of finfish (1988- 2010 in tone) in Ethiopia adopted from (FAO 2013)

1.1 Statement of the problems

The fish breeding and research centre in Ethiopia was initiated in 1975 by the Japanese government under the Ministry of Agriculture. The centre is part of the fish research (Aquatic Science) wing that belongs to the Ethiopian Institute of Agriculture Sebeta National Fishery and Other Aquaculture Research Centre. Even if favourable conditions are available, aquaculture development and production in Ethiopia tend to be relatively low. There are many factors that constrained aquaculture growth, such as lack of adequate feed; quality fingerlings scarcity and shortage of man power in aquaculture sector. Fish harvested from natural water bodies have no longer fulfilled the growing needs for fish consumption (Moehl et al. 2005). There is no available domestic fingerling producing hatchery as of now, and fingerlings are always obtained from the wild where the fish quality is low and the stock quantity is limited (Brummett 2007; Pouomogne 2007).

Besides such restricted criteria, wild captured *Clarias garipinus* strains are more prone to diseases, therefore could cause additional difficulties to cultivate commercially. As a result of high population growths in Ethiopia, competitions in fishing activities around the natural lakes remain high. Consequentially, most of domestic lakes are already overexploited, triggering an alarmingly decline rate in potential rooms of development. One of the best solutions for African aquaculture is to start from tackling natural lakes by resolving fishing related problems with projected sustainable development methods (Watanabe 2002; Yang et al. 2006). There are possibilities that inadequate study has been undertaken on quality seed production and selective breeding process. This study is introduced to observe the comparative effectiveness of Carp pituitary gland and Ovaprim hormones on African catfish (*C.gariepinus*) spawning efficiencies.

2 General objectives

The main objective of the study was to evaluate the effect of natural and synthetic hormones on African catfish (*C. gariepinus*) seed production performance.

To achieve the objective mentioned above, the investigations were conducted with the following focus:

- To compare the effect of natural and synthetic hormones on fecundity rate
- To examine the effect of different hormone administrations on fertility and hatchability rate of African catfish
- To study the survival rate of African catfish (*C.gariepinus*) larvae using different stimulator hormones.

2.1 Study hypothesis

To achieve the aforementioned objective the following null hypothesis is proposed:

1. There is no significant difference in catfish egg fertility rates by using natural and artificial hormones.

2. There is no difference in the hatchability rates by using natural and artificial hormones.

3. There is no difference in larvae survival rates by using natural and synthetic hormones

2.2 Research question

Is there any statistically significant difference in ovulation time, fertilization, hatching and survival rate in response to Ovaprim and Carp pituitary extract induced breeding?

Which hormonal treatment yields better results in ovulation, fertilization, hatching and survival rate?

3 Overall experimental plan

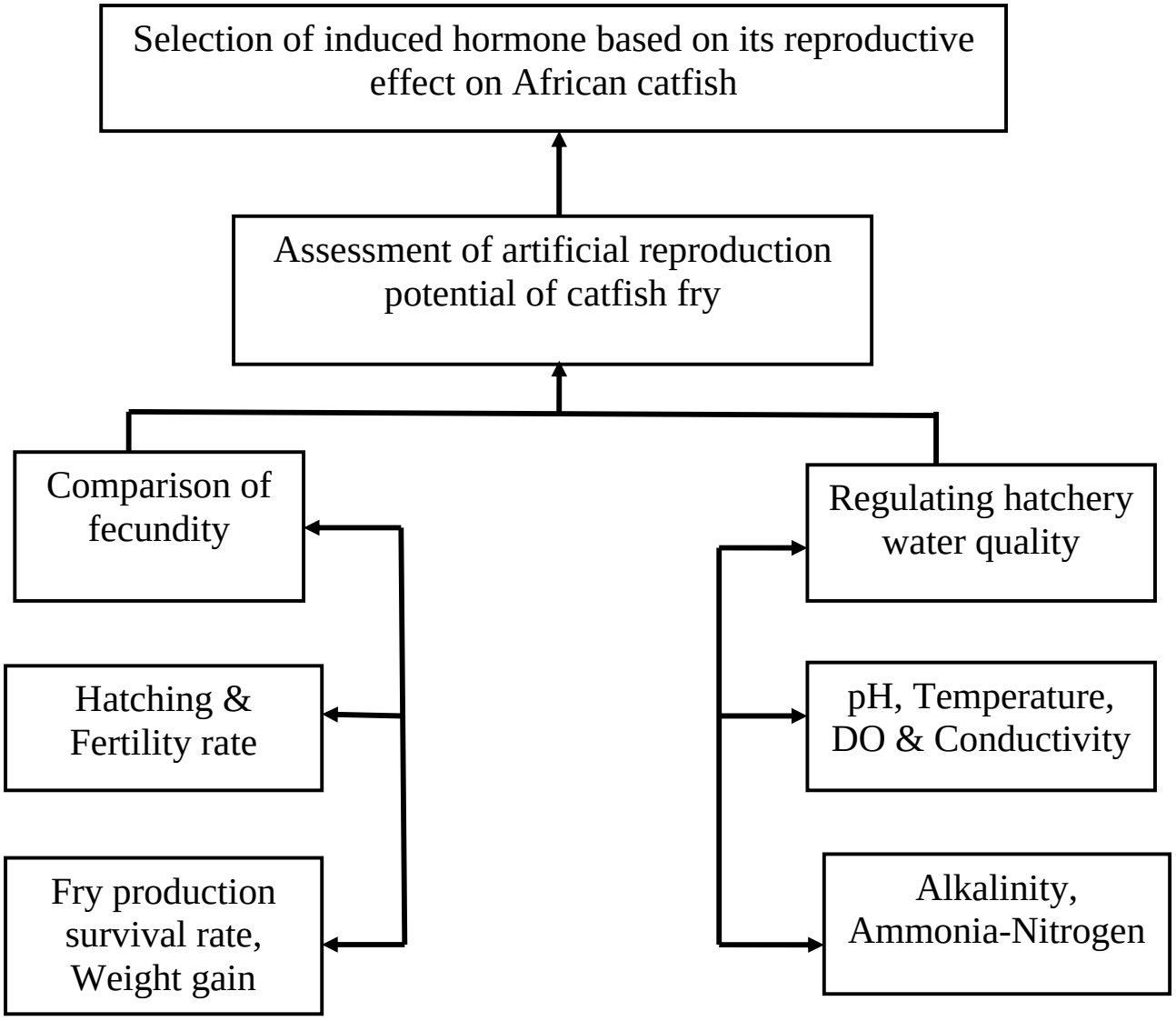


Figure 2: Schematic outline of overall experimental plan

4 Literature Review

4.1 State of World Aquaculture

Aquaculture is an ancient food production practice originally rooted from Asia. It is the fastest growing production sector with great potentials to meet the expanding demand for aquatic food (FAO 2011). The growth rate of global aquaculture production in 2004 was estimated to be 45.5 million tons (FAO 2009). Aquaculture is considered one of the best options that involves given natural resources to bridge the gap between world fish demand and production deficit. Carp (*Cyprinus carpio*), tilapia (*Oriochromis niloticus*) and catfish (*Clarias gariepinus*) are the main commercial fish species in the aquaculture developement. Large productions of aquatic plants and mollusks, together with the fishery, complete the activity infrastructure. Such models of implementation can act as self-sustaining, mini natural ecosystems (FAO 2006). For a world that faces rapid population growths, aquaculture with its promising outlooks to reduce environmental pressure and maintain ecologically balanced of natural ecosystem. It is one of the best answers to increase aquatic food production and job opportunities to the rural communities.

According to FAO (2006) report, out of the total amount of fish consumed in the world every year, almost half is produced in aquaculture farming. The remaining half is harvested from the oceans, natural lakes and rivers. Aquaculture has undergone development, expansion and intensification in almost all available regions of the world, except in sub-Saharan Africa. The global population demand for aquatic food products is expected to inevitable increased (FAO 2012).

4.2 Aquaculture in Africa

Aquaculture has been a farming practice in Africa since 1960s, where stocking of fingerlings in hydroelectric dams can be sourced from either natural or manmade water bodies (Meschkat 1967). However, aquaculture development in Africa has gone through a long and rough road. The current supply trends combined with the population rise results in low consumption per capita of fish in Africa (Muir e 2005; FAO 2011).

According to Huismas and Richter (1987), the average aquaculture growth rate has been 8.9% per year since 1970 compared to just 1.2% for capture fisheries and 2.8% for terrestrial farmed meat production over the same period. Since then, fishing industry has not progressed much further. In many cases, the lack of progress became declines, directly results in discouraged farmers abandoning their livelihood. According to Meschkat (1967), the main cause of failures can be attributed to over-harvesting on unmarketable sized fish from over-populated ponds. A high dependency on subsidized extension services and fingerling distribution centers also contributes as factors of obstacles. The gap between mutual interests is clearly visible between the local policy makers and the fish farmers. Foreign aquaculture practices are often implemented locally through the governmental enforcement without any willing fish farmers. The fish farmers find such intangible strategies and the policies were met with great resistance to expand the sector.

The African cat fish (*Clarias gariepinus*) is recognized as one of the most suitable aquaculture fish species in Africa. It is highly resistant to handling, stress, and able to remain a fast growth rate in pond culture condition (Micha 1975; Jocque 1975; Richer 1979; Hogendoorn 1979). Aquaculture research development methods for the production of catfish fingerlings has been regarded as one of the top priorities in Africa (Anonymous 1987a). African catfish hormone induced using deoxycorticosterone acetate, human chorionic gonadotropin and common carp pituitaries, has been carried out successfully (Hogendoor and Wieme 1976; Hogendoor and Vismans 1980; Micha 1976; Kelleher and Vincke 1976; EI Bolock 1976). Intensive farming system of African catfish fingerlings production has been successfully developed, using Artemia saline nauplii and commercial trout as a starter feed (Hogendoor 1980; Hogendoor and Vismans 1980). However, the existences of technically feasible farming methods and manuals do not guarantee implementation success (Viveen et al. 1985).

The main problem encountered with fingerling production in pond would be poor and erratic fish survival rate. Lack of appropriate feed and presence of predators would cause the production to vary from 0-60 fingerlings/m^2/cycle (Micha 1973; Hongendoor 1976; Kelleher and Vincker 1976). In the late eighties a simple and reliable method was developed within protected ponds for the nursing of *C.gariepinus* in the republic of Congo. This study indicated that competition for

food and cannibalism were the major factors affecting the pond nursing situation of *C.gariepinus* (Graaf et al. 1995).

4.3 Seed Production in Africa

Aquaculture begins with stocking of fry. Compare to the large arrays of fish species in natural water bodies, only very few strains were considered suitable aquaculture candidates. Nigeria is the first producer of African catfish in aquaculture. Considered a popular African culinary choice, catfish enjoys a celebrated status along with tilapia and carp. One must point out that it is difficult to reproduce catfish in captivity (Ponzani and Nguyen 2008). The fingerlings are collected mostly from the wild, where the availability varied greatly depends on seasonal changes (Brain and Army 1980). Insufficient number of eggs, along with shortage of quality fingerlings still imposes challenges in methodologies implied within controlled conditions (FAO 2003; Moehl and Halwart 2005). The current number of functional hatcheries plus the additional wild seed collection still do not meet the demand of the fingerlings for the farmers. On top of such issues, poor infrastructure for transportation causes additional fingerling mortalities before stocking (Yapi-Gnaore et. al. 2004).

4.4 Aquaculture in Ethiopia

Ethiopia is a landlocked country. The population solely depends on inland water-bodies in terms of fish supply. The country's water-bodies have a surface area estimated at 7,334 km^2 of major lakes and reservoirs, 275 km^2 of small water bodies and 7,185 km of rivers (Shibru Tedla 1973). The total fish yield from capture fisheries is roughly estimated at 40,000-50,000 tons (40-50 million kg) per year, where the contribution of aquaculture is almost negligible. In addition, actual annual aquaculture production was not well documented. FAO (2013) estimates that the aquaculture production is approximately around 15-25 tonnes (15,000-25,000 kg) per year in small scale fish farming. African aquaculture production totals to about 0.5% of the world overall production, while the highest portions of such production comes from the Mediterranean Sea. Egypt takes the lead of African aquacultural production with enough stock available to

export (FAO 1997). Of the 49 countries in Sub-Saharan Africa, 38 reported aquaculture production. Despite favourable physical conditions to cultivate and the comprehensive utilization of water resource for food production, aquaculture in Ethiopia is still considered at its beginner stage. The Sebeta National Fishery Research Centre has introduced different fish species under various aquaculture purposes in the country (Shibru and Fisseha 1981).

Table 1: Introduced species and purposes of introductions

Common name	**Species name**	**Origin**	**Purpose**
Grass carp,	*Ctenophyringdon idella*	Japan	Biological control & food
Common carp	*Cyprinus carpio*	Italy	Food
Carracius carp	*Carracius carracius*	Japan	Food
Golden fish	*Carracius auratus*	Japan	Entertainment
Silver carp	*Hypophthalmichthys molitrix*	Japan	Food
Tilapia zillii	*Tilapia zillii*	Uganda	Biological control & food

In addition to the table chart above, Sebeta research centre recruited Nile tilapia *Oreochromis niloticus,* another breed of indigenous fish. Currently, all fish species are circulating the market except the golden fish. The Centre has introduced about 2.5 million seeds of mostly Nile tilapia collected from the wild. The commercial and environmental profit envisioned by the project had declined and disappeared after the project had terminated. As of January 2009, the centre is working on approved five-year work plan both for Fisheries and aquaculture development/expansion. However, it faces severe constrains due to lack of quality seed particularly suitable for aquaculture growth.

4.5 **Taxonomy of African Catfish**

Species: *Clarias gariepinus* (Bruchell 1822)

Family: *Clariidae*

Order: *Siluriformes*

Class: *Actinopterygii*

Clarias gariepinus, commonly named African sharp tooth catfish, is scaleless and "air breathing". *Clariidae* are highly diverse; more than 100 different species have been studied in Africa based on their morphology and anatomical features (Teugels 1982a; 1982b; 1984). The genus *Clarias* was reviewed in the 1980s and number of species being synonymies (*C.lazera* of west and north Africa, *Clarias capensis* of southern Africa, *C. mossambicus* of central Africa) in the name *Clarias gariepinus* (Teugels 1986). *Clarias gariepinus* varies from dark to light brown in color and often spotted with shades of olive and grey (Skelton 2001). They are able to reach a maximum length of 170 cm (IGFA 2001) and weight of 60 kg (Robbins et al. 1991).

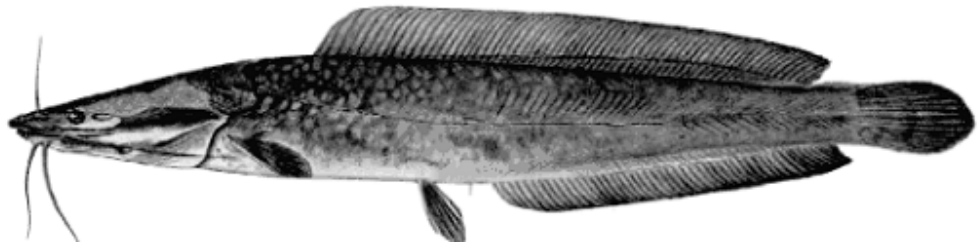


Figure 3: Lateral view of C.gariepinus (source: FAO 2012).

4.6 Natural Distribution of African Catfish

The aquaculture species that are regarded most economically valuable from the family *Clariidae* are: *Clarias lazera* from the North and Central part of Africa; *C.Mossambicus* from the central part; *C.gariepinus* from the southern part; *C. anguillaris* and *C. senegalensis* from the eastern part of Africa. Catfish (*C. gariepinus*) are commonly found in fresh and brackish water (Picker & Griffiths 2011). It is probably the most widely distributed fish species in Africa. *C. gariepinus* is tolerant of many different habitats, floodplains, lakes, slow flowing rivers and the upper reaches of estuaries. They are considered as fresh water fish species (Skelton 2001). In the natural aquatic systems *Clarias* can be matured at 6-9 months. However, the level of maturity varies in different water temperature condition (Schulz and Goos 1999). African catfish gonad maturation is generally related to water temperature and photoperiod. They showed aggressive behaviors among males during the courtship and spawning period. The male and female migrate upstream to still water body shores prior to spawning (de Moor & Burton 1988). The egg-laying takes place at night. Eggs are very sticky and are attached to aquatic vegetation that has been

submerged by seasonal flooding and water rise (Burton 1979). Hatching of eggs occurs after 24 to 36 hours depends on the water temperature (Burton 1979). Absolute fecundity estimated to be around 30,000–80,000 eggs per fish. Fecundity of a ripe female takes 15-20% of their total body weight (De Graaf et al. 1995). African catfish could be reproduced in natural ecosystem in response to water temperature fluctuation and water volume rising (Haylor 1992). Although *Clarias gariepinus* spawning tend take place in suitable sites that features favorable conditions, they take no parental care to ensure survival for their offspring (Haylor 1992). Catfish does not spawn in captivity even under favorable farming conditions. As a solution, several hormonal induced breeding techniques are developed for *C. gariepinus* farming (Richter and Van derHurk 1982). In Africa, the fish farming community mainly use natural pituitary suspension for catfish induced spawning (De Graaf and Janssen 1996). This method has proven to be highly reliable compared to synthetic hormone analogues in terms of cost and availability (Richter et al.1995).

4.7 Natural Food and Feeding

C. gariepinus has no specific food requirements; it could be feed mainly on aquatic insects, fish, higher plants, terrestrial insects, molluscs and fruits (Micha 1973). It may be considered as an omnivore - the one that eats almost everything. A catfish grabs its feed by orally sucking the prey. According to Bruton's (1976) study, catfish in Lake Sibaya (South Africa) were fed mainly on fish or crustacean. Those terrestrial and aquatic insects were important components of the diet for fish at both juvenile and adult stages. The feeding habits of *C. gariepinus* in Lake Mcilwaine (Zimbabwe) changed as the fish become larger in sizes: Diptera, particularly Chironomid pupae, dominates the diet of the smaller-sized fish, as the sizes of fish increase, the pupae consumption amount the grown fishes decreases (Munro 1967). Zooplankton gradually takes bigger portion in the diets of the larger fish (J. Janssen and Port Harcourt 1996). In scenarios where the previous diets cannot be realized, the cat fish will consume from an additional series of alternative food groups. The rate of consumption varies depends on the availability or the abundance of the resources. The further importance of zooplankton in the diet of larger fish was believed to be the cause of increase in the number of gill rakers (Jubb 1961; Groenewald; 1964). From the study of the feeding habits of *C.gariepinus* in Lake Kinneret (Israel) and according to Spataru's conclusion, preyed fish consist of the most abundant food component. In conclusion, *C.*

gariepinus is considered as a slow moving omnivorous predatory fish, who feeds on a variety of food items ranged from minute zooplankton to fish around 10% of its own body weight. In order to retain this variety level of organisms in the diet, *C. gariepinus* is equipped with a wide array of anatomical feeding adaptations (Bruton 1979), include;

1. A wide mouth capable of considerable vertical displacement for engulfing large prey or large volumes of water during filter feeding.
2. A broad band of curved teeth on the jaws and pharyngeal teeth preventing prey from escaping.
3. An abundant network of sensory organs of the body, head, lips and circumpolar barbells.
4. Long gill rakers on the five brachial arches.

In Lake Sibaya, South Africa, catfish tend to ignore or were incapable of catching prey during day light. They were feed mainly on invertebrates, which are abundant and relatively easy to capture. The catfish switch their feeding habits to fish prey when the prey become more vulnerable during the night, In general, fish prey provide far more energy per unit weight than any other prey items. Notably, the feeding habits of African catfish rely heavily on the existence of at least two alternative abundant preys (Bruton 1979).

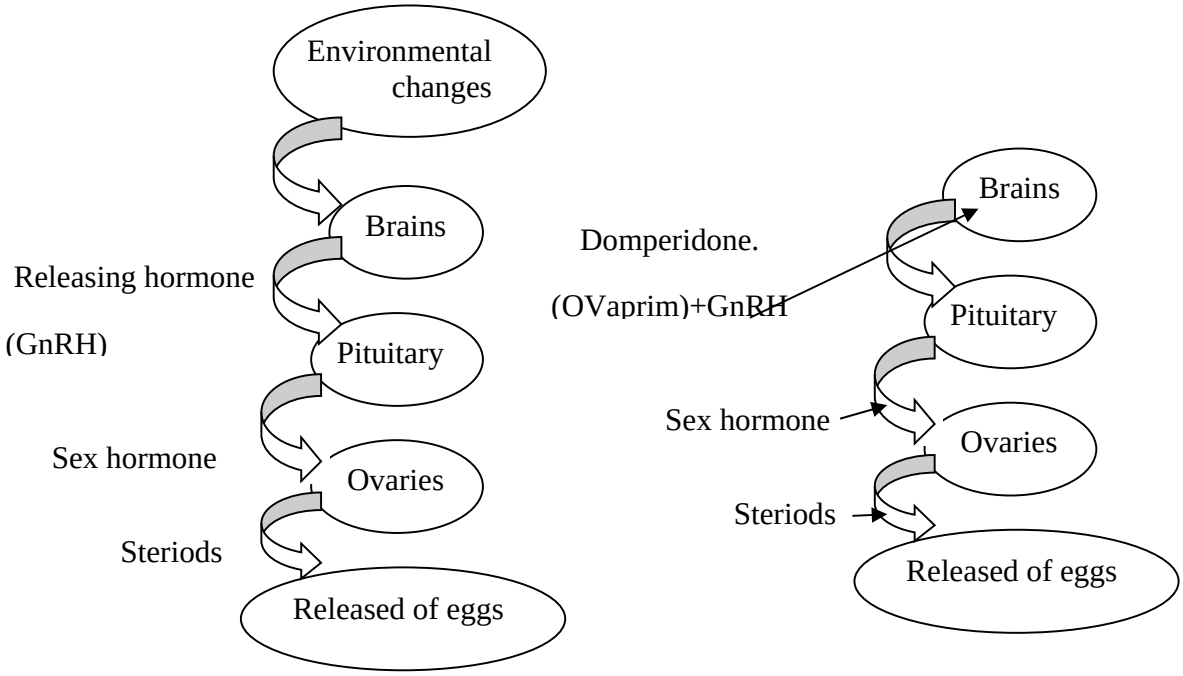
4.8 Natural Reproduction

African catfish (*C. gariepinus*) is known to breed during the summers, which usually associates with rainy season (Skelton 1993). The maturation stage of *C. gariepinus* is influenced by water temperature change. The final phase of spawning is triggered by a water level rise due to rainfall (de Graaf et.al. 1995). Given one example in Lake Victoria, Kenya, where the maturation and spawning of local *C. gariepinus* reproduction starts in March after the first heavy rain (Owiti and Dadzie 1989). Mature females have large ovaries that weigh up 15-20% in their total body weight, but the ovary weight decreases once the water temperature drops below 22^{o}C (de.Graaf et.al.1995). Natural reproduction is completed in July and the gonad somatic index remains low utill November. The Oocytes start growing slowly and ripens the next March. Spawning usually

takes place at night after heavy rain fall in the shallow areas of the rivers and lake margins (Bruton 1979). Before spawning the fish become aggregates, courtship can be preceded by highly aggressive encounters between males (de.Moor and Bruton, 1988). A batch of milt and eggs is released, followed by a vigorous swish of the female's tail to distribute the eggs over a wide area. Tiny fertilized eggs hatch out within 24 to 36 hours and attach themselves to surrounding water plants (Hecht et.al 1988).

4.9 **Induced Breeding**

The natural production process of *C. gariepinus* is closely associated to environmental factors such as rising water level and fluctuating water temperature. Fish ponds eliminated such factors from interfering the breeding (de Graaf et.al. 1995). The African catfish can be induced with pituitary glands or synthetic hormones injections in order to obtain a large amount of Oocytes. Induced ovulation and spawning can be achieved through hypophysation, which is a shortcut of natural process (Van der Waal 1974). There are two main strategies used in induced catfish reproduction: the first method is to provide an environment that imitates the natural condition that encourages natural spawning. Fish farmers can create simulations by placing milk cans in fish ponds. A hatchery photoperiod change can accelerate ovulation in many salmon, trout and catfish species. This method has not been reliable for mass fry production within the intensive fish farming system. Artificial propagation techniques have been developed by injecting natural and synthetic hormones. Artificial fertilization and incubation of eggs then follow (Woynarowich and Horvath 1980). This is only effective when brood fish are already in breeding condition, where matured eggs migrated to the germinal vesicle. Hormone treatment is employed to ensure large scale production of catfish fingerlings. Induced breeding allowed farmers to profitably breed and raise species that do not naturally reproduce in captivity (de.Graaf.et.al.1995).



Natural spawning cascade (a) Ovaprim-induced cascade (b)

Figure 4: Natural (a) and Ovaprim induced (b) reproductive cascade in fish (adopted from Rottmann et.al. 1991a)

Below is a list of hormones that have been used daily for fish induced reproduction:

1. DOCA (Desoxycorticosteriod Acetate), 2.5-5 mg per 100 gram for female.
2. HCG (Human chronics Gonadotrophin), 25 I.U. per 100g for female, effective but expensive
3. Common carp (*Cyprinus carpio*) pituitary gland materials, 3-4 mg per body weight of female fish
4. Pituitary of the African catfish (*C.gariepinus*)

The pituitary gland (PG) can be conserved in either alcohol or glycerin. It can be used in either fresh or dried form. It is grounded, dissolved, centrifuged and injected into the female fish. There is no standard dose; the dose used by fish breeders varies considerably. For a pituitary extract, a

general rule of thumb is to use one pituitary gland from a fish of equal size/injection. Others talk about 2 to 6 mg/kg of body weight. Experiments have been conducted by using anything from 0.5 to 8 mg of pituitary extract/kg of brood stock. The hormones may be administered in one single injection. If spawning within 24 hours does not follow, then there may be repeated injections. The dose and frequency of spawning depends on the quality of the pituitary extract and the particular maturity stage of the female.

4.10 **Brood Fish Management**

Brood stock management has critical impacts on health status and performance of the seed (Maire, 2002). Development of brood fish depends mainly on the protein portion content in their feed supplies. Catfish can be matured at the age of 12 months under good feed supply and farming (de.Graaf and Janssen 1996). The nutrition status of brood fish can significantly affect the reproductive physiology and egg/larvae quality. The brood stock should be maintained in decent standards so that ripe broods could be obtained during the whole breeding season. Feeding is carried out twice times a day with feed containing 40% crude protein, at 5% of biomass (Viveen et.al. 1986; Jassen 1987). To prepare for spawning, feeding has to be stopped one day prior to stripping. A seine net is used to gently capture the brood fish. After collection of the fish from the conditioned pond, fish are treated with formalin bath to prevent the transfer of pathogens from fish with eggs and fry.

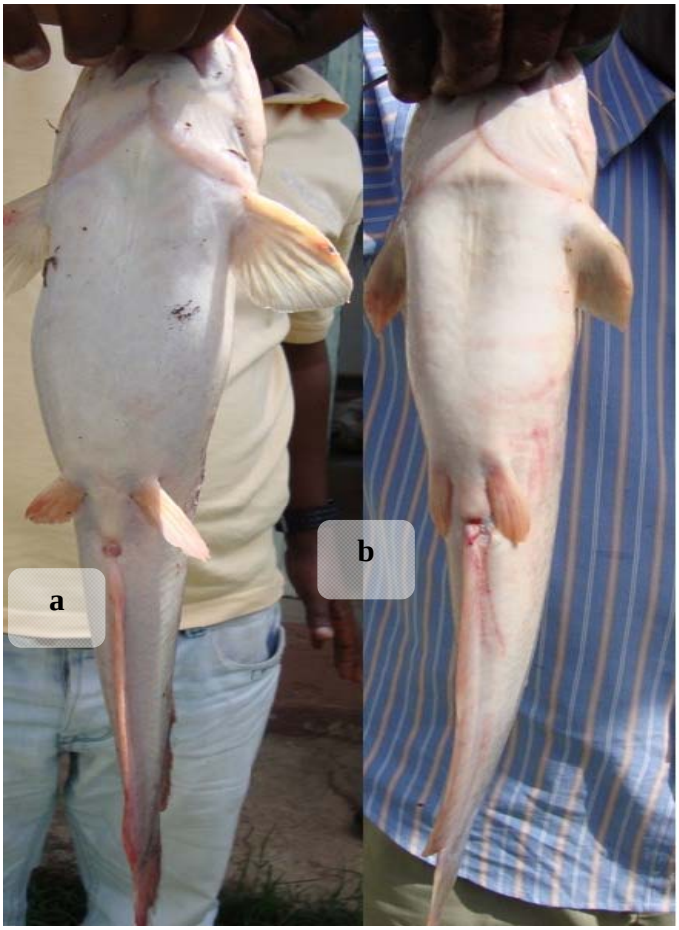


Figure 5: Sexual characteristics of female (a) and male (b) catfish (*C.gariepinus)*

4.11 **Pituitary Gland Collection and Administration**

The pituitary gland is collected from the donor, and then placed in a mortar containing 2 ml of physiological salt solution (9 g salt/1 liter of water) as solvent. Pituitary is grounded and mixed with the saline solution. Generally, 1ml pituitary gland solution per 500g body weight is recommended. Alternatively, the pituitary can be stored for later use up to several months in 1 ml acetone at a cool dry place. Synthetic hormone such as Luteinizing Hormone Releasing Hormone Analogue (LHRH-a) is used (10-30 μg) in combination with Domperidone (3-5 mg per kg of brood stock). The second injection normally takes place 6 hours after the initial injection. About 10 hours after the first injection, the fish are ready for egg stripping. For the injection of pituitary extract, a syringe with a needle (2.5 to 3 cm long with diameter of 0.7 mm) is used to draw the

pituitary suspension. The most commonly adopted technique to hormone solution administers is injecting intramuscular into the dorsal muscles above the lateral line, just below the anterior part of the dorsal fin using a graduated syringe (5ml). The needle is placed parallel to the fish, pointed posterior at an angle of approximately 30-45°. To distribute the hormone suspension evenly throughout the muscles, the injected area is rubbed with fingers afterwards. The head of the fish should be covered with a hand towel prior to injection. The fish is then released back into the tank. Females are generally injected in the evening. The injection time is calculated according to the water temperature and the desired time of stripping. Eggs are stripped gently from the female into a dry bowl and the number of eggs are estimated (1 g = approx. 600-700 eggs). Male gonads are removed and macerated, squeezed and immediately mixed with eggs, distributing the milt evenly with the help of a feather. Besides gently swirl the bowl and move the feather to facilitate the egg and sperm mix, clean water is also added to the bowl (Woynarovich and Horvath 1980).

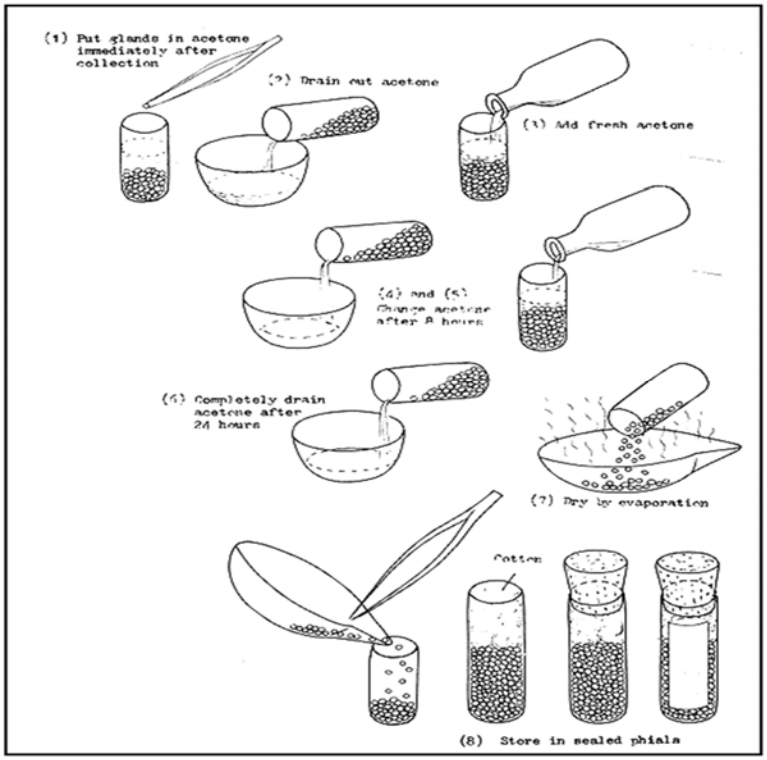


Figure 6: Preparation of acetone dried pituitary gland (Woynarovich and Horvath 1980)

4.12 **Fertilization and Hatching**

Once in contact with water, African catfish eggs became sticky. Such scenario can be prevented by salt rinsing or stirring (de.Graaf et.al.1995). The fertilized eggs are poured into an incubating tray in a single layer. Within a few minutes after fertilization, the eggs will absorb water and sticky attachment discs will develop. Eggs are often spread out on a screen of 1 mm mesh size, which is then placed in plastic basin. Eggs incubated in flowing water have a gentle flow-through rate of 1-3 liters per minute. Dead eggs, normally white in color, are removed immediately. Eggs hatch within 20-25 hours depend on the water temperature (Hogendoorn and Vismans 1980). Hatchings must be separated from the egg shells to avoid fungal infections that can lead to mortality. At this stage of development, the hatching rate will be about 50-60%. Hatched fry are 5-7 mm in length and weigh about 1.2-3.0 mg. They cluster together in dark places in the tank and require dark cover. Within 3 days the yolk sac will be absorbed, and the swim-up fry will start to search for food (de.Graaf et.al, 1995). Water is renewed to provide oxygen and to avoid fungal infection which causes mortality. The hatching and development rate mainly depend on water temperature like other biological process (Hogendoorn and Vismans 1980).

Table 2: Time of hatching rate of catfish eggs in relation to water temperature

Water temperature (^{o}C)	Time between hatching rate (hours)
20	57
21	46
22	38
23	33
24	29
25	27
26	25
27	23
28	22
29	21
30	20

4.13 **Larval Rearing**

Larvae are very vulnerable, especially in tanks with chlorine-less water supply. They require refined environmental conditions. Stocking density should be around 100 larvae per liter to optimize growth and survival. They are fed with rotifers or *Artemia* for the first 10-14 days. Fry can then transferred to ponds and begin their live zooplankton and supplementary feeds. Water should be maintained at a desired temperature of 25°C. Catfish larvae start feeding on newly hatched brine shrimp (*Artemia nuaplii*), a very small saltwater crustacean known as an ideal feed for catfish and carp larvae. Manufactured dry feeds should be supplemented with *Artemia nauplii* or rotifers, and 10 to 12 daily feeding rations should be provided for first three to four days of feeding. Feeding with live feeds has advantages over dry feed. *Artemia nauplii* or rotifer feeding is stopped on the second or third day after external feeding starts. The larvae should then grow rapidly after the beginning of external feeding (up to 100% body weight/day).

4.14 **Hatchery Water Quality**

Both spring water and ground water can be appropriate for hatchery use. But ground water is considered to have better water quality, usually free from floating materials, contaminants and pathogens that might cause fish diseases. Physical, biological and chemical parameters of water can affect the growth and welfare of cultured organisms. Successful aquaculture production depends on optimum environmental condition for their growth and expansion.

Water can regulate the growth rate and internal body temperature of the organisms. Its regulation also is shown through dissolved material and waste product exchange within the system. Before aquaculture production starts, the source and quality of water serves as the main determining factor. PH, ammonia, nitrite, alkalinity, temperature, suspended substances, heavy metal and concentrations of dissolved oxygen can all be counted as the key factors for healthy fish growth. Dissolved oxygen is considered the most important parameter. Such condition requires continuous monitoring in artificial fish production systems

5 Materials and Methods

5.1 Study site

The study took place between October 2014 to January 2015 at Sebeta National Fisheries and Other Aquatic Life Research Centre (NFALRC). It is located 25 km southwest of Addis Ababa, Ethiopia, an altitude of 2250 m a.s.l, 08 54' N latitude and 38º38' E longitude. The mean monthly minimum and maximum temperature varies between 24ºC to 27.9ºC throughout the year. The institute was built in 1975. It has a total area of 5 hectares and consists of buildings (offices, laboratory, hatchery and stores) and outdoor 26 experimental ponds. Theoretically, the production capacity of the hatchery could reach up to 2 to 3 million fingerlings per year. The most commercial fish species in the research centre are Tilapia (*Oreochromis niloticus*), African catfish (*Clarias gariepinus), Carp (cyprinid carpio*) and gold fish (Center profile leaflet 2012).

Figure 7: The pictures show the study site fish pond and hatchery (NFALRC)

5.2 Statistical Analysis

The data of fertilization, hatching and survival rate over 14 days were analyzed by two methods: A one way analysis of variance (ANOVA) and least significant difference (LSD) test was used to determine mean differences between doses of Ovaprim (OV) and Carp Pituitary (PG) extract at different hormone administration levels.

5.3 **Brood fish collection**

The African catfish, *Clarias gariepius* brood were collected from the Lake Koka hydroelectric dam and transported to the centre in plastic containers. All brood fish were stocked and acclimatized in a fish pond for 15 days. The female fish were kept separated from the male fish in concrete pond ($10\times10\times1.5m^3$). They were fed Sebeta feed 5% of their total fish biomass with 35% of crude protein twice a day at (10 am and 15 pm). The brood stock would be checked regularly based on external morphological features associated to ripeness, e.g. swollen reddish genital papilla, soft abdominal region, and comparatively large size. In order to check egg maturation, Oocytes samples were taken from each brood fish by inserting canule 4-5 cm inside the papilla. Each Oocytes diameter was measured under dissecting microscope in the laboratory. If 90 % of the total Oocytes have diameters larger than 1.05 mm, then the fish is considered ready for artificial incubation. Ripe ovaries are green-brown in color and generally uniform in size (Ayinla et.al.1994).

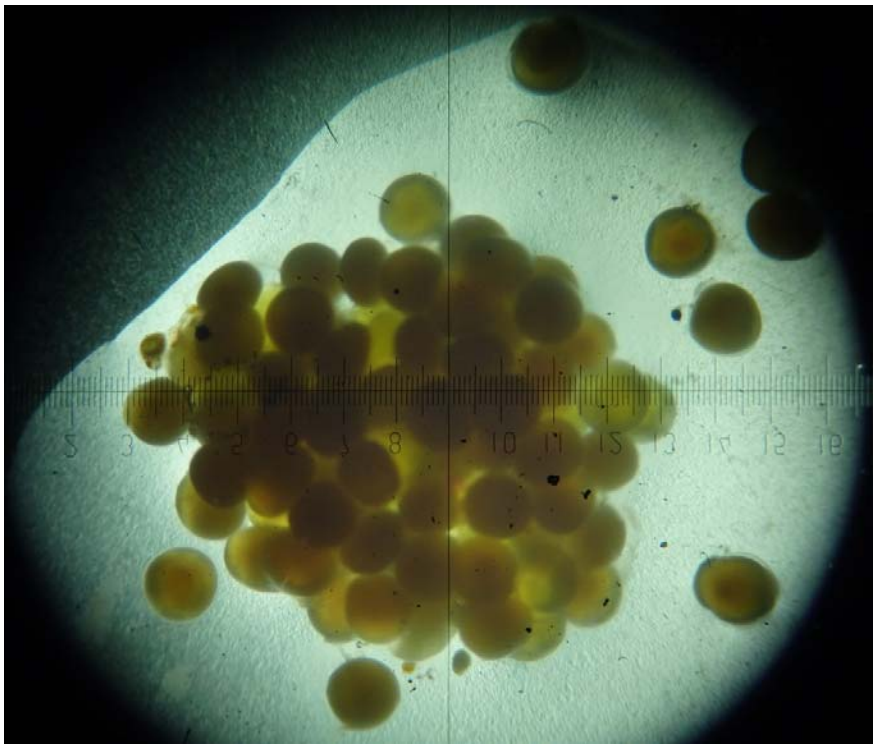


Figure 8: Ripe Oocytes of *C.gariepinus* under dissecting microscope

5.4 Synthetic hormones Ovaprim

Synthetic hormones Ovaprim (OV) is a commercial product that contains 10mg/ml of a salmon gonadotrophin releasing hormone (sGnRHa). The synthetic hormone, Ovaprim used for the study from the Aqua life syndial laboratories LTD. Nanaimo, B.C. Canada. Ovaprim is marketed ready to inject in a liquid form. It is used as spawning aid to induce ovulation in well conditioned brood fish. It is recommended to be stored below 25ºC and has a shelf life of 2 years

Figure 9: Synthetic hormone Ovaprim solution which was used as spawning aid in catfish breeding (Source: Syndel Laboratories. Ltd)

5.5 Pituitary gland collection

The Carp pituitary gland (PG) was collected from the fisher men leftover in Lake Koka reservoirs. It was removed from sexually matured (female or male) Carp fish. In order to access the pituitary gland the top of the skull is removed with a knife. Pituitary gland is left behind on the basis of the skull. Each pituitary glade was preserved in alcohol or with acetone immediately after collection then transferred in a vial. The acetone was decanted after 8hours and then refilled with fresh acetone. This was kept in a cool place for 24 hours after which it was poured acetone completely, dried by evaporation and stored in a sealed vial for use (Woynarovich and Horvath 1980).

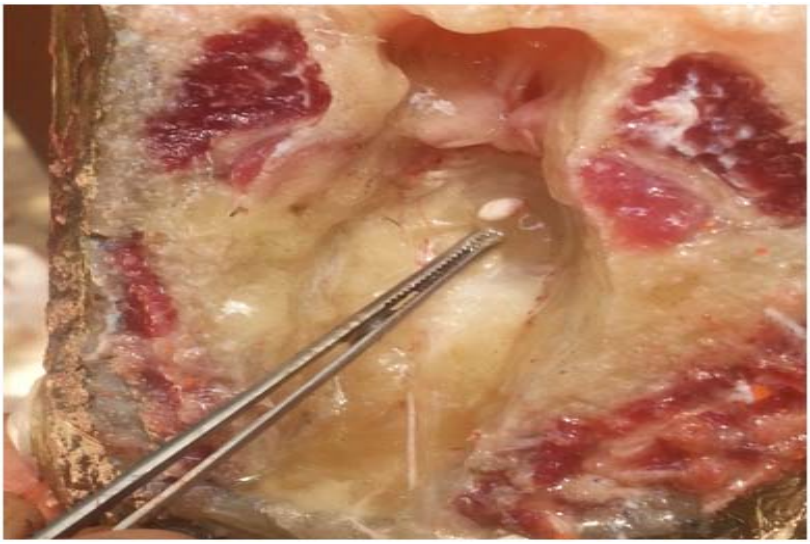


Figure 10: Pituitary gland collection from Carp head in Lake Koka hydroelectric dam

The dose of pituitary gland used was calculated in relation to total fish body weight to be injected using the following formula:

$$\text{Weight of of PG (mg)} = \frac{\text{Wt} \times \text{Pt}}{1000}$$

Where Wt represents fish body weight in (g) and Pt represent weight of pituitary gland in mg to be injected/kg body weight under particular treatment. PG=the total Carp pituitary gland to be used for injection.

5.6 **Experimental design**

Three factors: Ovaprim (OV), Carp pituitary gland (PG), and the control group without any treatment.

Three levels: one time injection total dose at once; two times injection (10 %, and 90% at 6 hours time interval between the first and second injection; three times injection (10%, after 4 hours second injection 10% and then 2 hours later 80 % was injected) and the control has no injection.

The experimental was design in a complete block random design (CBRD). All hormones were administered by intra-muscular injection on the muscle beneath the dorsal fin. The method of injection to both ovaprim and pituitary hormones were the same methods.

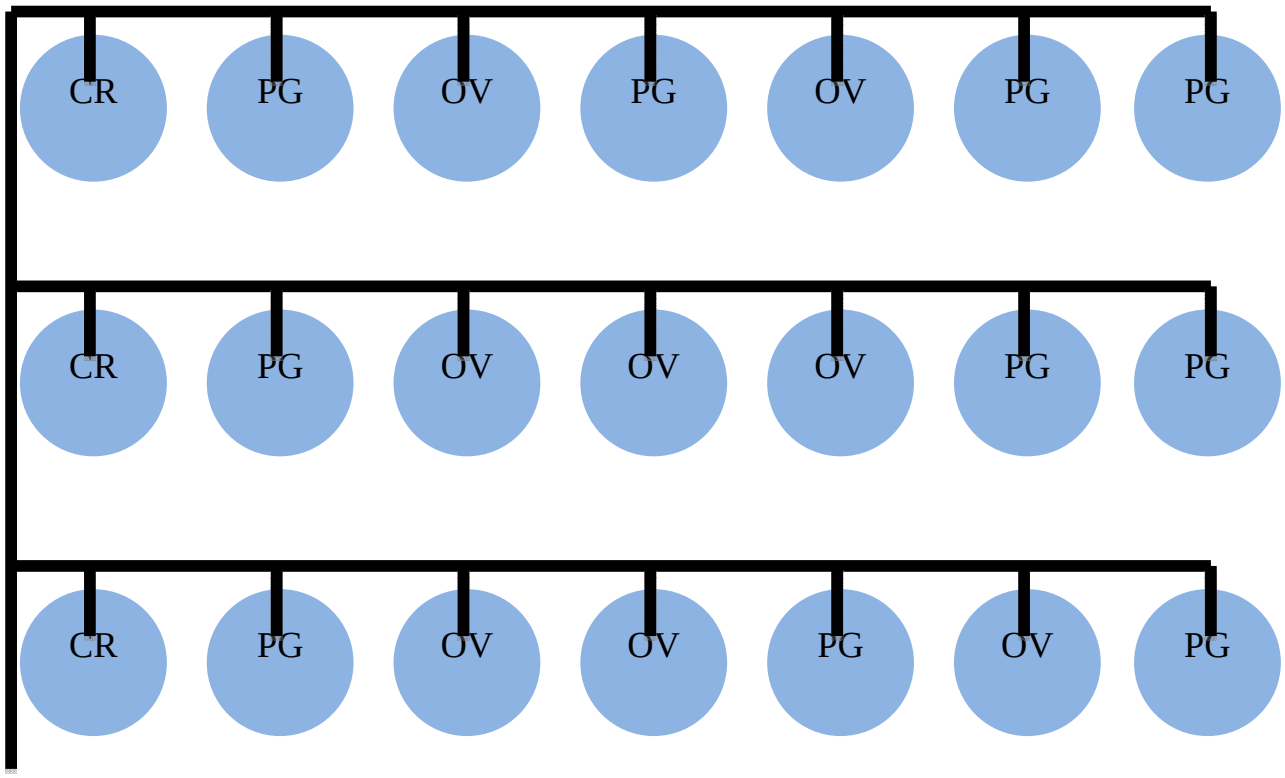


Figure 11: Experimental tank layout for incubation of eggs (where CR=control, PG=pituitary gland and OV=Ovaprim)

Figure 12: Catfish larval rearing basin layout in the hatchery

There are two treatment groups, one with Ovaprim hormone (OV) injection, the other one with carp pituitary gland (PG) injection. The injections were given to three fish at a time, and one fish in each replica was used. Prior to injection, the female brood fish were kept without fed for 24 hours in a tank size ($4\times5\times3$ m^3) from male fish. The purpose is to release the fecal matter outside the body and prepare for spawning. During the experiment the water temperature in the tanks will be kept between 25 to 28^{o}c.

Table 3: Hormone administration (Dose, Latency time

Brood stocks	Hormones used	Trail codes	Nr of injections and dosage		
			One time	Two times	Three times
1	Ovaprim	OV1R1	0.5 ml kg^{-1}		
2	Ovaprim	OV1R2	0.5 ml kg^{-1}		
3	Ovaprim	OV1R3	0.5 ml kg^{-1}		
4	Ovaprim	OV2R1	0.05 ml kg^{-1}	0.45 ml kg^{-1}	
5	Ovaprim	OV2R2	0.05 ml kg^{-1}	0.45 ml kg^{-1}	
6	Ovaprim	OV2R3	0.05 ml kg^{-1}	0.45 ml kg^{-1}	
7	Ovaprim	OV3R1	0.05 ml kg^{-1}	0.05 ml kg^{-1}	0.4 ml kg^{-1}
8	Ovaprim	OV3R2	0.05 ml kg^{-1}	0.05 ml kg^{-1}	0.4 ml kg^{-1}
9	Ovaprim	OV3R3	0.05 mlkg^{-1}	0.05 mlkg^{-1}	0.4 ml kg^{-1}
10	Pituitary gland	PG1R1	3 mg kg^{-1}		

11	Pituitary gland	PG1R2	3 mg kg^{-1}		
12	Pituitary gland	PG1R3	3 mg kg^{-1}		
13	Pituitary gland	PG2R1	0.3 mg kg^{-1}	2.7 mg kg^{-1}	
14	Pituitary gland	PG2R2	0.3 mg kg^{-1}	2.7 mg kg^{-1}	
15	Pituitary gland	PG2R3	0.3 mg kg^{-1}	2.7 mg kg^{-1}	
16	Pituitary gland	PG3R1	0.3 mg kg^{-1}	0.3 mg kg^{-1}	2.4 mg kg^{-1}
17	Pituitary gland	PG3R2	0.3 mg kg^{-1}	0.3 mg kg^{-1}	2.4 mg kg^{-1}
18	Pituitary gland	PG3R3	0.3 mg kg^{-1}	0.3 mg kg^{-1}	2.4 mg kg^{-1}
19	Control	CR1	No injection	No injection	No injection
20	Control	CR2	No injection	No injection	No injection
21	Control	CR3	No injection	No injection	No injection

In total, 21 females and 9 males were used for this study. To stimulate ovulation of the first group (1 to 9 females; Table 3), gravid females was weighed individually. Then the fish was covered with towel and given intramuscular injection. When pulling the needle back, set back the needle slightly to create small space in the fish muscle for hormones. Ovaprim (OV) was injected at dose rate of 0.5 ml kg^{-1} of body weight of female fish (Legender 1986; Madu 1989). Injection frequencies vary between one, two and three times with different time interval mentioned above. The time interval between two successive injections was a 6-hour difference. The injected fish were promptly returned to water tanks where they wait to be stripped.

In the second treatment group (10 to 18; Table3), similar gravid females were weighed individually and intramuscular injection with acetone dried Carp pituitary gland extract (PG) at a dosage of 3 mg kg^{-1} of body weight (Hogendoorn and Vismans 1980). Injection frequencies vary between one, two and three times with different time interval mentioned above. The fish were caught carefully with towel and kept in sponge. During injection, fish receives finger rubs to prevent back flow of fluid. Once they were injected, each treatment fish was kept in different water holding tanks for 14-16 hours under a hatchery. The control group, which contains three similar size female catfish were weighed and kept in 25 to 28 ºC water temperature without hormone injection. The hormone is administered with a 5ml syringe and inserted at 30-45º angles into the fish at the anterior part of the dorsal fin. The ovulation of the brood fish was checked every time by pressing the abdomen (Richter et .al. 1987). The male was scarified to obtain their sperm and dissected, the milt sac was removed before artificial spawning.

The milt sac was opened with sharp scissors, and the milt then washed into a vial with 0.9% saline solution. For this study vials with milt were prepared to cater for fish spawned. Stripping was taken place 14-16 hours after the first injection at temperature range of 25-28^{o}C. This was carried out by an assistant holding the fish at the head and tail. The ovulated eggs were squeezed into a plastic bowl with thumb pressure. The process shall continue until traces of blood appear at this point the stripping can stop, and the fish can be returned back to the water.

The eggs obtained from stripping were weighed, followed by several drop of creamy milt squeezed over the eggs with dry hands. The bowl is then gently swirled to mix the eggs and milt. A 0.9% (NaCl) saline solution (Woynarovich and Horvath 1980) was added, causing gentle movement of sperms, activates fertilization. After 45 seconds to one minute, no further fertilization can take place as the sperms are no longer motile. The fertilized eggs were then spread over the nylon net in each incubation plastic basins.

Number of eggs released in tray was estimated using gravimetric methods (Bagenal and Braum 1978). Total weighing of the Oocytes using electronic balance and small subsample of ovary was taken from the total and weighed gravimetric method (Legender 1986). Then it is preserved with Gilson's fluid, where the number of eggs was counted. Thus fecundity was calculated using the following formula.

(1). $F = \frac{\text{Total Oocytes Weight} \times N}{\text{Weight of subsample Oocytes}}$

Where F represent fish fecundity and N represent number of eggs counted in the subsample.

Measurement of the egg size will be done by measuring the egg diameter (mm) using digital microscope. For each female fish, ten eggs were collected from the total Oocytes and the diameters were measured.

The fertilization rate was calculated through random sampling by examining 3 samples from each breeding basin (trail). About five hundred (500) or two hundred (200) eggs were used for for each fertilization trail. The number of eggs was estimated using the gravimetric method (Bangenal and Braum 1978) (i.e no.of eggs/g). The translucent eggs that contain embryonic eyes

at the time of polar cap formation (about 20 minutes after fertilization) were considered fertilized, while dead eggs appeared whitish and opaque within 8 to 10 hours of fertilization. The fertilized eggs were then counted in order to calculate the percentage fertilization.

(2). $Fertilization\ rate\ (\%) = \frac{\text{No.of fertilized eggs}}{\text{Total no.of eggs sampled}} \times 100$ (Alam et.al. 2006).

Immediately after the fertilization, the fertilized eggs were spread over in a single layer of nylon mosquito net (mesh size 0.5mm) that is submerged in water, but suspended a few centimeters above the base of the tray. Incubation follows in well aerated flow water in plastic basin filled with 40 L of water. Water flows slowly in and out of the basin to obtain well oxygenated medium and not to disturb the eggs. The outer flow end of the basin was screened to prevent the young larvae from being swept out. Incubated eggs were kept in separate plastic basin with water temperature between 25 to 28^{o}c. After 24 hours of incubation, the nylon mesh was removed. Spoiled or dead eggs attached to the net, along with shells and other particles were discarded. The hatched larvae were clustered at the dark corners of the incubation basins. Three to four days after the hatched, *Artemia nuplii* were fed to the larvae.

Figure 13: Spreading of fertilized eggs over the nylon net in the incubation basins

After the larvae hatch, the percentage of dead eggs of each basin was calculated. The percentage of hatched larvae was counted several hours after the incubation processes. The hatching rate was calculated using the following formula.

(3). Hatching rate (%) $= \frac{\text{No.of hatchlings}}{\text{Total no.of fertilized eggs}} \times 100$

The fry was fed with *Artemia* for 14 days and then take a commercial feed in a size of 0.2 mm. The *Artemia nauplii* were hatched in the salt water (20 ppt) indoor hatchery. *Artemia* is tiny salt water shrimps that are live food for African catfish larvae. Each replica has been fed uniformly at 10 % of their body weight twice daily, regularly from 7.00 hours to 23.00 hours. Larvae stomach content is checked after every feeding. The duration of this indoor experiment took 14 days.

5.7 **Survival rate:**

After 14 days of rearing, the percentage of live embryo was calculated for each female brood fish. Each experimental parental fry was stocked in three groups with plastic basins. The pooled weight of fry will be taken in continuous flowing water. Siphoning of uneaten food and dead fry was picked up daily to determine the mortality rate. The survival rate of fry was obtained through visual counting at the end of the experimental period.

$$\text{Survival rate (\%)} = \frac{\text{No. of larvae at the end of study}}{\text{No. of larvae at the beginning of study}} \times 100$$

5.8 **Water quality requirement**

Water used for cultivation deteriorates rapidly and needs to be frequently refreshed. The water supply was stopped during feeding to avoid food thinning. The experimental water quality was monitored daily, parameters like pH, DO, conductivity and temperature during the trials using a multi-meter (HQ) electronic probe.

Table 4: Water quality requirements of African cat fish hatchery (FAO 1992).

Chemical and physical features	Desired level
Dissolved oxygen (DO)	6 mg L^{-1}
Water pH	7-7.5
Temperature	27-30 $^{\circ}C$
Ammonium(NH4)	0.5mg L^{-1}

5.9 Ammonia-Nitrogen (NH_4^{+3}-N)

NH^{+4}-N was determined by using the Indo-Phenol blue method (Krom et.al.1989). A stock solution of 1g L^{-1} NH_4^{+3}-N was prepared by dissolving 3.819 g NH_4Cl in 1000 ml of distilled water. Intermediate solution of 10 mg L^{-1} was prepared by diluting 10 ml of stock solution to 1000 ml of distilled water. Working solution of 250 µg L^{-1} was prepared by diluting 25 ml of intermediate solution into 1000 ml of distilled water. A volume of 25 ml of sample was used. The non-ingested food and fecal particles should be washed out once a day by exchanging about 20% of the water volume of the container to keep clean the basins (Parry 1960).

5.10 Reagents

5.10.1 Sodium salicylate solution

130 g Sodium-Salycylate and 130 g of Trisodiumcitrat-Dihydrat was dissolved in 800 ml of distilled water, then 0.97 g of sodium nitropruside was added and the volume made to 1L.

5.10.2 Hypochloride solution

32 g of NaOH was dissolved in 1000 ml of distilled water just before use. Then 0.2 g Sodium dichloroisocyanurat was dissolved in 100 ml of the base (reagent B). 2.5 ml of reagent A were added to 25 ml of filtered sample and standard series. After shaking, 2.5 ml of reagent B were immediately added. Eventually the samples were stored in dark for 1.30 hours at 25ºC for color development, and then the absorbance was measured at 655nm.

6 Results

6.1 Physicochemical Parameters

During the study period water qualities parameters were measured except temperature all others parameters remains in the appropriate range set for *C.gariepinus* cultivations (Stone and Thomford 2002).

Table 5: The water quality parameters measured at different treatments over 14 days period *C.gariepinus* induced breeding. Values are mean ± SE of three replicates.

Water quality parameters	Carp pituitary gland treated basins	Ovaprim hormone treated basins
Temperature (ºC)	25.12±1.54	25.1 ± 1.37
pH	7.77 ± 0.4	7.6 ± 0.19
Oxygen (mg L^{-1})	4.95 ± 0.59	5.43 ± 0.57
Oxygen Saturation (%)	78.38 ±0.19	85.97 ± 0.52
Conductivity (µs cm^{-1})	287.9 ±27.33	285.4 ± 17.08
Ammonia-Nitrogen (NH_4-N) (mg L^{-1})	0.104 ± 0.026	0.12 ± 0.025

6.1.1 Temperature

Temperature was measured every day and there was slight fluctuation during the study period. The value was in the ranged between 23-28 ºC in over all treatments. While the mean temperature of 25.12 ± 1.54 ºC and 25.1 ± 1.37 ºC in Carp pituitary and Ovaprim treated basin was recorded respectively. The mean water temperature values in all trials fell below the recommended ranges.

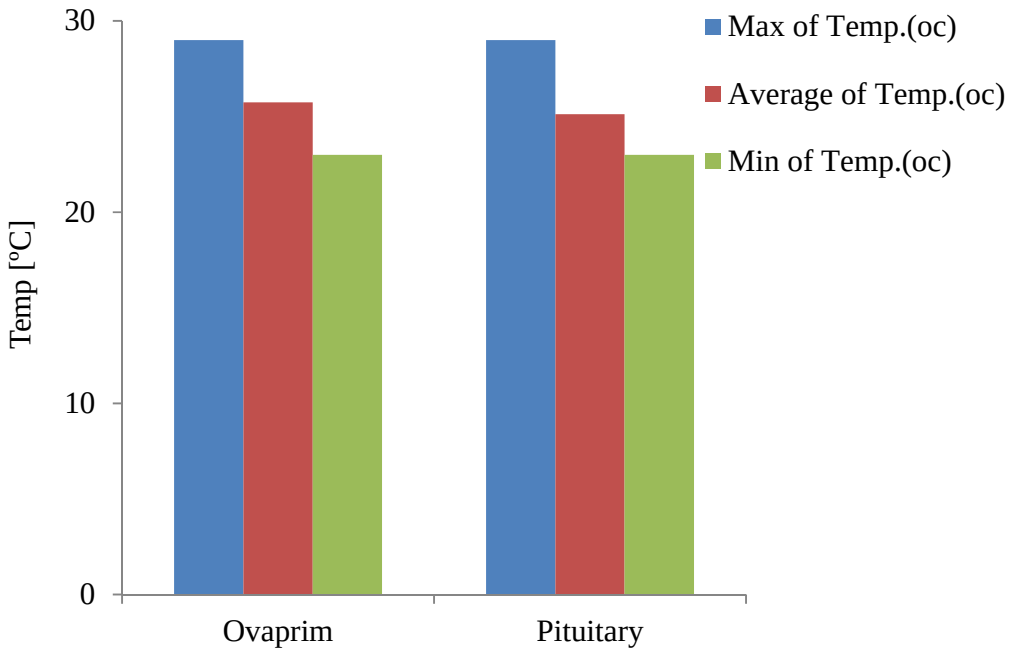


Figure 14: The water temperature measured at different treatments over 14 days for C.gariepinus induced breeding in a hatchery

6.1.2 **Dissolved oxygen**

The dissolved oxygen (DO) concentration was measured in the range between 3.7 - 6.4 mg L^{-1} and mean value was 4.95 ± 0.59 for Carp pituitary gland and 5.43 ± 0.57 mg L^{-1} for synthetic (Ovaprim) hormone treated basins during the experimental period. The maximum dissolved oxygen (DO) concentration was ranged between 5.9 - 6.4 mg L^{-1} in this study period. There was no significant difference ($p>0.05$) in dissolved oxygen concentration among all treatments during the study periods. The range oxygen recorded in this study was slightly varies but fell within the recommended values. This may be contributed by continues flow of water in all experimental basins.

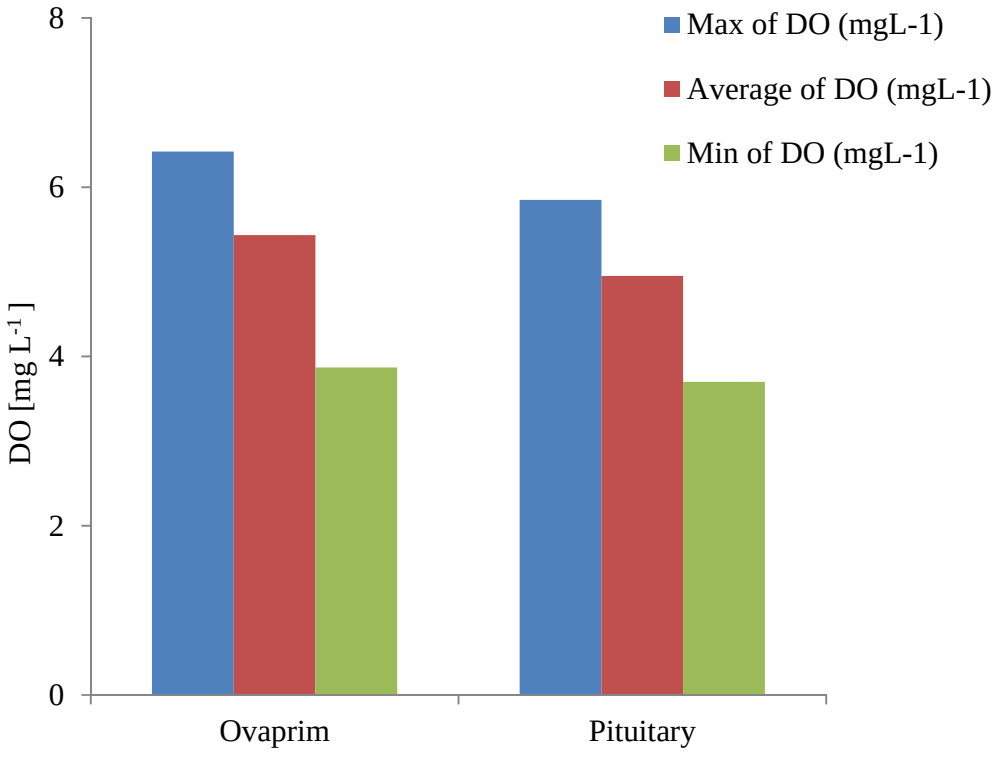


Figure 15: Maximum, average and minimum dissolved oxygen (DO) concentration measured at different treatments over 14 days period *C.gariepinus* induced breeding in a hatchery

6.1.3 **PH**

The pH value of each treatment basin was in range between 7.1 - 8.8 with mean of 7.77 ± 0.4 for Carp pituitary hormone treated basins while Synthetic hormone (Ovaprim) treated basin was mean of 7.7 ± 0.19 for study period.

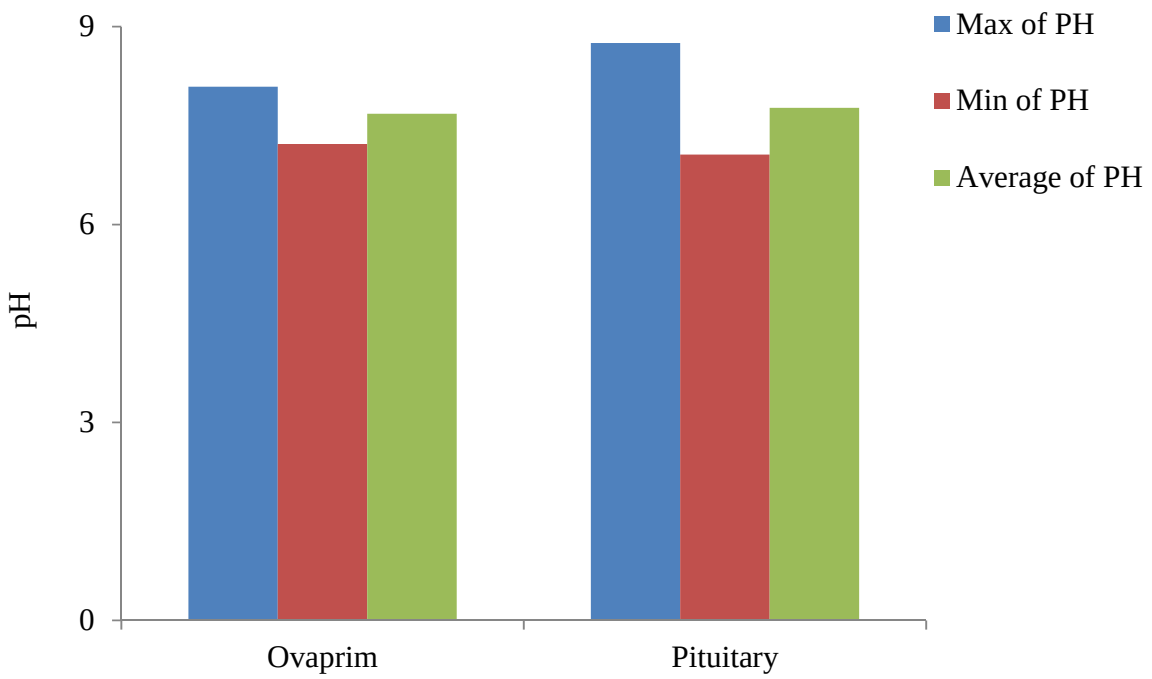


Figure 16: Maximum, mean and minimum water pH value measured at different treatments over 14 days for *C.gariepinus* artificial breeding in a hatchery

6.1.4 **Conductivity**

Conductivity can be varies from 50 to 1500 µs/cm in unpolluted fresh water. The mean conductivity measurement for each treatment was 285.36 ± 27.33 µs $cm^{-1}$and 287.93 ± 17.08 µs cm^{-1} for Ovaprim and Carp pituitary gland treated basins in a hatchery respectively.

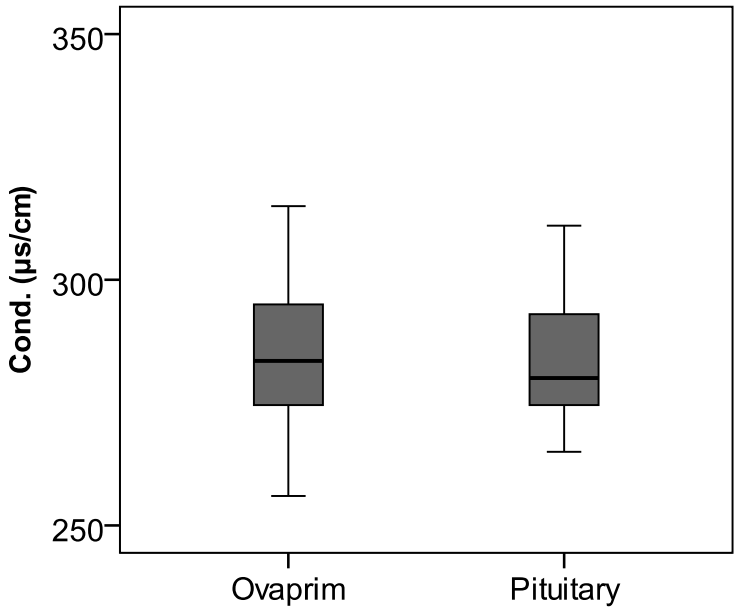


Figure 17: Mean conductivity values measured at different treatments over 14 days for C.gariepinus artificial breeding in a hatchery

6.1.5 **Ammonia (NH_4-N)**

Un-ionized ammonia concentration was in measured using phenol-hypochlorite methods. Maximum concentration of NH_4-N that allowed is about 0.05 ppm for fish ponds. The concentration of NH_4-N was measured early morning at the end of 14 days. The mean value of ammonia-nitrogen (NH_4-N) concentration was 0.12 ± 0.024 mg L^{-1} and 0.104 ± 0.026 mg L^{-1} recorded in the Ovaprim and Carp pituitary gland basins respectively.

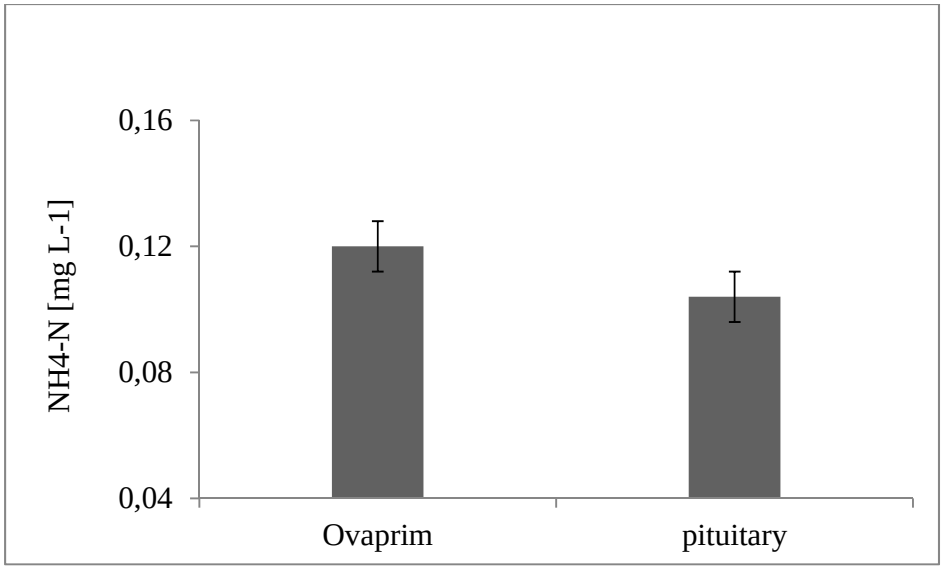


Figure 18: The mean NH4-N (mg L^{-1}) value recorded during experimental period in catfish artificial rearing in a hatchery (Mean ± SE) for each replicates.

6.2 Ovulation of catfish

6.2.1 Body weight to gonad weight relationship

The gonad weight increased according to fish body weight is presented in the figure 17. It shows that the gonad weight of catfish (*C.gariepinus)* is related to fish body weight. The Pearson correlation coefficient (R^2=0.74) values indicate that there is a strong positive correlation (linear relationship) between gonad weight and fish body weight.

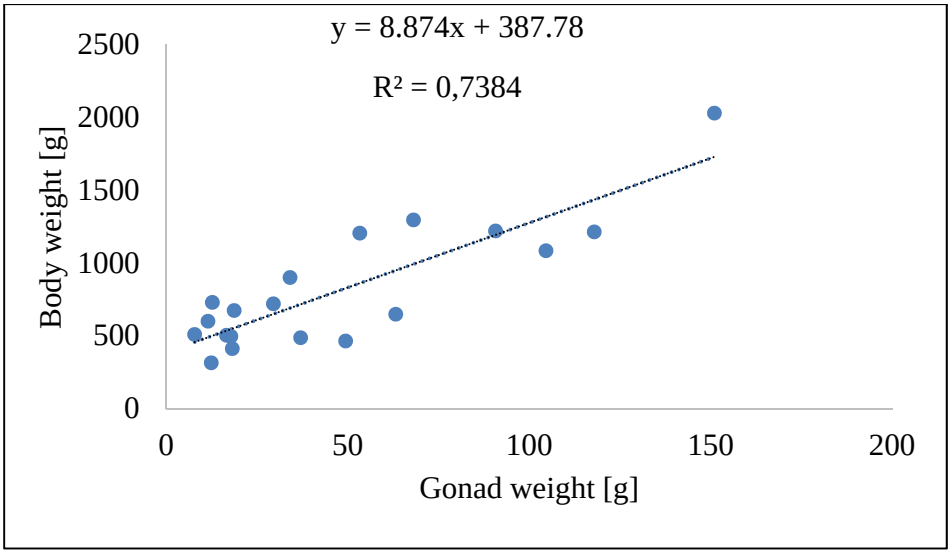


Figure 19: The scatter line shows the relationship between body weight and gonad weight for *C.gariepinus* induced production under a hatchery. Each dot refers to one fish.

6.2.2 **Total length and gonad weight relationship**

The Pearson correlation coefficient (R^2=0.67) values indicate that there is positive linear correlation between gonad weight and fish total length. The gonad weight increases with increasing in fish length.

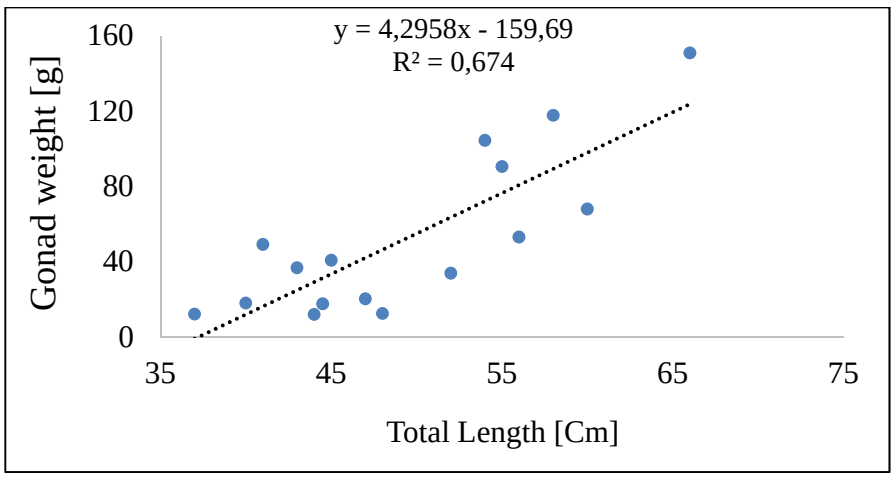


Figure 20: The scatter line shows the relationship between total length and gonad weight for C.gariepinus induced breeding in a hatchery. Each dot indicates to one fish.

6.2.3 **Mean fecundity**

Comparison of the average ovulation rate treated with synthetic hormone and Carp pituitary gland was shown in figure 19. The highest mean ovulated (36829) eggs was found in Pituitary gland twice injected brood fish and lowest number of (22353) eggs was recorded in brood fish injected once with Ovaprim. There was no significant difference (P>0.05) in the mean number of eggs released with different hormone administration rate.

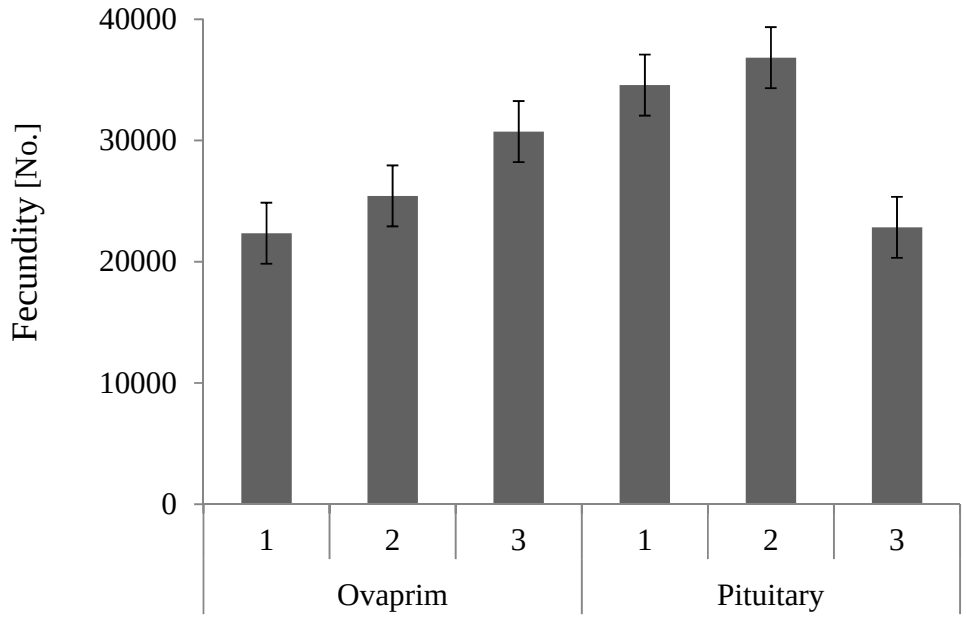


Figure 21: Shows mean number of ovulated eggs of *C. gariepinus* injected synthetic hormone (Ovaprim) and Carp pituitary gland with different hormone administration rate.

Table 6: The effects of synthetic and pituitary hormone on catfish egg fertility, hatching and survival rate of fry. Values are (Mean ± SE) of the three replicates.

Parameters	Synthetic hormone			Carp Pituitary gland		
Replicates	R1	R2	R3	R1	R2	R3
Mean body weight (g)	961± 257.5	785 ± 150.7	1142.8 ± 457	864.7± 178.5	723.6± 244.7	730.2±283
Number of fecundity	22353±8923	25436±18405	30731±10075	34576±13820	36829±19530	22838±5511
No. of eggs fertilized	13113±6704.9	14380±10031.5	16763±4793	17136±7045.4	20558±10051.5	12680±2062.8
Mean No. of dead eggs	9238 ± 2608.7	10354 ±7638.9	13914 ±5227	17440±7037.4	16388 ±9314.7	10054±3378
Fertility Rate (%)	52 ± 3.9	56.7 ± 8.8	56.8 ± 3.5	52 ± 5.67	59 ± 3.5	58.8 ± 6.7
Hatching rate (%)	18±9.1	64±19.24	32±1.3	34±17.5	25±7.5	26±106
Fry survival rate from total fertilized eggs	22.85 ± 3.56	44.96 ± 14.68	27.26 ± 1.24	24.92 ± 14.73	18.71 ± 4.64	19.14 ± 7.8
Fry survival rate from hatching larvae (%)	85.5 ± 3.3	69 ± 3.7	84 ± 1.2	68 ± 6.8	77 ± 5.2	79 ± 1.5

6.2.4 **Fertilization rate**

The brood fish (*C.gariepinus)* injected with Carp pituitary gland the fertilization rate was in the range between 61.3-72.2 % while catfish treated with synthetic hormone (Ovaprim) had mean fertilization rate range between 63.6 - 72.6% was recorded. Both Ovaprim and pituitary gland hormones were found to be equal effect on fertility successes of catfish induced breeding in (Figure 19). However, fertilization rate was slightly higher (72.6%) fish which was injected two times with synthetic hormone Ovaprim in different doses with 6 hours time intervals between the first and second injection. in Figure 20.

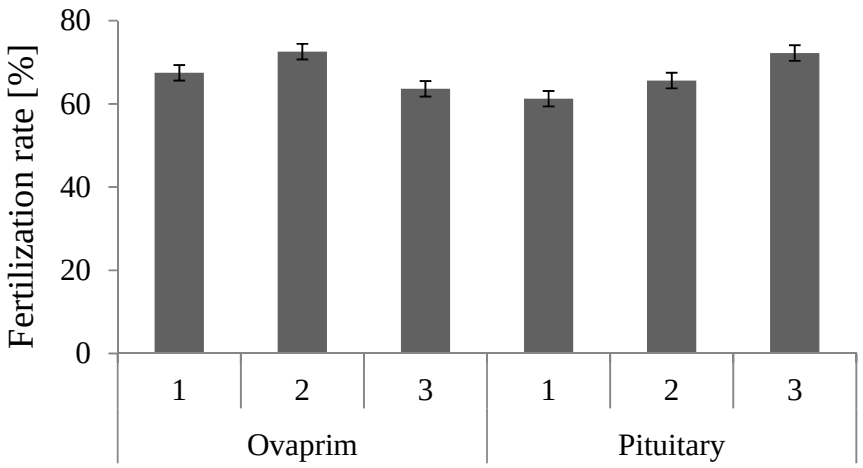


Figure 22: Percentage highest fertilization of *C. gariepinus* from each treatment with synthetic hormones and Carp pituitary glands with different administration time

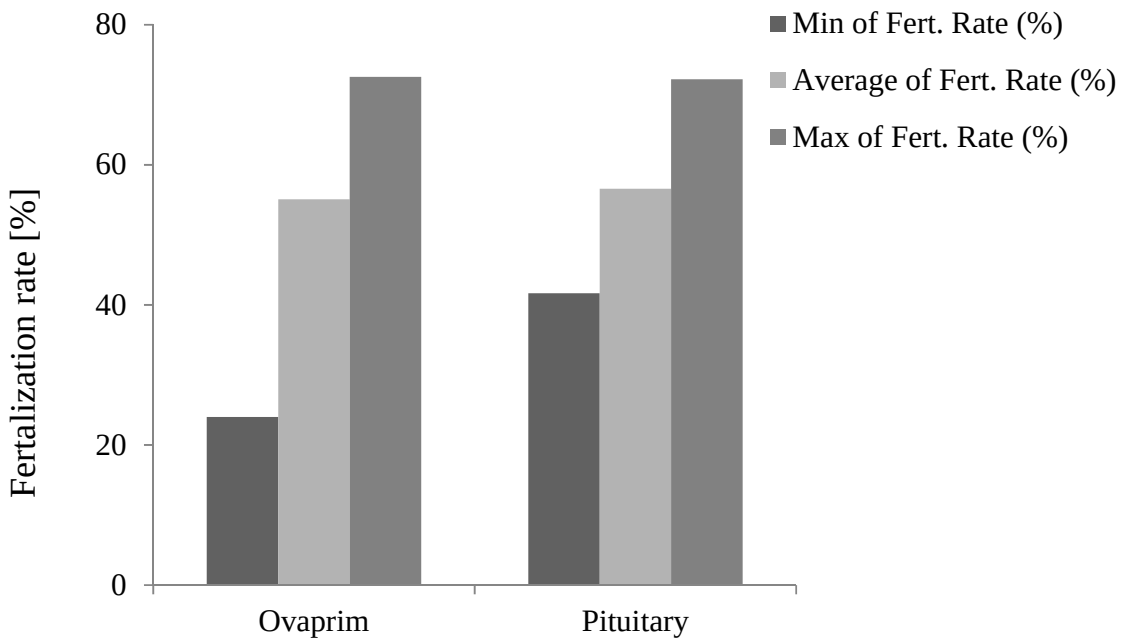


Figure 23: Mean, maximum and minimum fertilization rate of *C.gariepinus* eggs treated with Synthetic hormone and Carp pituitary gland.

6.2.5 **Hatching rate**

Significant hatching rates (89%) was recorded in OV2 (Ovaprim two times injected) fish as compared to all other treatments except Pituitary gland 1(one time injected) fish. Similarly, Pituitary gland 1 has significantly higher hatching rates than Ovaprim 1, Ovaprim 3 and Pituitary gland 2 treated catfish respectively

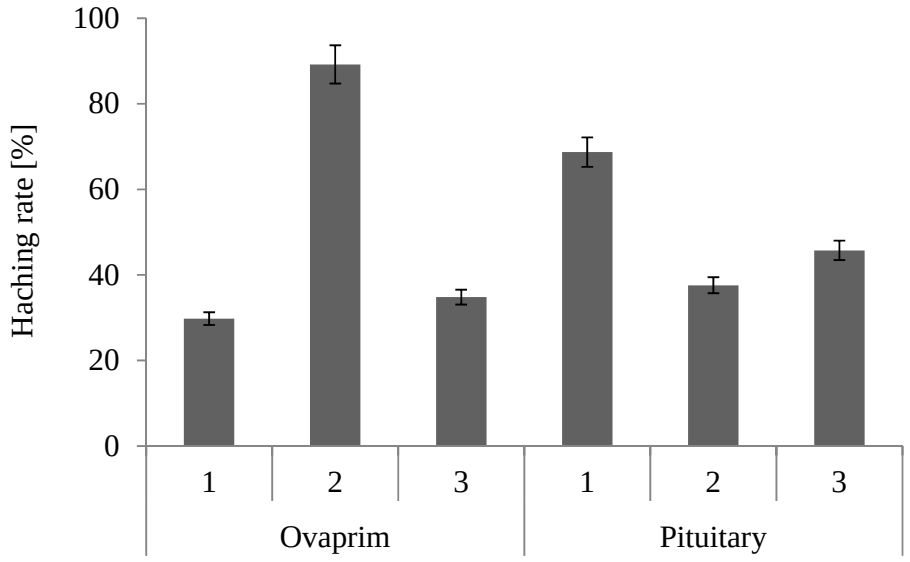


Figure 24: Maximum hatching rate *C.gariepinus* eggs at Ovaprim and Carp pituitary gland treated fish with different hormone administration rate.

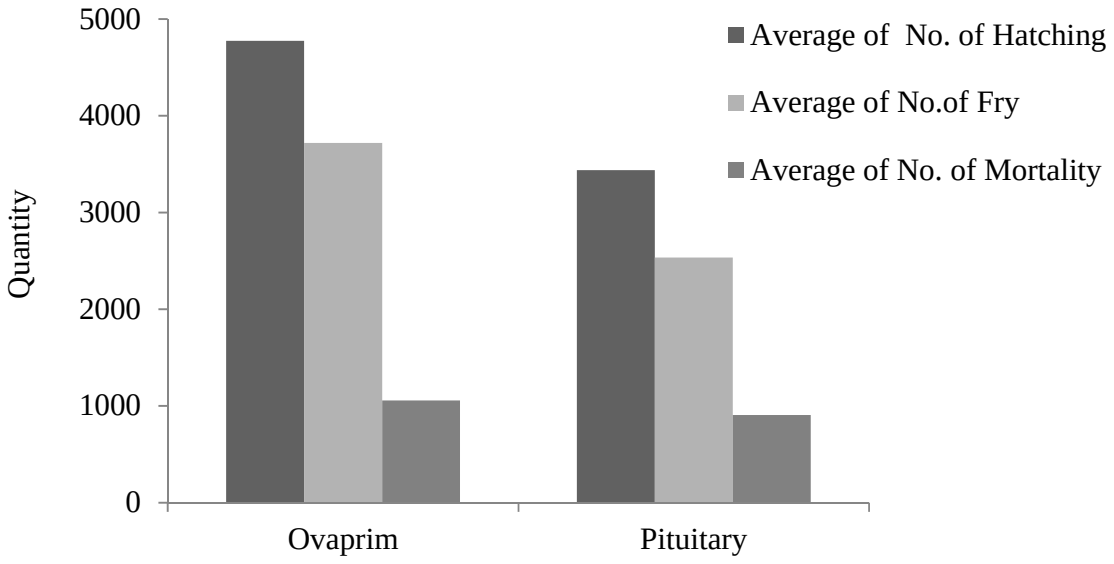


Figure 25: Comparison of hatched eggs, number of mortality and survived of *C.gariepinus* fry after 14 days experimental period in a hatchery

6.2.6 **Survival rate**

Dead fry were counted every day and the survived fry was calculated at the end of the 14 days. From the presented study results, the lower survival of fry (67.8 %) in Ovaprim 2 (twice injected) catfish and the higher survival of fry in the Ovaprim 1 (one time injected) (85.5%) was found in (Figure 23.). However, there is no significant variation ($p > 0.05$) on survived fry among treatments during the study.

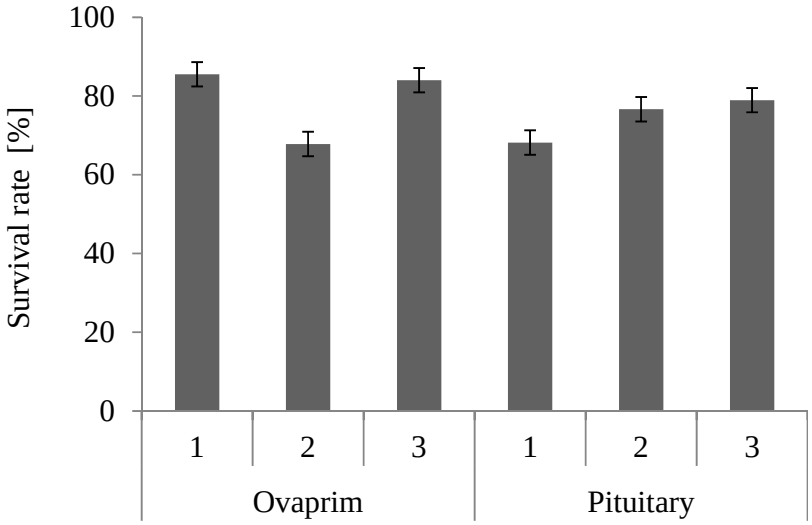


Figure 26: Mean survival rate of 14 days old catfish (*C.gariepinus*) fry spawning artificially using Ovaprim and Carp pituitary glands.

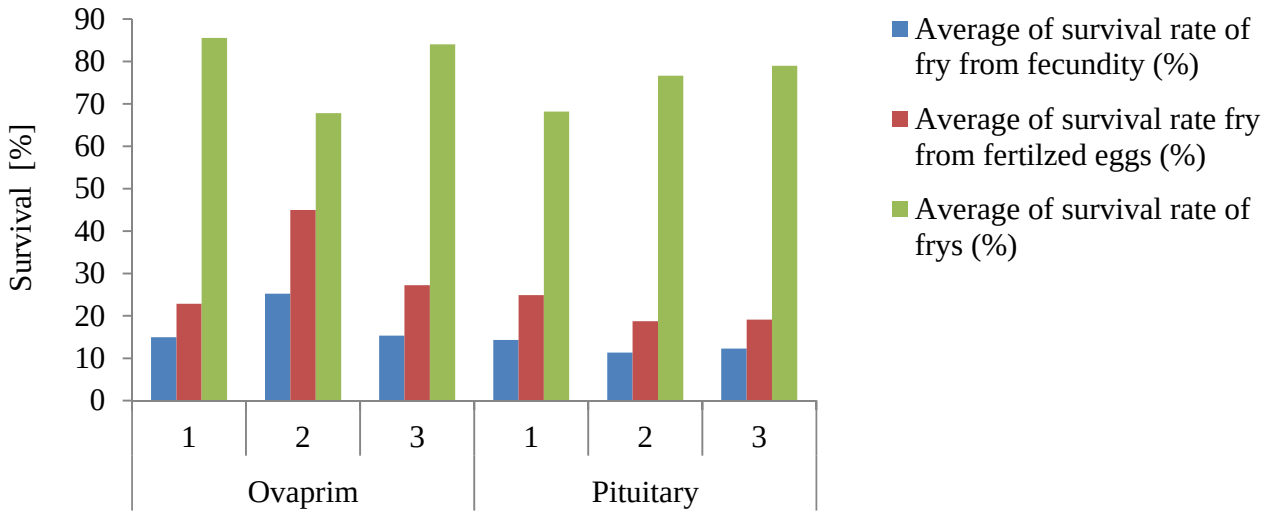


Figure 27: Comparison of survival of *C.gariepinus* fry produced from the total number of fecundity, fertilized eggs and hatched larvae using Ovaprim and Carp pituitary glands under a hatchery.

6.2.7 **Growth performance**

The fry were feed same live *Artemia nauplii* for all treatment and the growth rate was measured at the end of the experiment. The result showed that the higher mean growth rate was found in ovaprim 2 (fish injected two times) (14.18± 1.84) and the smallest mean weight was recorded in Ovaprim 1(fish injected once) with Ovaprim hormone (10.76 ± 0.24). However, the growth rate of all replicates has significantly similar growth performance.

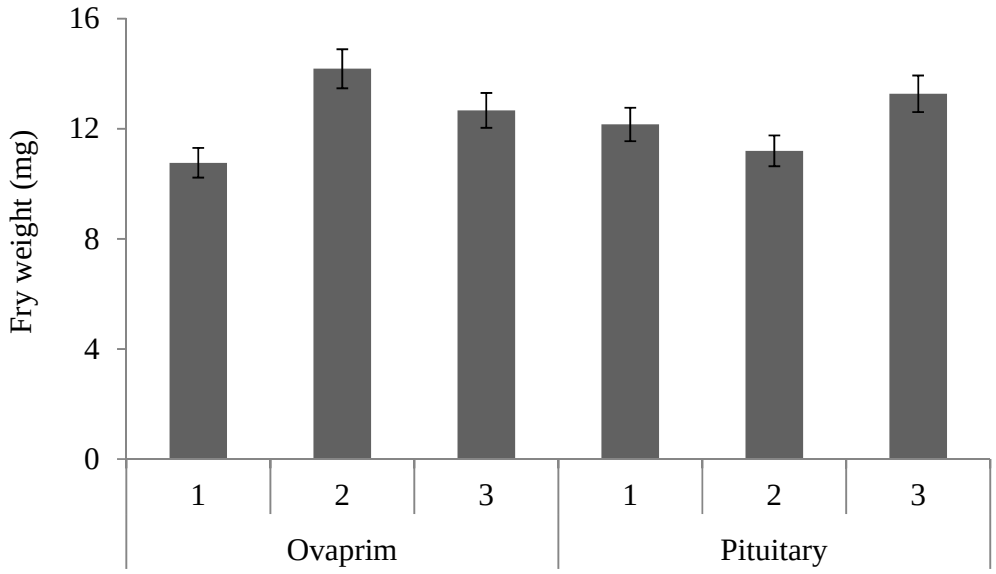


Figure 28: *C.gariepinus* fry weight gain after14 day experimental period in a hatchery

7 Discussion

7.1 Temperature

All water quality parameters are important for the growth; development of the fry and fingerlings. During the study period, the mean water temperature value recorded ranges from 25.12±1.54 ºC to 25.1±1.37 ºC in the Carp pituitary gland and the synthetic hormone Ovaprim basins respectively. During the experiments, the difference between water temperature changes from 23 to 28ºC, which falls slightly below the recommended values. According to Akinyemi, water temperature between 27-30ºC is suitable for *Clarias garipinus* larvae rearing (Akinyemi 1988). Fish are cold blooded animal and internal body temperature will change in adjacent to changes in external environment. Water temperature fluctuation affects the fish's metabolism activity, physiology and growth rate. It could be potentially lethal to the fish population (Lucinda and Martin 1999). As a result, fish do not respond positively to the frequent fluctuation of the water temperature (Pruthi 1932; Lucinda and Martin 1999; Bhatnagar and Singh 2010).

7.2 Dissolved oxygen

The result of this study showed that each treatment basins had sufficient amount of dissolved oxygen. The mean dissolved oxygen (DO) concentration in Carp pituitary gland treated basin and Ovaprim hormone treated basin were 5.0 ± 0.5 mg L^{-1} and 5.4 ± 0.57 mg L^{-1}. There was no significant difference ($p>0.05$) in dissolved oxygen level between treatments during the study period. Dissolved oxygen in the range of 4.5 - 8 mg L^{-1} is generally considered suitable for fresh water fish cultivation (Bhatnagar 2009). During this study, the dissolved oxygen concentration recorded was slightly fluctuated in the range of (3.7 - 6.4 mg L^{-1}), but the mean recorded values falls within the recommended range. This may be contributed by the continuous flowing and flushing of water in and out of all experimental basins. Lower level in water dissolved oxygen leads to poor feeding efficiency, starvation, slow growth rate and even mortality (Bhatnagar and Grag 2000). On the other end of the spectrum, very high level of dissolved oxygen in water will result in super saturation of the oxygen content, which also leads to mortality during the fish fry rearing period (Alikunhi et.al. 1995).

7.3 **Water pH**

pH is used to express the intensity of the acidity or basic character of the water. The water pH values recorded for *C.gariepinus* egg incubation and fry rearing was between 7.5-9. According to Santhosth and Singh (2007) the suitable pH range for fish cultivation is between 6.7-9.5, a water pH level above and below this range will be stressful to the fish strain. Water PH of between 6.7-8.5 is considered enough for fresh water fish cultivation (Adeniji 1987; Vivven et.al. 1985). Our water pH results showed no effect on fish fry mortality hence it was in a desirable range according to the data above.

7.4 **Conductivity**

Conductivity is an index of total concentration of ions (Ca^{2+}, Mg^{2+}, HCO_3^-, CO_3^-, NO_3^-, and PO_4) in the water (Ogebeibu and Victor 1995). The optimum concentration of ions for fish growth and production varies depends on species (Sikoki and Veen 2004). During the study, mean conductivity was measured, with results from 287.9± 27.33 to 285.4±17.08 μscm^{-1} in Carp pituitary and Ovaprim treated basins. According to Stone and Thomsone (2004), the desirable range of conductivity for commercial pond culture is between 30 - 5000 μscm^{-1}. Therefore, our collected measurements do fall within the desirable range.

7.5 **Ammonia**

Un-ionized ammonia concentrations could be quite toxic to catfish fry. The maximum limit of ammonia concentration in the aquatic system is 0.5 mg L^{-1} (Meade 1985; Santhosh and Singh 2007). In the present study, the maximum concentration of NH_4-N was 0.12 mg L^{-1} and 0.104 mg L^{-1} in Ovaprim and Carp pituitary treated basins respectively. The concentration of NH_4-N was within the recommended range which was not causes for fry mortality.

7.6 **Gonad weight to body weight and total length relation**

In the present study, it was observed that the fish within the same size categories could have different ovaries weights, and number of eggs in each individual ovary from the controlled group

is also subjected to changes. The relationship between the gonad weight, body weight and total length of the fish is linearly connected. This finding agrees with Yalcin's claim, that a correlated relationship between gonad weight, body weight and the total length of the fish (Yalcin, K. Solak 2001). Mc Fadden Copper and Anderson (1965) found a direct relationship between egg weight and fish weight. The R values in the correlation between gonad weight and body weight was higher than the relationship of gonad weight to fish total length (figure 19). Meanwhile, the gonad weight to body weight relationship serves as better prediction of fish fecundity than the relationship between gonad weights to fish total length. The bigger fish produce more eggs than the smaller ones. The number of eggs in the ovary increases in direct proportion to the fish weight and its gonad weight, but could not be dependent on the ovulation stimulators used.

7.7 **Fecundity**

Fecundity generally defined as the number of ripening eggs found in the female fish just prior to spawning (Bagenal 1978). In order to evaluate induced maturation and spawning performance of African catfish, the conducted experiment used Ovaprim hormone and Carp pituitary gland. The fecundity was estimated from 21 ripening African female catfish, while three of them serve as control group, the other two teams of nine were placed under Ovaprim hormone and Carp pituitary gland. The calculation was estimated by examining of all brood fish total length (TL), body weight (BW), gonad weight (GW) and egg counts. The mean fecundity of PG (pituitary gland) injected *C.gariepinus* gland ranges between 22838 - 33799 eggs. And from OV (Ovaprim) injected *C. gariepinus*, the obtained mean fecundity is 22352 - 29440 eggs.

The highest and least mean number fecundity was recorded in fish injected pituitary 2 (two times injection) and Ovaprim 1 (One time injection) respectively in (Figure 21). The maximum amount of spawned eggs (75220) was found in fish that were injected twice with PG and minimum amount of eggs (4899) was recorded in fish that were injected twice with OV. In this study, the brood fish injected with PG solution gives the highest mean number of eggs (36829) followed by fish injected with OV hormone (30730) ripe eggs were released. In both PG and OV injected catfish, all ovulated with varied egg amounts. In the control group, among three treated brood catfish, two of them did not demonstrate ovary development, the one left produced eggs that

were poor in quality and quantity. The eggs were attached together by muscle tissue, which results in no hatching during incubation period. From this study we conclude that catfish (*C.gariepinus)* would not spawn in captivity unless it is induced by different hormones in the hatchery.

The overall fecundity pattern has shown that there is wide variation in the number of eggs released. The higher mean number of ovulated egg was recorded in PG treated fish than those injected with OV. There is no significant differences observed on the ovulation stimulator ($P>0.05$) in the number of ovulated eggs from all treatments.

The study by (Britz PJ.and Hecht T 1988) using PG extract spawned (76 500) eggs, and my study result, also with PG injection, ended up with comparatively similar number of yields (75220). The earlier study done by (Khan et.al. 2006 and Shano et.al. 2005) who found high fecundity on *C.gariepinus*, *C.batrachus*, and *Labeo rohita* when OV was induced at the rate of 0.2 mg kg^{-1}.

This result indicates that the dose and administration rate of synthetic hormone and pituitary gland do not influence in mean number of eggs released. This result however disagrees with the report of (Hill, et.al. 2009) the increasing in dose and administration rate of breeding hormones results in more eggs being produced.

The variation in fecundity within a common trail of similar-sized fish species could be attributed to hormone administration rate, breeding history, maturity stage, and other external environmental factors (Lager 1986; Schulz et.al. 2007; Ataguba et .al. 2009). Use of hormones may produce poor results if the brood fish are not well conditioned. Under such conditions a partial spawn or no spawning at all may occur, and others may not respond to hormone treatment even if they are in relatively good condition (Piper et.al. 1982).

7.8 **Fertilization rate**

The results of this study shown the higher percentage of fertilization rate were in OV 2 (fish injected twice) with a value of 72.6 %, and the lowest rate 61.3 % was recorded in PG 1(fish

injected once). There was still no significant difference (P>0.05) in fertilization rate in all treatments. This study agreed with earlier reports of (Nandeesha et.al. 1990; More et.al. 2010; Adebayo O.T. and O.A Afagbenro 2004), whom claimed that OV injected *C. gariepinus* had higher fertilization rate followed by the PG injected group. Haniffa and Sridhar (2002) reported (70%) fertilization rate of *Heteropneustes fossilis* (Stinging Catfish, *Clariidae*) treated also with synthetic hormone Ovaprim at dose rate of 0.3mlkg^{-1} body weight, the same size of our study targets. The highest fertilization rate (98%) was recorded in *H.fossilis* with a dose of 75 mgkg^{-1} PG injection. This number is much higher than the present study result (Begun et.al. 2001). The variation in fertilization rate might be attributed due to varied egg and sperm quality, physiological difference of brood stocks, seasonal variation, as well as difference in hormone dosage (Gheyas et.al. 2002; Haniffa and sridhar 2002; Nwokoye et.al. 2007). Environmental factors, water quality parameters (pH, oxygen concentration, hardness and temperature of water) and handling procedure of the brood fish also are determining criteria (Khan et.al. 2006). Artificial breeding of African catfish or Salmon fish prolonged exposure of both sperm and eggs to the water, which will reduce the fertility and hatching potential (Piper et.al. 1982).

7.9 **Hatching rate**

The present study shows that, the mean hatching rate has generally lowered in all treatments. The maximum hatching rate was (89%) and the minimum hatching rate was (30%) recorded in *C.gariepinus* OV 2 (injected twice) and OV 1 (one time injection) study groups respectively. The OV 2 group had shown a significant lead in maximum ($p<0.05$) hatching rate not only than both OV 1 and OV 3 (one time and three time injection), but also ahead of group PG 1 and PG 3 (one time and three times injection) (Figure.24). The mean hatching rate of OV 1 demonstrated significantly lower (P<.0.05) value than OV 2 and OV 3. The poor hatching rate of spawned eggs could be linked to the exposure of fungus infection, where amount of dead eggs risked at being nutritional bases for fungal growth (Tucker and Robinson 1990). Additional interferences that would affect the hatching rate are breeding history, age of fish and hatchery water quality according to (Ataguba et. al.2009). Other disturbances to hatching rate would be water temperature fluctuation and egg hatching lengths (Piper et.al. 1982). The higher fertilization rate and the consequent lower hatching rate could be an outcome of the over-ripped eggs, which

might have result in egg mortality post fertilization. This was similar to the finding of (Sahoo et. al. 2005).

7.10 **Fry survival rate**

The catfish fry were feed *Artemia nauplii* twice per day for 14 days. Remaining or leftover feeds were siphoned every day from each basin to prevent water quality deterioration. The percentage of survival rate was higher (85%) in group OV 1 (figure 26). In a similar study, where Ovaprim was also used for breeding the stinging catfish *H. fossilis*, (Haniffa and Sridhar 2002) stated that Ovaprim treated fish had better fry survival rate than other hormonal materials used. In our study group PG 1 had a lower fry survival rate of 68.2 % recorded at the end of 14 days indoor rearing period. In a similar *Heteropneustes longifilis* induced breeding study by (Nwadukwe 1993), the use of frog pituitary extract had a lower fry survival rate. The survived rate ranged between 84-85 % was recorded in Ovaprim treated catfish (*C.gariepinus*) after the 14-day experimental period (Figure 26). The higher survival rate of *C.gariepinus* fry might occur due to the appropriate water management at the duration. The overall results yield comparatively higher (68.2-85%) survival rates in all treatments after 14 days. However, there were no statistically significant variations ($P > 0.05$) in all the treatments.

Physical observation during the study indicated yolk sacs disappearance within the first three to four days post hatching. During this period no mortality occurred, the reason might be limited food seeking activities and social competitions within groups. However, mortality begun on the 5^{th} day of the experiment, where the live feed (*Artemia nauplii*) was also given. The reason of fatality could be nutrition deficiencies; physical injury; over feeding; competition for food and high movement in confined spaces. From day 10 to 14 cannibalism surfaced: attacks among the same size begun. The mostly attacked parts are tails, mostly broken with body wounds, eventually dead (Figure. 29A). Baras and Almeida (2001) stated that by grading catfish fry every 3 days will result in better survival rate. During the study, separating and giving pure live feed (*Artemia nauplii)* was difficult, due to the amount of cysts and shell mixed in feed which requires a lot of time and effort to remove. Microscope examination from the laboratory revealed

that some *C.gariepinus* fry had died of eventual digestion failure from accidental consumption of cysts (Figure. 29B).

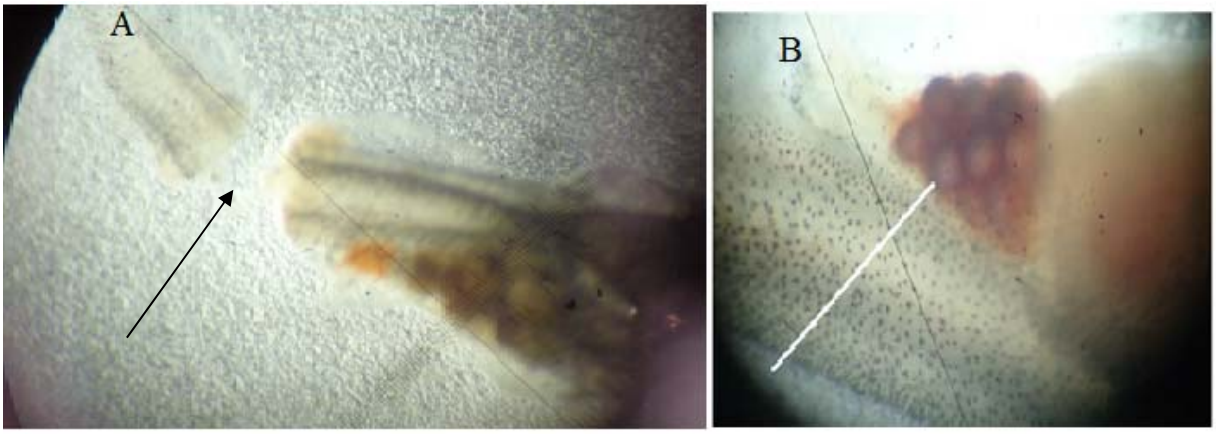


Figure 29: The picture shows (A) cannibalism and (B) cysts blocking fry stomach under a microscope in the laboratory.

7.11 **Growth performance**

At end of study, 15 fry samples were weighed from each experimental plastic basin, growth rates were also examined. The maximum and minimum growth rates were achieved by group OV2 (14.18 mg) and OV1 (10.76 mg) respectively. In all treatments, the final result showed a rather similar growth rate of fry. This implies the use of different breeding hormones, regardless whether natural or artificial, when at different administration and dose rates, had no significant effect in *C.gariepinus* fry growth rate. Catfish breeding hormones had neither promoting nor inhibiting effect on fry growth rate. Instead, growth rate of fish could be affected negatively by water quality and stocking density. The growth rate was affected positively by supplementary feed (Borghetti and Canzi 1993). The optimum growth rate of *C.gariepinus* can be obtained at water temperature ranging from 27 to 31ºC (Hoogendoor et.al. 1983). However, the average water temperature in the study was below the optimum level for maximum growth rate. According to Halver and Hardy (2002) the water temperature below the desirable range could affect the physiological function and metabolic activity as well as the growth of the fish.

8 Conclusion and recommendation

Even though the experiment was carried out with limited facilities, the result was promising. The study result has the potential to be a guide line for further researches to improve artificial breeding of African catfish in Ethiopia. It also aims to combine terms of optimization between environmental parameters and hormone application. In terms of accessibility, OV being imported could be pricier, while PG extract is relatively affordable and available locally. Hence, Carp pituitary gland (PG) is highly recommended for the benefit of small scale aquaculture farmers and commercial hatchery users.

1. Additional study is needed for the cause and prevention of cannibalism within controlled system.
2. Further study on fry stocking density is recommended.
3. Research is needed to determine the optimum number of days to leave the fry in the fish ponds.
4. Alternative options to replace the imported catfish larvae live feed *Artemia nauplii* are necessary.
5. The need of governmental funding to increase and promote potential hatchery production. And the need of funding for fry nursing and breeding under the aquaculture research centre supervision.

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