# DETERMINATION OF THE EFFICIENCY OF 17-α METHYLTESTOSTERONE ON SEX REVERSAL OF NILE TILAPIA, *OREOCHROMIS NILOTICUS* AT DIFFERENT DEVELOPMENTAL STAGES

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## DECLARATION

I declare that this thesis is my original work and has not been presented in any other University. No part of this thesis may be reproduced in any form without prior written permission of the author and/or University of Eldoret.

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## DEDICATION

I dedicate this thesis to my wife Janet and beloved sons and daughter Benny, Henry and Emily.

## ABSTRACT

The aim of the study was to determinate the efficiency of 17-α methyltestosterone on sex reversal of Nile tilapia, *Oreochromis niloticus* at different developmental stages. Fish were seined from Eldoret fish farm and those brooding eggs and fry were selected. The brood was emptied into a bucket containing aerated water. The fry and eggs were transported to the laboratory for further processing. Eggs were incubated in the incubation jar while fry were transferred into aquaria. The fry were sorted out according to age stages and sex reversed using 17-α Methyl-testosterone incorporated into a diet at 40mg per kilogram of feed. The hormone supplemented feed was prepared by weighing, using digital weighing balance the desired quantity of the hormone, dissolving it in 95% ethanol and spraying it on the feed. The feed was then dried under shade. The age stages included 8 days, 23 days and 40 days after hatching. The fry were fed on food containing methyl-testosterone for a period of 21 days, 30 days, 42 days and 60 days. Sex of fingerlings was determined through microscopy and sex identification was facilitated by staining the gonads using aceto-carmine. Water quality parameters were measured weekly and room temperature was controlled using adjustable fan heater and thermostat dipped in water. Female fingerlings were not observed while male and intersex percentages were 92.2% and 7.8%, respectively. The age of fingerlings was significant in determining fingerlings' sex. Pearson and Spearman's rho correlations was highly significant at ($P<0.05$) while hormone supplemented food in days and sex of fish showed no significant. Pearson Correlation between hormone supplemented food and age of fish showed high significance at ($P<0.05$). According to this study, is recommended that sex reversal for *O. niloticus* should start at the age of eight days after hatching for a period of twenty one days of hormone supplemented food feed.

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## LIST OF ABBREVIATIONS AND ACRONYMS

MT – Methyltestosterone

DO – Dissolved oxygen

pH – Potential hydrogen

ANOVA - Analysis of Variance

FAO – Food and Agriculture Organization of the United Nations

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# CHAPTER ONE

# INTRODUCTION

## 1.1 BACKGROUND TO THE STUDY

Tilapia is often cultured primarily in freshwater ponds without supplementary feeding. Research has been conducted to determine the influence monoculture and polyculture on growth tilapia nilotica and tilapia aurea in intensive culture by using low cost raw material as protein source for tilapia diets (El Saidy & Gaber, 2002; Gaber et al., 2012). Nile tilapia, *Oreochromis niloticus,* is one of the most important fish species for aquaculture worldwide. It represents the species of choice due to its high growth rate, significant tolerance to environmental stress, ease of reproduction, and high market demand (El-Sayed 2006). *Oreochromis niloticus* is therefore best suited for the study of sex determination of the efficiency of 17-α methyltestosterone on sex reversal of Nile tilapia at different developmental stages.

The increasing human population pressure, and the ensuing land demarcation in Kenya have stimulated use of alternative farming methods and animal species in rural development efforts, which were previously ignored (MoLFD 2007). With this, the role of aquaculture in food production, economic development and food security is increasingly becoming important in the country and the whole world. It is evident that aquaculture is currently the fastest growing segment of food production in the world. According to FAO (2012), capture fisheries and aquaculture supplied the world with about 148 million tonnes of fish in the year 2010, of which about 128 million tonnes was utilized as food for people, and preliminary data for 2011 indicate increased production of 154 million tonnes, of which 131 million tonnes was destined as food. With sustained growth in fish production and improved distribution channels, world

fish food supply has grown dramatically in the last five decades, with an average growth rate of 3.2 percent per year in the period 1961–2009, outpacing the increase of 1.7 percent per year in the world's population (FAO 2012). However, fish consumption in Africa is still lowest relative to other parts of the world.

From the foregoing, aquaculture holds promise to help provide a growing human population with food as many of the world's capture fisheries have reached their biological limits of production or have been depleted through over-fishing and habitat degradation (United Nations 2005; MoLFD 2007; FAO 2012). To meet the increased demand for food, fish and aquaculture production should increase by 50 million metric tons by 2050 (Tacon and Forster 2001), especially from smallholder production.

*Oreochromis niloticus* is yet to reach its full potential for aquaculture because of the problems associated with precocious maturity and uncontrolled reproduction, which often results in overpopulation in production ponds. Overpopulation in the pond leads to stunting of fish because of nutritional and environmental constraints. To increase the yield of this species, there is a need for sex control in the ponds. Sex control in farmed *O. niloticus* has been reviewed by Guerrero (1982), Mair and Little (1991) and Fagbenro (2002). The authors and others (Suman *et al*., 2009) reviewed various methods of sex control including sex reversal by steroids like androgenic hormones, hybridization, hand-sexing and genetic manipulations, which involved production of super YY males. However, all these sex control methods have their limitations; e.g. expensive technology, need for hatchery facilities, need for skilled labour and expensive hormones, which are difficult to obtain (Suman *et al*., 2009).

Artificial sex reversal in fishes like tilapia is the process by which the physical sex direction (male or female) can be manipulated through the feeding of synthetic sex hormones (e.g.,

methyl testosterone) prior to and during the "sexless stage" of the fry. The technique was first developed in Japan in the 1950s for sex reversal of the aquarium fish (*Oryzias medaka*) and the common carp (*Cyprinus carpio*) and was first demonstrated to be commercially feasible for sex reversal of the Mozambique tilapia in the Philippines in the 1970s. Sex reversal of tilapias is now applied worldwide (Fagbenro, 2002).

One of the most common methods is use of androgens, especially 17-α Methyltestosterone which is used to produce all male population of *O. niloticus* (Remedios *et al*., 2008). The hormone is administered in two ways; through immersion method or oral administration (Utete *et al.*, 2012). In the first method, fry or eggs are immersed in a solution of hormone for 10 minutes. The treatment is repeated about three times at a 10 day interval (El-Sakhawy *et al.* 2011, Neves *et al.* 2009 and Gaber *et al.* 2012). In oral administration method, fry are given feed treated with hormone at 40mg/kg of feed for about 21 days. The factors influencing the efficiency of sex reversal include fry age and set up used for feeding. For example *O. niloticus* may be fed in the pond, tank, or aquaria. This will either restrict *O. niloticus* from eating any other food within its surrounding or may give it a room for finding some unwanted food which finally reduces the efficiency of 17-α methyltestosterone on sex reversal.

## 1.2 PROBLEM STATEMENT

There was a need to study the efficiency of 17-α methyltestosterone on sex reversal of Nile tilapia, *Oreochromis niloticus* to ascertain its percentage success to improve food security in Kenya and around the world.

## 1.3 JUSTIFICATION

Tilapia yield in Kenya currently is very low to meet its market demand. This factor is more acerbated by low tilapia population in Lake Victoria, which has drastically gone down while human population keeps on growing thus leading to malnutrition and lack of enough protein. The area required for food production is gradually becoming smaller every day. A type of farming that requires smaller space is necessary. Tilapia farming and especially *O. niloticus* is one of the options. However, there are inerrant problems associated with overpopulation of culture facilities therefore leading to stunting of individuals. Several strategies have been adopted to create mono-sex population of *O. niloticus* but the results have given mixed results with low efficiency of mono-sex conversion. To deal with this problem, this current study aimed at improving the efficiency of 17-α methyltestosterone on sex reversal of Nile tilapia, *Oreochromis niloticus* so as to reduce food insecurity in Kenya and around the globe. It also provides important information that is required for future reference in management of hatcheries, efficiency and sex reversal of *O. niloticus*.

## 1.4 OBJECTIVES OF THE STUDY

### 1.4.1 MAIN OBJECTIVE

To determine the efficiency of 17-α methyltestosterone on sex reversal of Nile tilapia, *Oreochromis niloticus* at different developmental stages.

### 1.4.2 SPECIFIC OBJECTIVES

1. To determine age of *Oreochromis niloticus* fry after hatching for efficiency of 17-α methyltestosterone on sex reversal.
2. To determine the right stage of exposure to hormone.

### 1.4.3 STUDY HYPOTHESIS

1. $H_o$: The age of *Oreochromis niloticus* fry after hatching and the period of exposure to hormone do not significantly influence the efficiency of 17-α methyltestosterone on sex reversal.
2. $H_1$: The age of *Oreochromis niloticus* fry after hatching and the period of exposure to hormone significantly influence the efficiency of 17-α methyltestosterone on sex reversal.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 PRODUCTION TRENDS FOR TILAPIA

Nile tilapia, *Oreochromis niloticus* (L) is presently widely distributed in many parts of the word (Trewavas, 1983). It occurs together with *Sarotherodon galilaeus* (L) and *Tilapia zillii* (Gervais) throughout much of its natural range in Palestine, the Nile, and across West Africa, and Lakes Turkana, Edward, George, Tanganyika and Albert in East Africa (Lowe McConnell, 1958; Trewavas, 1983). The herbivorous tilapiine, the Nile tilapia, *Oreochromis niloticus* (L.) was introduced in Lake Victoria in 1950s and 1960s to boost the then declining fishery (Welcomme 1967; Ogutu-Ohwayo 1990a). Currently Nile tilapia is the most commercially important tilapiine in Lake Victoria (Cowx *et al.* 2003; Njiru *et al*., 2005). This is in sharp contrast with the native species of 1950s and 1960s of *Oreochromis esculentus* (Graham) and *Oreochromis variabilis* (Boulenger) Today *O. niloticus* constitutes the third most important fishery in Lake Victoria, after Nile perch, *Lates niloticus* (L.) and a native cyprinind, *Rastrineobola argentea* (Pellegrin). Increase in *O. niloticus* is attributed to over fishing of endemic tilapiines thus reducing competition, while swamps clearance could have increased it spawning areas (Balirwa, 1998). Nile tilapia can also survive a wide range of pH, resists low levels of dissolved oxygen and feeds on a variety of food items (Balirwa, 1998; Njiru *et al*., 2004). The paper evaluates possible factors which have led to the dominance of introduced Nile tilapia in Lake Victoria.. *Oreochromis niloticus*, a member of the *Cichlids* family is one of the largest freshwater *Oreochromis species* found in most tropical waters (Thistleton 1986; Lamtane 2008). This is the most common artificially raised species of fish,

because it can be easily managed by farmers, it has indiscriminate appetite, highly prolific and is tolerant even to poor water quality (de Graaf 2004; FAO, 2005).

## 2.2 HISTORY AND DISTRIBUTION OF *OREOCHROMIS NILOTICUS* CULTURE

The culture of *O. niloticus* can be traced to ancient Egyptian which dates back to over 4000 years (FAO, 2006). Evidence is demonstrated by pictures showing ornamental fish in ponds. There is significant worldwide distribution of tilapias. *Oreochromis mossambicus*, distribution occurred during the 1940s and 1950s, while that of the more desirable *O. niloticus* occurred during the 1960s up to the 1980s (FAO, 2006). *Oreochromis niloticus* from Japan was introduced to Thailand in 1965, and from Thailand they were sent to the Philippines. *Oreochromis niloticus* from Cote d'Ivoire were introduced to Brazil in 1971, and from Brazil they were sent to the United States in 1974. In 1978, *Oreochromis niloticus* was introduced to China, which leads the world in tilapia production and consistently produced more than half of the global production in every year from 1992 to present (FAO, 2006). The uncontrolled breeding of tilapia in ponds, which led to excessive recruitment, stunting and a low percentage of marketable-sized fish, dampened the initial enthusiasm for tilapia as a food fish. The development of hormonal sex-reversal techniques in the 1970s represented a major breakthrough that allowed male mono-sex populations to be raised to uniform, marketable sizes. In addition, research on nutrition and culture systems, along with market development and processing advances, led to rapid expansion of the industry since the mid 1980s. Several species of tilapia are cultured commercially, but *O. niloticus* is the predominant cultured species worldwide (FAO, 2006).

## 2.3 CULTURE SYSTEMS FOR TILAPIA

The culture practices of tilapia can be extensive, semi intensive and intensive (Tsadik, *et al* 2007). There has been a gradual shift in tilapia culture from traditional semi-intensive to non traditional intensive farm systems (El-Sayed, *et al* 2008). But, deciding the optimal culture method for tilapia farming can be quite complex (Graaf, *et a*l 2005). Traditionally, tilapia is often cultured in earthen ponds without supplemental feeding (Liti, e*t al* 2005). Intensive monoculture of the fish in concrete tanks is carried out in a few countries (Green *et al*. 1997). Although practiced in some countries, cage culture of tilapia is yet to be commercialized on a wide scale basis. Pen culture of tilapia in open waters of lakes is practiced in the Philippines on an appreciable scale (Pillay, 1993). Flow-through culture of tilapia is also done on a very limited scale, for producing marketable fish. Extensive evaluations of various management strategies are required to select the optimal culture procedure under certain eco-socio-economic conditions.

## 2.4 FEEDING AND ENVIRONMENTAL CONDITIONS FOR *O. NILOTICUS*

Nile tilapia is a tropical species that prefers to live in shallow water. The reason for the preference is because this fish can tolerate a wide range of environmental conditions. The lower and upper lethal temperatures ranges for *Oreochromis niloticus* are 11-12 °C and 38 - 42 °C, respectively, while the optimum growth temperature ranges from 31 - 36 °C. It is an opportunistic feeder with diets ranging from phytoplankton, periphyton, small invertebrates, benthic fauna, detritus and bacterial films associated with detritus (Varadaraj and Pandian, 1987). *O. niloticus* can filter feed by entrapping suspended particles, including phytoplankton and bacteria, on mucous in the buccal cavity, although its main source of nutrition is obtained by surface grazing on periphyton mats.(Ridha *at el.* 1990) Sexual maturity in ponds is reached

at an age of 5-6 months. Spawning ceases when the water temperature reaches 15.5 – 17.5°C (Harrison *et al.* 2009). Better growth performance in terms of average weight gain (g) and average length gain (cm) was seen in treatment containing highest salinity level (4000 ppm) while the lowest was observed in 800 ppm, Iqbal *et al*. (2012). *Oreochromis niloticus* is able to tolerate poor water quality and a wide range of environmental conditions compared to many other species. *Oreochromis niloticus* has ability to tolerate extremely low dissolved oxygen (DO) concentration at the level of less than 1 mg/L in early morning and can survive when DO drops briefly (for at most 6 hours) as low as 0.1 mg/L. However, the fish will die if they are exposure to low DO for a long time i.e. not more than 6 hours (Yang Yi and Kwei Lin, 2008).

**2.5 BREEDING OF *OREOCHROMIS NILOTICUS***

The breeding process starts when the male establishes a territory, digs a craterlike spawning nest and guards his territory. The ripe female spawns in the nest, and immediately after fertilization by the male, collects the eggs into her mouth and moves to quiet and safe places for mouth brooding. The female incubates the eggs in her mouth and broods the fry after hatching until the yolk sac is absorbed. Incubating and brooding is accomplished in 1 to 2 weeks, depending on temperature (FAO, 2006). After fry are released, they may swim back into her mouth if danger threatens. Being a maternal mouth brooder, the number of eggs per spawn is small in comparison with most other pond fishes. Egg number is proportional to the body weight of the female. A 100 g female may produce about 100 eggs per spawn, while a female weighing 600-1 000 g can produce 1 000 to 1 500 eggs. The male remains in his territory, guarding the nest, and is able to fertilize eggs from a succession of females. If there is no cold period, during which spawning is suppressed, the female may spawn after every 2

to 3 weeks. While the female is brooding, feeding ceases. Nile tilapia can live longer than 10 years and reach a weight exceeding 5 kg (FAO, 2006).

## 2.6 PHYSIOLOGICAL EFFECTS OF TEMPERATURE FLUCTUATION ON TILAPIA

In fish, the degree of tolerance to lethal temperatures is dependent upon environmental effects, history of the fish and genetic effects (Cnaani *et al.*, 2000) as well as fish health and nutrition status. It has been reported for many ectotherms that animals can extend their thermal tolerance range through acclimatization and acclimation (Cossins and Bowler, 1987). In tilapia, prior acclimation temperature and rate of temperature reduction are considered important factors in determining mortality at a given temperature (Stauffer, 1986; Stauffer *et al*., 1988). It is thought that the ability of fish to adapt to different temperatures is closely linked to the lipid composition in their muscles (Hazel, 1984, Greene and Selivonchick, 1987). Fatty acid composition is in turn influenced by the fish's diet (Henderson and Tocher, 1987).

Sex determination is controlled by the actions of a variety of biochemical pathways involving many different proteins (e.g. transcription factors, steroidogenic enzymes, receptors and second messenger systems, etc.). Since it is well known that temperature can dramatically influence the structure and function of proteins and other macromolecules, temperature fluctuations as are encountered by fish in different habitats could alter sex-determination pathways and influence the probability that development would be male or female. Temperature-dependent sex determination has been extensively studied in reptiles, where exposure to elevated temperature results in female development in some species (Bull and Vogt, 1979; Vogt and Bull, 1982). These temperature-dependent effects appear to be

mediated in part by influencing aromatase activity and estradiol synthesis in females, and by steroid receptors in both sexes (Crews and Bergeron, 1994; Crews, 1996). Such effects may also occur in fish: Estradiol secretion has been shown to range as much as 20-fold over just a 5 jC temperature range in common carp (Manning and Kime, 1984), and temperature also affects steroid production testis in trout, carp and tilapia (Kime and Hyder, 1983; Manning and Kime, 1985; Kime and Manning, 1986). In Nile tilapia (*O. niloticus*) and Japanese flounder (P. olivaceus), elevated temperatures (which cause masculinization) are associated with reduced aromatase mRNA levels and lower estradiol levels (Kitano *et al*., 1999; D'Cotta et al., 2001), and treatment with an aromatase inhibitor is able to counter the masculinizing effects of high temperature (Kwon *et al*., 2000).

In the Atlantic silverside *Menidia menidia*, incubation of larvae at higher temperatures increases the proportions that differentiate as males (Conover and Kynard, 1981). The temperature-sensitive period was during the mid-larval stage, and subsequent temperature fluxes had no effect on sex ratio, suggesting a switch-type mechanism operates to control sex in this species (Conover and Fleisher, 1986). The temperature responsiveness of M. menidia also has a genetic component since progeny from different females respond differently to temperature influences (Conover and Kynard, 1981), and different sires also can have a strong effect on temperature responsiveness (Conover and Heins, 1987a). In nature, ocean temperatures are suspected to affect sex ratio in *Menidia* species such that females are produced from earlier, colder spawning conditions, allowing additional time for ovarian growth (Conover, 1984; Middaugh and Hemmer, 1987). This temperature responsiveness is affected by latitude (Conover and Heins, 1987b), such that northern populations from Canada do not respond to temperature, whereas those from South Carolina do (Lagomarsino and

Conover, 1993). These genetic differences allow distinct populations of M. menidia to adjust sex ratios appropriately at different latitudes to maximize fitness.

In *Oreochromis niloticus*, elevated temperature generally has a masculinizing effect that is affected by, and can override, genetic influences on sex determination (Baroiller et al., 1995, 1996; Baras et al., 2001), but a feminizing effect has also been observed in all-male and YY strains of *O. niloticus*, particularly in inbred vs. outbred strains (Abucay et al., 1999).

## 2.7 OVERPOPULATION OF CULTURE FACILITIES

Despite brooding few eggs, *O. niloticus* is known to reproduce within short period i.e. After every 14 days leading to overpopulation of culture facilities. Popma and Green (1990) discussed how the presence of 3 to 5% females in tilapia production ponds can result in excessive reproduction and reduced growth. The major drawback in culture of *Oreochromis niloticus* is their early sexual maturity, which results in excessive recruitment in ponds. *Oreochromis niloticus* reproduce when they are only 2-3 months old; often well below the preferred market size. Uncontrolled spawning in production ponds often causes overpopulation resulting in competition for food, reduced growth, and lower yields of marketable size fish (Yang Yi and C. Kwei Lin, 2008). Production of all-male fish therefore became essential.

## 2.8 TECHNIQUES FOR MONO-SEX PRODUCTION

Techniques to produce all-male fingerlings are now an established part of tilapia culture and the use of mixed-sex groups has almost entirely been abandoned (Nicholas, 2012). Mixed-sex culture was tried in many tropical countries, but even good quality stock that matured late in the wild started reproducing at an early age in aquaculture ponds, and commercial culture failed time and again. Various methods of sex control including, hand-sexing, hybridization

and genetic manipulations sex reversal by steroids like androgenic hormones (Nicholas, 2012).

**2.9 HYBRIDIZATION**

Initial attempts used closely related species such as *Oreochromis hornorum, O. niloticus, O. aureus* and *O. mossambicus* crosses. Some of these hybrids produced nearly 98% male fish, but hatchery production of large numbers of fry was inconsistent and unreliable (Mabarerehe, 1992). Many *Oreochromis* species utilized in aquaculture were extensively introduced outside their native range in Africa. Given their recent evolutionary radiation, these species hybridize easily, posing a threat to the integrity of local adaptation, Marı´a *et al.* (2005).

Interspecific hybrid fish have been produced for aquaculture and stocking programmes to increase growth rate, transfer desirable traits between species, combine desirable traits of two species into a single group of fishes, reduce unwanted reproduction through production of sterile fish or mono-sex offspring, take advantage of sexual dimorphism, increase harvest ability, increase environmental tolerances, and to increase overall hardiness in culture conditions (Lahav and Ra'anan, 1997; Stickney, 2000; Bartley *et al.*, 2001; Hallerman, 2002) cited by Samy *et al.* (2012).

**2.10 GENETIC MANIPULATION**

One other sex reversal method is the production of ‘super male’ fish. These are fish that have been bred to have YY-chromosomes, as opposed to the normal XY males (females are XX). It is only used in a few hatcheries worldwide, but the advantage is that the actual fish eaten are never hormone-treated. For those who want to farm tilapia commercially and successfully, obtaining good quality all-male fingerlings from a competent hatchery is an essential first step (Nicholas, 2012). In Africa, where very little has been done in terms of genetic improvement

of Nile tilapia, one may safely assume that the productivity of the current stock is at the level of the GIFT (Genetically Improved Farmed Tilapia) base population or lower (Brummett *et al*., 2004) cited by Paul *et al.* (2008). The integration of a physical and a genetic map has been hindered due to the absence of specific chromosome markers for this species, Paul *et al.* (2008).

**2.11 SEX DETERMINATION**

Among mammals sex is usually defined by the presence or absence of the sex specific chromosome Y. In many, but not all, fish species there is also a chromosomal background to sex determination. Several fishes, including most salmonids, have heterogametic males and homogametic females, similar to the mammalian XY/XX-system (Thorgaard G.H., 1977; Thorgaard G.H.,1978; Phillips RB and Ihssen PE 1985). Other species, such as *Poecilia*, have homogametic males and heterogametic females (ZZ/ZW), which also is the case for birds (Volff JN and Schartl M. 2001). Some species of the Poecilid platyfish *Xiphophorus*, utilize a system with three sex chromosomes (Kallman KD 1968). In yet other species sex determination is influenced by environmental factors such as the temperature surrounding the developing embryo (Bull JJ and Vogt RC 1979; Conover DO and Heins SW 1987; Pavlidis M. *et al.* 2000). Hermaphroditism is also a common feature of several fish species. Several studies have shown that species with genetic sex determination can be directed to produce genetically sex reversed offspring. This is accomplished either by treating the fish with hormones, which can induce sex reversal in synchronous hermaphroditic fish (Tang F. *et. al.* 1974; Yeung WS 1993) and masculinization/ feminization in gonochoristic species, or by incubating embryos in certain temperatures or pH (Baroiller JF 1999). The proportion of males usually increases with temperature whereas lower temperatures favour females. In the

case of pH, species differences have been observed. There are few studies of sex determination in fish and the genetic mechanisms behind sex determination in fish remain largely unknown.

Cichlid fishes of the East African Great Lakes Malawi (LM), Victoria (LV), and Tanganyika (LT) are a prime model system in evolutionary biology and provide an exceptional opportunity to study organismal diversification (Kocher 2004). As for the majority of fish species, the triggers of sex determination in cichlids are largely unknown. Yet, it becomes clear that also in this group various mechanisms exist, including genetic systems and environmental triggers such as water pH and temperature (Baroiller 2009; Reddon and Hurd 2013). The known genetic factors in cichlids include, for example, sex determination via B-chromosomes (small supernumerary/accessory chromosomes, Yoshida *et al*. 2011) and male and female heterogametic sex chromosome systems, with the possibility of both systems co-existing within a single species (Roberts *et al.* 2009). The best-studied cichlid species with respect to sexual development is the Nile tilapia (*Oreochromis niloticus*), a member of a more basal lineage, widely distributed in rivers and lakes of Africa. The Nile tilapia has an XX-XY sex-determining system, which can substantially be influenced by temperature (Baroiller 2009). For this species, expression profiles of key genes of sexual development are available (Ijiri *et al.* 2008), which is not the case for other cichlid species such as the radiating lineages in East Africa.

Fishes have the most plastic system of germ and somatic cells in comparison with other animals. For them, the plasticity is maintained throughout the life cycle. It describes the impact on the process of such factors as temperature, pH, and population density. Temperature sex determination (TSD) at fish is less common than previously thought. The

effect of estrogen acting through estrogen receptor (ER) directly or indirectly regulates P450arom and Anti-M¨ullerian hormone (AMH) (Kobayashi Y *et al*., 2013).

The brain-pituitary-gonad (BPG) axis is the key regulator of sexual maturation. Neuron stimulation of the brain leads to stimulation of the pituitary through gonadotropin releasing hormone (GnRH) which releases relevant hormones, such as follicle stimulating hormone (FSH) and luteinising hormone (LH) into the blood plasma for transport to the effector tissue. In the testes LH induces the production of testosterone which then affects various aspects of male physiology, secondary sexual characteristics and behavior (http://www.salmongenome.no/cgi-bin/)

**2.12 HORMONAL SEX REVERSAL**

The process of gender manipulation through treatment with androgen or estrogen has been called sex reversal or sex inversion. Two methods of sex control using steroids like androgenic hormones have been applied in tilapia production (El-Sakhawy *et al.,* 2011). The hormonal sex reversal involves use of synthetic Methyltestosterone to muscularinize the culture population (Gaber *et al.* 2012). In some cases, natural crude hormones have been tried to a limited extend. The testosterone level of the freeze-dried bull testes, for example, was found to be 11.4 µg/g by Jay-Yoon *et al.*, (1988). The authors found that when the bull testes was mixed half and half with the commercial feed ration, the effective hormone dose on *O. niloticus* sex reversal was reduced to approximately 5.7 mg/kg of diet. The authors further reported that when *O. niloticus* was fed diets containing 11.4 µg/g, the percentage of males was increased from 89 to 97%.

A mono-sex population of males avoids recruitment of fingerlings into the ponds, while creating a faster growing male sex. The use of hormones in food fish has raised concerns

about eating hormone treated fish (Ronald *et al*., 2004). To alleviate these concerns, genetic manipulations have been used to create the YY super male which can produce mono-sex populations without use of hormones. Two methods **of sex control** using the hormones are commonly used including immersion of fry in a hormone solution and oral administration.

**2.13 IMMERSION OF FRY IN HORMONIZED SOLUTION**

Immersion of tilapia fry in androgen solutions may be an alternative to oral administration of androgen. Induction of masculinization by immersion has several advantages over feeding because all fish are exposed to the hormone and a shorter exposure period is required. Besides, the hormone can be destroyed using formalin and disposed safely to avoid environmental contamination. The disadvantages of immersion treatment include the need for an enclosed tank for treatment; however, this need also results in the androgen being contained for easy filtration (e.g. carbon filtration) and removal. The main impediment to the adoption of this technique is that minimum effective treatments have yet been established for many species. This technique is only well developed in salmonid aquaculture (Piferrer and Donaldson, 1989) but remains largely experimental in tilapia culture. Results of immersion of *O. aureus* fry in mibolerone at 0.6 mg/L for 5 weeks resulted in populations that were 82% male, while 0.3 mg/L mibolerone immersion for 5 weeks resulted in less than 1% functional females (Torrans *et al*., 1988). Immersion of *O. niloticus* fry of 10 and 13 day post-fertilization in 17 α – methyldihydro-testoterone at a concentration level of 0.5 mg/L for 3 hrs resulted in population that were between 93 and 100% males (Gale *et al*., 1995).

## 2.14 17α-METHYLTESTOSTERONE (MT) MODE OF ACTION AND ITS ADVANTAGE ON SEX REVERSAL

The synthetic steroid 17α- methyltestosterone is a male specific hormone commonly used to induce sex reversal in teleost fish. 17α-Methyltestosterone (MT) is a synthetically produced anabolic and androgenic steroid hormone; i.e. it promotes both muscle growth and the development of male sexual characters (Al-ablani and Phelps, 2002).

Androgens are commonly applied for hormonal stimulation of growth or sex reversal in fish (Colborn *et al*., 1993; Damstra *et al.*, 2002). Among the androgens, MT is most commonly used. It is easily absorbed, does not accumulate in fish body and is readily excreted (Sumpter, 2005).

Sex reversed tilapia showed a better growth rates than normal because administration of androgen have both an androgenic and anabolic effect. There are several studies comparing the growth of sex reversed, near all male populations, to that of a mixed sex population after hormone treatment showed the improved growth of sex reversed fish than non-treated because the presence of females reduces the growth rate due to their slower growth rate or reproduction (Macintosh *et al.* 1985). Commercial tilapia production generally requires the use of male monosex populations. Male tilapia grows approximately twice as fast as females (FAO, 2006).

## 2.15 ORAL HORMONAL ADMINISTRATION

Oral administration procedure of sex reversal involves feeding fish with hormone treated diet. Fish exposure to androgens usually occurs through dietary treatment, and the most commonly used androgen is 17 α – methyltestosterone (MT). Feed preparation has been described by Popma and Green (1990), Neves *at al.,* (2009). Feeds that contain 25 to 45% protein are

recommended, although a lower level of 20% protein feed has been used successfully (Popma and Green, 1990). Androgen-treated feed is prepared by mixing androgen that has been dissolved in solvent, usually 80 to 95% ethanol with fine grounded feed or the hormone treated feed may be prepared by spraying a solution of androgen in 95% ethanol onto the feed. Mixing the components of feed and hormone minimizes atomization of androgen solution, which reduces the risk of contamination of workers. However, the spray method is advantageous in that the volume of solvent can be greatly reduced. In both cases, the alcohol is allowed to evaporate and the dried feed stored in a cool, well-ventilated area or refrigerated until used; good air circulation around the complete feed container helps maintain feed quality during storage. Because MT is photosensitive, pure MT should be protected from sunlight, and treated feed should not be dried or stored exposed to direct sunlight. Feed that was prepared with MT and stored exposed to light was found to be ineffective in sex inversion of *O. mossambicus* (Varadaraj *et al*., 1994).

The age of *O. niloticus* fry is an important factor that influences the efficiency of sex reversal efficiency and should be considered. Treatment duration of 3 to 4 weeks consistently produced male tilapia populations comprising ≥95% males, while periods that exceed 4 weeks did not further improve the efficacy of the treatment (Tayamen and Shelton, 1978; Owusu-Frimpong and Nijjhar, 1981; Nakamura and Takahashi, 1985). However, at lower water temperatures of about $20\pm2^{0}$C, increasing the duration of treatment from 20 to 40 days increased the efficiency from 69 to 95% males (Mabarerehe, 1992). Treatment with higher doses of MT for 19 or 28 days did not result in successful sex reversal (Nakamura, 1975; Okoko and Phelps, 1995). Ridha Lone (1990) reported no significant differences in tilapia fry survival, and final weights after a 38 day trial where fry were fed androgen-free diet

containing 30, 50, and 70 mg MT/kg. From the ongoing it is clear that 100% all male population has not been attained. For successful culture of tilapia, there must not be females in the population because presence of a few female would negate the gains achieved in sex reversal. The current study was aimed at determining the age and duration of hormone exposure which would lead to 100% conversion efficiency of 17-α methyltestosterone on sex reversal of Nile tilapia**.**

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 STUDY AREA

The study was carried out in Zoology Laboratory, University of Eldoret. The University is located at Latitude of $00^0$ and Longitude $035^0$, and 2154m above mean sea Level.

## 3.2 SOURCES OF EXPERIMENTAL FRY

Fish were seined from Eldoret fish farm and those brooding eggs and fry were selected. The eggs and fry were transferred from the mouths into a bucket containing aerated water. The fry and eggs were then transported to the laboratory for further processing. The fry were placed in the aquarium while the eggs were incubated in incubation jars until hatching (see Figure 1).

## 3.3 MINI HATCHERY SYSTEM

Mini hatchery System consists of two – 3 litre jars fitted in two slots on a wooden board. On top of the jars was a head basin of 10 litres which supplied the jars with aerated water, through 2 cm horse pipes. Drain horse pipes were fitted to the jars near the upper brim. The inlet pipe was connected to a supply tap. A thermostat was immersed in the supply basin to regulate the water temperature to range between 31 - $32^0$C.

**( Source: Author, 2014)**

**Figure 1: Mini hatchery System where the incubation jars were installed for the purpose of incubating the tilapia eggs**

### 3.4 HORMONE PREPARATION

The hormone feed was prepared by weighing the desired quantity of hormone dissolving it in 95% ethanol and spraying it on the feed. Methyltestosterone was incorporated at 40mg per kilogram of feed. The feed was then dried under shade and stored in a well ventilated container in a cool place.

### 3.5 EXPERIMENTAL SET-UP

The fry were sorted into two categories according to whether they had yolk sac or not. Fry which had no yolk sac were stoked in two aquaria, each measuring 45 x 30 x 30 cm length, width and depth, respectively at a rate of 150 fry per aquarium. The yolk sac fry were stoked in four aquaria of similar size as above and also at the same stoking rate. Room temperature was maintained at $25^0$C using fan heater. Water temperature ranged from 28 - $31^0$C. The

higher temperature of the water was due to higher specific heat capacity of water compared to air.

### 3.6 FEEDING

Fry were fed using the feed containing 17 α-methyltestosterone for varying periods of days. The period of exposure to hormone feed were distributed into 21 days, 30 days, 42 days and 60 days. And at the end of each period, the fry were then sampled in three replicates of ten fry for dissection and microscopic examination.

### 3.7 WATER QUALITY

The water in the aquaria and incubation jars was monitored for various water quality parameters, which included temperature, pH, DO, salinity, TDS and Conductivity. Measurements were taken weekly using YSI 3D Interactive model Professional Multi-parameter meter. The egg development time was also determined at two temperature ranges.

### 3.8 MICROSCOPIC EXAMINATION

Sex of fry was determined through microscopy using compound microscope. Identification was aided by staining the gonads using aceto-carmine. A readymade solution of aceto-carmine was purchased and used to stain the gonads in the staining jar for six hours. The stained gonads were mounted on new microscopic slides and covered with a cover slip. The microscopic slides and cover slips containing stained gonads were then observed under compound microscope using oil immersion at 100x objective power, and microscopic slide photos were taken using digital camera mounted on a microscope.

### 3.9 STATISTICAL ANALYSIS

The data was statistically analyzed using Two-way Analysis of Variance and correlations, Statistical Programme for Social Scientists and Excel Statistical package.

# CHAPTER FOUR

# RESULTS

## 4.1 MICROSCOPIC OBSERVATION FOR SEX DETERMINATION OF *O. NILOTICUS*

The results of microscopic observations are shown in Table 1. For all the treatment no pure females were observed but intersex of varying number of ova was observed. The number of ova in the intersex fry ranged from a minimum of one to a maximum of eight. After 21 days of hormone exposure of fry from brooding mothers which had absorbed the yolk sac were 50% converted into males and when the period was increased to 42 days, the conversion efficiency into males increased to 80%. Fry from the mouth of brooding mothers which had yolk sac and exposed to hormone for 21 days resulted into 60% conversion efficiency. When the period for same fry was extended for 30 days, the conversion efficiency into males increased to 70%. The yolk sac fry from the incubator had 100% conversion efficiency into males both at 21 and 60 days of exposure to hormone.

**Table 1: Microscopic observation for sex determination of *Oreochromis niloticus***

| Category (Age) | Hormone period in days | No. of Males | No. of Females | No. of Intersex | Total |
|---|---|---|---|---|---|
| Fry from mouth having no yolk sac | 42 | 9 | 0 | 1 | **10** |
| | | 10 | 0 | 0 | **10** |
| | | 9 | 0 | 1 | **10** |
| | | **Total** | | | **30** |
| | 21 | 7 | 0 | 3 | **10** |
| | | 9 | 0 | 1 | **10** |
| | | 9 | 0 | 1 | **10** |
| | | **Total** | | | **30** |
| Fry from the mouth with yolk sac | 21 | 9 | 0 | 1 | **10** |
| | | 8 | 0 | 2 | **10** |
| | | 9 | 0 | 1 | **10** |
| | | **Total** | | | **30** |
| | 30 | 8 | 0 | 2 | **10** |
| | | 9 | 0 | 1 | **10** |
| | | 10 | 0 | 0 | **10** |
| | | **Total** | | | **30** |
| Yolk sac fry hatched in incubators | 60 | 10 | 0 | 0 | **10** |
| | | 10 | 0 | 0 | **10** |
| | | 10 | 0 | 0 | **10** |
| | | **Total** | | | **30** |
| | 21 | 10 | 0 | 0 | **10** |
| | | 10 | 0 | 0 | **10** |
| | | 10 | 0 | 0 | **10** |
| | | **Total** | | | **30** |

Table 1 above shows the number of males, females and intersex observed during slide microscopic examination.

The results from Pearson correlation are shown in Table 2. According to Pearson Correlations, category and sex of fish was significant at ($P<0.05$), as showed in table 2. Hormone period and category (age) was highly significant at ($P<0.05$). Pearson correlation showed that there was no significance between hormone period and sex of fish. Category was significantly correlated to period of exposure. The interaction between the category of fry and

period of exposure was significant (P<0.05). Category was also significantly correlated to sex reversal inversion. The period of exposure to hormone was not significantly correlated to sex inversion.

**Table 2: Pearson Correlations of sex of fish, category and period of exposure to hormone**

| | | Category | Period of exposure to hormone | sex of fish | Temperature |
|---|---|---|---|---|---|
| Category | Pearson Correlation | 1 | -.242(**) | .174(*) | -.008 |
| | P-value | . | .001 | .019 | .912 |
| | N | 180 | 180 | 180 | 180 |
| Period of exposure to hormone | Pearson Correlation | -.242(**) | 1 | -.132 | -.009 |
| | P-value | .001 | . | .077 | .904 |
| | N | 180 | 180 | 180 | 180 |
| sex of fish | Pearson Correlation | .174(*) | -.132 | 1 | .014 |
| | P-value | .019 | .077 | . | .852 |
| | N | 180 | 180 | 180 | 180 |
| Temperature | Pearson Correlation | -.008 | -.009 | .014 | 1 |
| | P-value | .912 | .904 | .852 | . |
| | N | 180 | 180 | 180 | 180 |

** Correlation is significant at the 0.01 level .

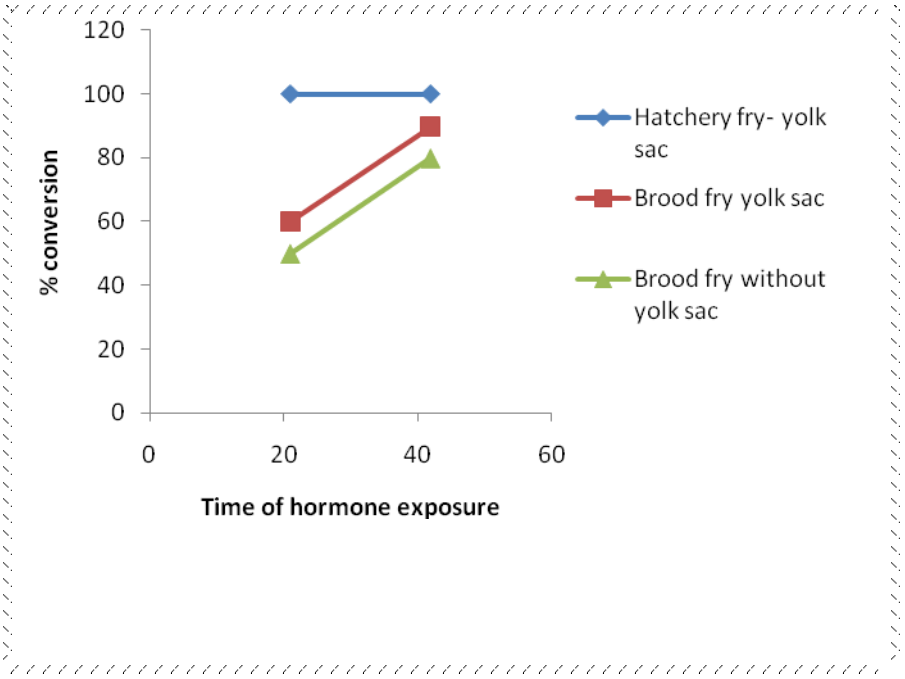
* Correlation is significant at the 0.05 level .

**Table 3: Spearman's rho Correlation Coefficient for sex of fish in relation to category and period of exposure to hormone**

| | | | Category | Period of exposure to hormone | Sex of fish | Temperature |
|---|---|---|---|---|---|---|
| Spearman's rho | Category | Correlation Coefficient | 1.000 | -.127 | .178(*) | -.007 |
| | | P-value | . | .089 | .017 | .931 |
| | | N | 180 | 180 | 180 | 180 |
| | Period of exposure to hormone | Correlation Coefficient | -.127 | 1.000 | -.116 | -.002 |
| | | P-value | .089 | . | .120 | .984 |
| | | N | 180 | 180 | 180 | 180 |
| | Sex of fish | Correlation Coefficient | .178(*) | -.116 | 1.000 | .020 |
| | | P-value | .017 | .120 | . | .792 |
| | | N | 180 | 180 | 180 | 180 |
| | Temperature | Correlation Coefficient | -.007 | -.002 | .020 | 1.000 |
| | | P-value | .931 | .984 | .792 | . |
| | | N | 180 | 180 | 180 | 180 |

* Correlation is significant at the 0.05 level.

Comparing Pearson correlation and Spearman's rho Correlation Coefficient, they both showed that there was significance between age in days and sex of fish, and no significance between period of exposure to hormone and sex of fish as demonstrated in table 2 and table 3. Interaction between hormone exposure and category of fry was highly significant. The curves for brood fry sac and brood fry without yolk sac were more or less parallel. The curve for hatchery based fry had steep increase to 100% conversion.




**(Source: Author, 2014)**

**Figure 2: Interaction between fry category and hormone exposure**

**Table 4: Analysis of Variance for sex of fish in relation to Category of fry**

| | Sum of Squares | Df | Mean Square | F | P-value |
|---|---|---|---|---|---|
| Between Groups | 2.178 | 2 | 1.089 | 3.896 | .022 |
| Within Groups | 49.467 | 177 | .279 | | |
| Total | 51.644 | 179 | | | |

Analysis of Variance for sex of fish in relation to age in days showed significance at (P< 0.05).

**Table 5: Paired Samples Correlations of sex of fish, category and period of exposure to hormone**

| | | N | Correlation | P-value |
|---|---|---|---|---|
| | category & sex of fish | 180 | .174 | .019 |
| | Period of exposure to hormone & sex of fish | 180 | -.132 | .077 |

Correlations of category and sex of fish showed high significant at (P<0.05). But in the same table, period of exposure to hormone and sex of fish did not show any significance. In percentages, the sex of fish showed 92.2% male, 7.8% intersex and 0% female.

**4.2 Physiochemical parameters and egg development time**

The results of physicochemical parameters and egg development time are shown in Tables 9a and b.

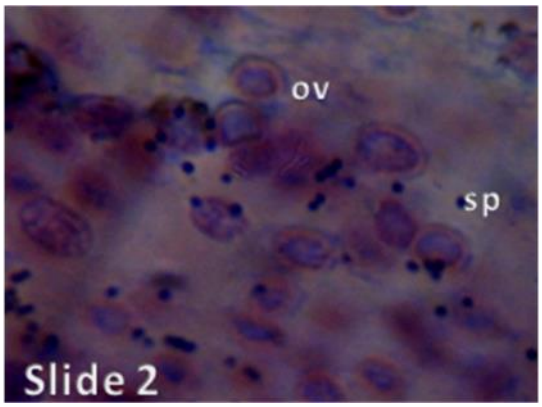
Temperature ranged from 23.2 – 31.4$^0$C, pH ranged from 6.02 -8.58, DO ranged from 0.1 ppm, salinity ranged from 0.19 - 0.28 ppt., TDS ranged from 217.1 - 383.6 mg/l and conductivity ranged from 334.4 - 588 µS/cm. The embryonic development time varied with temperature with an inverse relationship. At a temperature range of 19$^0$ – 20$^0$C it took 6 days for the eggs to hatch while the period was reduced to a half when the range was 31$^0$ – 32$^0$C. The yolk absorption period was also inversely proportional to temperature. It took almost a half of the time to absorb the yolk when the temperature was increased by 10$^0$C.

**Table 6: Physicochemical mean water quality parameter**

| Environmental water quality parameters | | | | | |
|---|---|---|---|---|---|
| **Temperature** | **pH** | **DO** | **Salinity** | **TDS** | **Conductivity** |
| 28.3±3.7$^0$C | 7.3±1 | 1.95±2mg/l | 0.24±0.11ppm | 300.4±3mg/l | 461.2±4 µS/cm. |

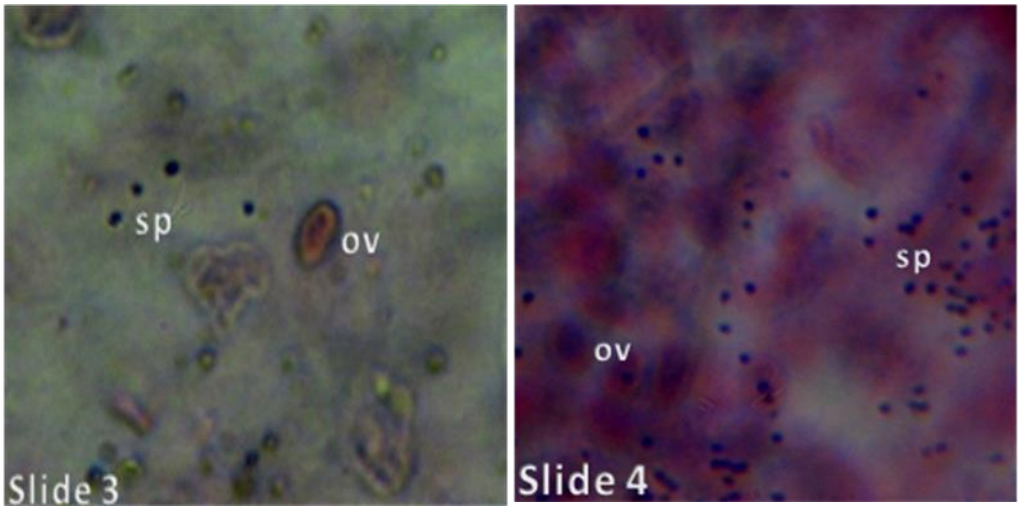
**Table 7: Effects of temperature on embryonic development time and yolk absorption period**

| **Hatching of eggs and yolk absorption time in the hatchery in relation to temperature** | | |
|---|---|---|
| **Hatch/ Yolk Fry absorption** | **Days** | **Temperature** |
| Embryonic development time | **6** | 19$^0$C – 20$^0$C |
| | **3** | 31$^0$C – 32$^0$C |
| Yolk Fry absorption | **12** | 20$^0$C – 23$^0$C |
| | **5** | 31$^0$C – 32$^0$C |



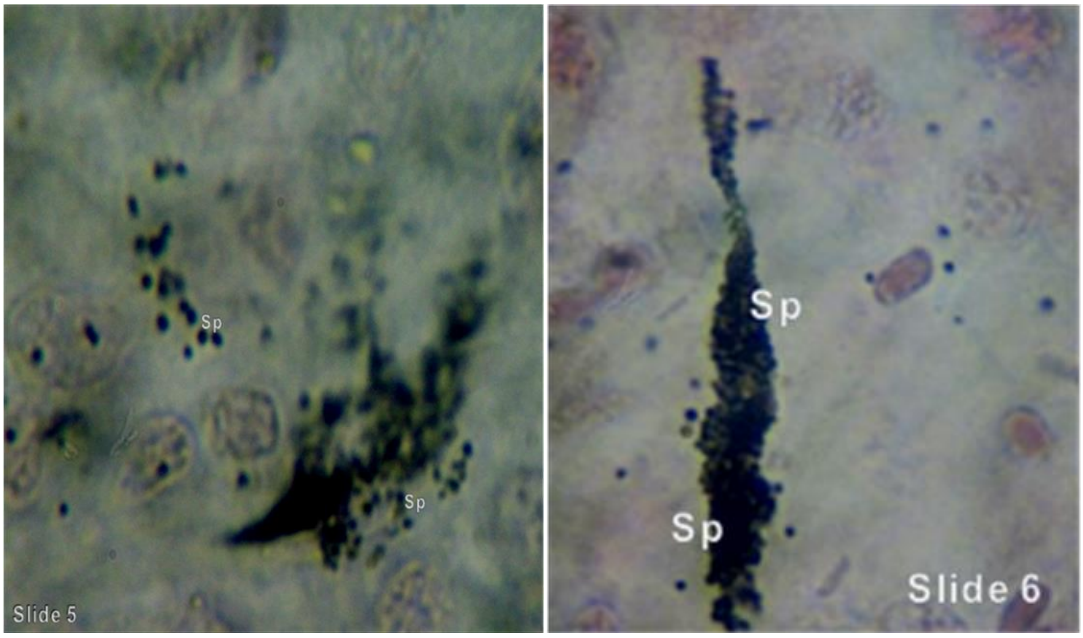

**(Source: Author, 2014)**

**Figure 3: Slide 2: showing intersex,  sp. means sperm and ov. means ova**



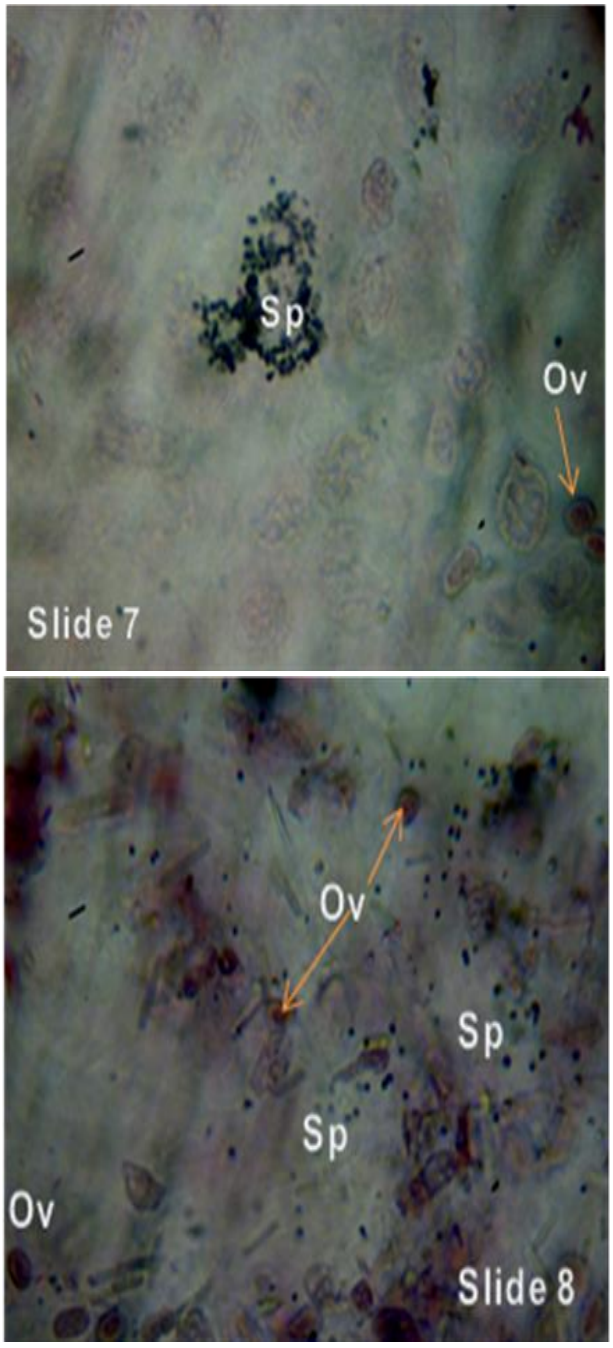

**(Source: Author, 2014)**

**Figure 4: Slides 3 and 4 showing intersex, sp. means sperm and ov. means ova**




**(Source: Author, 2014)**

**Figure 5: Slides 5 and 6 showing sperms (sp)**




**(Source: Author, 2014)**

**Figure 6: Slides 7 and 8 showing intersex sp. means sperm and ov. means ova**

# CHAPTER FIVE

# DISCUSSION

In the present study, an inversion rate of 100% was observed in fry that were hatched from eggs in the laboratory. Both yolk-sac-fry and those lacking the yolk from the mouths of brooding mothers did not achieve 100% sex inversion. Further, no pure females were observed during microscopic examination and all departures from pure males were inter-sex individuals. This observation suggests that all fry had access to the hormone treated feed in one way or the other. Others authors found that producing a monosex population of *O. niloticus* for aquaculture is high priority since males have a higher growth rate as compared to females and their observations revealed male percentage above 90% but less than 100% Utete and Victor (2012). The results as indicated by others here meant that the methods used were crude and need refinement. This may lead to lack of pure female. Most experimental evidence only partially supports a monofactorial model for sex determination in tilapia; autosomal and environmental factors also are thought to influence sex determination (Wohlfarth and Hulata, 1981; Majumdar and McAndrew, 1983; Shelton *et al*., 1983, Mair *et al*. 1991b; Trombka and Avtalion, 1993) cited by Green et al., (1997).

Pearson correlation and Spearman's rho Correlation Coefficients between period of exposure to hormone and rate of sex inversion to males were not significant although there was a tendency for increasing efficiency of sex inversion with the time of exposure as demonstrated in Tables 2 and 3. Several authors have reported increased conversion efficiency in to males with extended period of hormone exposure (Gale *et al*. 1995, Nakaruma and Takahashi 1985, Ronald and Robert 2004). Several authors found that hormone exposure time and rate of sex conversion into males were not significant, but increasing efficiency of sex conversion was

evidence with the time of exposure (Raul *et al.,* 2007; Remedios B. B., 2008; Suman B. C. and Samir B., 2009).

The present study revealed that after 21 and 42 days of hormone exposure of *O. niloticus* fry from brooding mothers without the yolk sac produced both males and intersex. *O. niloticus* fry from the mouth of brooding mothers which had yolk sac and exposed to hormone for the same period resulted into more males and less intersex. However, the extension of time exposure to the hormone to 60 days slightly reduced the number of intersex fry. It is likely that from this result, the fry with yolk sac performed better than those without. This is because the fry without yolk sac were higher age as compared to fry with yolk sac. It is well established in the literature that fry of lower age respond better than an advanced aged fry (Piferrer *et al*. 1989). It is more likely that the hormone was absorbed by yolk which was intern a simulated by the fry since the sole nutrition of the fry dependent on the yolk. This result agrees with (Ana *et al*, 2011) on their results which showed that yolk sac fry exposed for a longer period to hormone feed led to higher rate of male conversion with very few intersex observed.

The result of the present study indicated that the yolk sac fry from the incubator had 100% conversion efficiency into males both at 21, 42 and 60 days of exposure to hormone. In this set up, eggs were incubated at a higher temperature which decreased embryonic development time and the same time, the swim-up fry had the first encounter to only hormonal feed. Given that sex determination conversion efficiency is dependent on age of fry; such fry are relatively younger and are more likely to be transformed with higher conversion efficiency into males. Gonadal tissue differentiation is presumed to occur between 8 to 25 days post-hatch, which is influenced by environmental conditions (Eckstain and Spira, 1965; Nakamura and Takahashi,

1973; Alvendia-Casauay and Carno, 1988) cited by Green, *at el*. (1997). In the present study, fry were exposed to hormone one day post-hatch. It is likely that masculinization hormonal stated its influence before the on-set of gonadal differentiation thus culminating into 100% conversion efficiency. This result did not conform to any result of other authors (Owusu-Frimpong and Nijjhar 1981, McAndrew and Majumbar 1983). The reason for the non conformity was because the current experiment was carried out within a confined aquaria environment which exposed all fry to hormone feed.

One striking observation was that fry which had yolk sac from mouth brooding mothers and those hatched from eggs in the lab differed in their rates of conversions. Despite both having yolk sac they yielded different results in terms of male conversion efficiency. Metabolic rates are increased by increasing temperature. Those fry in the mouth were incubated in much lower temperature, therefore the fry might have surpassed the minimum time of 8 days for on-set of gonad differentiation. This is supported by results observed during the study where it took 12 days before completion of the yolk sac at $19^0$C. This gives a plausible reason why most sex reversal trials (El-Sayed and Kawanna 2008) fail to achieve 100% efficiency.

The results of the present study indicated that high temperature reduced the fry development rate by half. At the temperature of $19^0$C embryonic development time was 6 days while when the temperature was increased to $31^0$C the development time was reduced to 3 days. This observation demonstrates that temperature is an important factor in sex reversal. The observation is in agreement the reports in the literature (Mabarerehe, 1992, FAO 2006, McGeachin *et al*., 1987). The relationship can be used to accelerate the rate of fingerlings production by regulating temperature. This experiment also revealed that yolk fry absorption varies with temperature with high temperature leading to faster yolk absorption.

The values of physicochemical water quality parameters measured in the present study were low in the beginning of the experiment. Temperature was close to $19^0$C, a very low value below the range of 25 – $30^0$C, which *O. niloticus* is known to grow optimally (Okoko and Phelps 1995) At the temperature of $19^0$C fish were observed to be in active and had slow response to offered diets. When temperature was increased fish became active and responded well to the feed. In the present experiment *O. niloticus* was exposed low dissolved oxygen at 0.1 mg/l for at most six hours. This finding agrees with that of Kamal e*t al.* (2010) and Yang Yi and Kwei Lin (2008) who stated the lower DO limit of *O. niloticus* is 0.1 mg/l of DO. Other physicochemical water quality parameters in the current experiment occurred within the ranges which have been reported for good growth of *O. niloticus* (Kamal e*t al.* 2010).

# CONCLUSION

The present study demonstrated that it is possible to achieve 100% all male mono-sex population. However to achieve this result fry must not be beyond the lower limit of 8 days post-hatch before exposure to hormone. For lower age fry conversion was independent of duration of exposure whereas for fry beyond 8 days post-hatch, the rate of male conversion was positively related to duration of exposure. Incubator hatched fry gave 100% male conversion rate whereas those snatched from the mouth did not attain 100% conversion efficiency at the duration of the experimental examination. Fry which had yolk sac from mouth brooding mothers and those hatched from eggs in the laboratory differed in their rates of conversions despite both having yolk sac they yielded different results in terms of male conversion efficiency. It was found that temperature was a key factor for faster egg and fry development.

## RECOMMENDATIONS

To produce all male population, fry should be under controlled conditions and at optimal temperature of 31 – $32^0$C. The age of fry is highly significant for efficiency of 17 α-methyltestosterone on sex reversal and a target below 8 days post-hatch could be taken into account. Sex reversal should start with fry of below 8 days old after hatching and duration of exposure for twenty one (21) days would be sufficient. Further research should be carried out to determine the possibility of producing YY Nile tilapia males so as to stop using hormone for sex reversal which is very expensive and labour intensive.

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# APPENDIX I: MONITORING AQUARIA, METHYLTESTOSTERONE MIXING WITH FOOD AND SLIDE OBSERVATIONS


Monitoring the aquaria in the laboratory


Mixing food with Methyl Testosterone


Slide observations in the laboratory

**(Source: Author, 2014)**