University of Natural Resources and Life Sciences, Vienna

Comparative assessment of growth performance of sex reversed Nile tilapia (*Oreochromis niloticus*) by fry immersion in freshwater and full seawater

Thesis submitted for the award of the title

"Master of Science"

by

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supervised by

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DECLARATIONS

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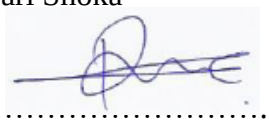
I, Remmy Safari Shoka, solemnly declare that this thesis is my original work and has not been presented for an award of a degree in any university or institution, and the sources of information used in the production of this work have been properly cited and acknowledged.

Remmy Safari Shoka

Signature  …….....

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We confirm that the work presented in this thesis was carried out by the candidate under our supervision and has been submitted with our approval as the university supervisors.

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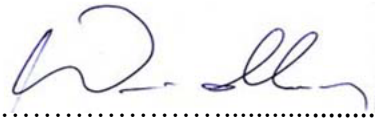
Signature  …….....

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I hereby declare that I am the sole author of this work. No assistance other than that which is permitted has been used. Ideas and quotes taken directly or indirectly from other sources are identified as such. This written work has not yet been submitted in any part.

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Dedication

I dedicate this work to my family

Acknowledgement

I wish to express my sincere gratitude to IPGL programme, University of Natural Resources and Life Sciences, BOKU, Vienna, Centre for International Relations (ZIB) under the Erasmus + programme, Joint Study Free Mover and KUWI, LWM programme Egerton University and IHE-Delft for giving me the opportunity and facilitating me through the study.

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Abstract

Tilapia production for economic and food security has led to improved efficiencies in the industry with primary focus in all-male sex production. This study was conducted to compare growth performance of sex reversed Nile tilapia by fry immersion technique in both fresh and marine water. Survival rate of fry, sex reversal and growth rate of Nile tilapia was determined. Brood stock for fry production were selected at their respective grow out farms (Bamba water for freshwater and Kibokoni for marine water) with monitoring done at KMFRI-Mombasa. Sex reversal was conducted through fry immersion in a solution of 17-α-methyltestosterone (MT) at different concentrations (0, 100 and 400 μg L^{-1}), which served as control and treated groups. Fry immersion was conducted for 3 hours on the 1st and 3rd day post egg-yolk absorption. After immersion, 15 L aquaria were used and fry fed on artemia supplemented with *omena* dust. The laboratory phase was conducted for 28 days for both setups and 49 days for field setup for the freshwater. The marine field phase could not proceed due to setup breakdown. Liner pond was used for the freshwater setup and hapa nets of 1 m^3 were randomly installed. Commercial feeds (35 % CP) were fed three times a day at 5 % body weight. The freshwater setup had a survival rate of 100 % while the marine setup had very low survival rates (5 %). Sex reversal was highest (91.5 %) in 400 μg MT L^{-1} and lowest in control (58.5 %). Treatment two showed better growth results for both the freshwater and marine setup with weight showing an interaction effect on the setups and treatments. The potential of the fry immersion technique is higher and different variables need to be considered for its improvement, efficiency and better results. With ever growing interest in the culture of tilapia in marine environment, and the findings of this study, key focus should be directed towards development of a breeding programme and capacity development on the same.

Keywords: fry immersion, sex reversal, hormone treatment, tilapia saline culture

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List of Abbreviations

C – Control

CP – Crude Protein

ESP – Economic Stimulus Programme

FAO – Food and Agriculture Organization

FCR – Feed Conversion Ratio

g – Grams

KMFRI – Kenya Marine and Fisheries Research Institute

Kn – Relative Condition Factor

KSh – Kenya Shilling

L – Length

MB – Mibolerone

MDHT - 17-α methyldihydrotestosterone

ml - milliliter

MT - 17α-Methyltestosterone

ppm – parts per million

ppt – parts per thousand

SR – Survival Rate

SGR – Specific Growth Rate

T1 - Treatment One

T2 - Treatment Two

TBA - Trenbolone Acetate

US $ - United States Dollar

W – Weight

WG – Weight Gain

1.0 INTRODUCTION

Aquaculture is an important sector which contributes to food security, income generation (FAO, 2020), poverty reduction and provide nutritional benefits in developing countries (Rothius et al., 2014). In 2018, the world aquaculture contribution to global fish production was 46.0 %. In Africa, aquaculture accounted for 17.9 % of total fish production. Inland aquaculture, mainly freshwater produces most farmed aquatic animals. The freshwater practice is highly advanced and most developed in terms of the culture methods, facilities, and integration practices thus leading to increased productivity, resource use efficiency and reduced environmental impacts. According to FAO (2020), the production was 51.3 million tonnes of aquatic animals accounting for 62.5% of the worlds production. Coastal aquaculture is key in providing for livelihoods, employment and boosting local economy among the coastal communities as observed in many developing countries. Coastal aquaculture is highly advanced in Asia and Latin America in terms of expertise and support institutions for marine and coastal aquaculture. In Africa, most of the countries are far much behind despite significant efforts and projections of the initiative at both regional and national levels. Investment in infrastructural, technical support and policies is needed to support development of marine aquaculture in Africa. According to FAO (2020), the global coastal and mariculture production amounted to 30.8 million tonnes of aquatic animals.

In Africa, the progress of aquaculture has been slow due to institutional, biotechnical and economic factors (Hecht, 2006). In Kenya, several campaigns have been steered by the government to promote aquaculture growth. The first one was in 1960, “Eat More Fish Campaign”, which increased the participation of small-scale farmers and the production to around 1000 tonnes (Aloo and Ngugi, 2005). Another programme launched by the government to boost aquaculture uptake is the Economic Stimulus Programme (ESP) in 2009. Key targets of the programme were improving fish rearing facilities, establishment of research programmes, initiating training programs and construction of fishponds to farmers (Musa et al., 2012). Another key policy which is aiming at transforming the aquaculture sector is the Kenya Vision 2030, which has identified aquaculture as one of the flagship projects and aims to increase the production by 10 % annually achieving 450000 tonnes by the year 2030. In Kenya, fish farming has mainly focused on inland aquaculture of freshwater fish accounting entirely for its aquaculture production since the 2000’s, with earthen ponds commonly used culture systems due

to their cost-effectiveness (FAO, 2004). Tilapia forms the major cultured species in Kenya according to Ngugi et al. (2017).

Tilapiine species are euryhaline with some restricted to freshwater or low salinity water (Fridman, 2012). Nile tilapia (*Oreochromis niloticus*) dominates tilapia aquaculture due to its adaptability and fast growth rate (Shelton, 2002). Despite several reviews on salinity tolerance as highlighted by Fridman (2012), *O. niloticus* is not considered to be the most tolerant cultured tilapia species, however, according to Suresh and Lin 1992, *O. niloticus* offers a considerable potential for culture in low saline water. According to Loya and Fishelson (1969), the culture of euryhaline species in brackish water or marine systems could potentially offer cheap animal protein in areas with minimal freshwater resources and land mainly for agriculture. Along the Kenyan coast, 70% of aquaculture activities are done on a small scale and mainly by community groups who venture into brackish water aquaculture (Mirera, 2011). However, marine aquaculture has depended on wild seed collection due to lack of established hatcheries (Mirera, 2007), leading to the introduction of Nile tilapia in brackish environments. The ability of tilapia to grow and breed in saline environment (Watanabe et al., 1989a) could be a solution to the lack of seeds for stocking marine culture facilities.

A major concern of *O. niloticus* in pond culture systems is the rapid reproduction resulting to over-population. This in turn results to reduced growth rate, lower harvesting yields and higher chances of inbreeding. This has called for all-male tilapia production as a control of the reproductive activity and to realize better yields (Omasaki, 2017). Male tilapias grow faster than females as they have better feed conversion ratio and relatively higher survival (Angienda et al., 2010) and also have a faster growth since metabolic energy is channeled towards growth by benefiting from anabolism enhancing androgens (Tran-Duy et al., 2008; Angienda et al., 2010; Khater et al., 2017). All-male productions is made possible through treating fry with methyltestosterone hormone (Fuentes-Silva et al., 2013). Several studies on sex reversal techniques have been conducted and proven to be working (Gale et al., 1995, 1999). Mostly widely used hormone, 17α-Methyltestosterone (MT) (Singh et al., 2018) and common feeding technique of incorporating the MT hormone in feeds and administered to fry have been extensively established. However, due to the shortcomings of the feeding technique of health and environmental hazards (Gale et al., 1999), focus has been diverted to fry immersion in achieving

sex reversal (Torrans et al., 1988; Gale et al., 1995, 1999; Fitzpatrick et al., 1998; Srisakultiew and Kamonrat, 2013; Singh et al., 2018).

The growing popularity of tilapia among consumers and the ever increasing need to improve food production, impose the need to seek production alternatives to culture tilapia. Such is the use of saline environments and even marine waters. The popularity is due to its market acceptability and tolerance to a wide range of physico-chemical parameters (Balcazaar et al., 2002). Thus, the aim of this study was to do a comparative assessment of growth performance of sex reversed Nile Tilapia (*O. niloticus*) grown in freshwater and marine water.

1.1 Study Objectives

1.1.1 General Objective

To determine the influence of methyltestosterone hormone in sex reversal of Nile tilapia in fresh and marine water environment through fry immersion

1.1.2 Specific Objectives

1. To determine survival rate of fry after immersion in different concentrations of methyltestosterone hormone
2. To determine percentage sex inversion of Nile tilapia in fresh and marine water
3. To determine growth rate of sex reversed Nile tilapia in fresh and marine water

1.2 Research Questions

1. What is the effect of different concentrations of Methyltestosterone hormone on survival of Nile tilapia fry after immersion?
2. How effective is the fry immersion technique on sex reversal of Nile tilapia in freshwater and marine water?
3. How is the growth performance of sex reversed Nile tilapia cultured in freshwater and marine water?

1.3 Study Hypotheses

H1: Survival rate of fry is higher in control with significant differences among the MT treatments for freshwater and marine water

H2: Sex inversion is higher with high male percentage in the MT treatments compared to the controls, with significant differences among MT treatments in both freshwater and marine water

H3: Growth rate of sex reversed Nile tilapia is higher in the treatment with high MT concentration in freshwater compared to both the controls and marine water

1.4 Problem Statement

Aquaculture has a huge potential in poverty alleviation and promoting food security by enhancing economic growth through production and sale of aquaculture products. In Kenya, most of aquaculture practices are conducted in the upcountry regions with low or minimal uptake along the Kenyan coast. One of the possible reasons is the influence of and preference to marine food and cultural set up or bias towards freshwater fish, however, the region is highly characterized by poverty cases and limited access to freshwater. This has led to few groups organized into small-scale aquaculture practices in brackish waters, known as mariculture. However, this practice since its inception has faced numerous challenges with key ones being lack of established hatcheries for the provision of quality seeds and developed mariculture feeds. Most of its operation is dependent on tidal flushing and the supply of seeds being seasonal with collection from wild. The wild harvesting of the seeds has experienced decline in the quantities let alone the deteriorating quality of the same. This is highly attributed to the changing climate scenarios like heavy rains resulting to turbid waters and higher temperatures. The growing interest and need to provide for food security, nutrition and economic gains has pushed for other culture alternatives. Research has turned its focus into the euryhaline tilapia species, mainly *Oreochromis niloticus,* as it is easily available and ease of propagation within the region. This is also supported by the fact of early sightings of the same species growing in the brackish environments within the region's tidal creeks and riverine estuaries. However, a key challenge to realizing economic benefits from the target culture species is the higher fecundity and inbreeding capabilities of the target species. To counter this, an all-male sex culture is encouraged to realize the economic, food security and nutrition benefits of the practice through reduction of over production, enhanced growth and higher survival rate of the all-male tilapias. Thus, the aim of the study was to compare the growth performance of sex reversed Nile tilapia (*O. niloticus)* through fry immersion cultured in freshwater and marine water.

1.5 Justification

The growth of aquaculture is becoming of huge interest both from the farmers and scientific perspective. With the farmers aiming at realizing economic and nutrition growth while the scientists aiming on invention and innovations for aquaculture development. Several reviews have highlighted the great potential of achieving culture of target species under different environments. This has geared to trials in the aquaculture sector ranging from feeds to seeds. Advancement in equipment and facilities has made it possible for these great innovations and the thirst for knowledge. Culture techniques, such as attaining all-male sex culture, improved feeds formulation through proximate analysis, improved culture units and increased expertise has made it possible to achieve and justify any aquaculture undertaking. Several sex reversal techniques have been established to achieve all-male sex. Trials have been done on fry immersion and there is still more room to experiment on the same, and the results are worthy the technique. Achieving the aim of this study will steer and improve the uptake of the aquaculture initiative at the coastal region of Kenya, both the freshwater and the marine environment. This will help to improve on nutrition and promote food security and economic growth through employment opportunities and returns of the aquaculture production. Information and knowledge generated from the study will form a basis to further polish the findings and efficiency of the technique through testing for different parameters.

2.0 LITERATURE REVIEW

2.1 Kenyan Aquaculture

Aquaculture plays an important contribution to livelihoods, economic development and food security in Africa (Quagrainie et al., 2009). The effective start of aquaculture in most of sub-Saharan Africa was, in the 1950's, established through the colonial administrations. It is increasingly recognized that promoting aquaculture as a business could yield adequate and solid benefits from the sector, and thereby leading to its sustainable development (Kaliba et al., 2007). Like many countries in Africa, aquaculture production in Kenya has been low and stagnated over the past decades (Hetch, 2006). The slow progress of aquaculture growth in sub-Saharan Africa has been attributed to institutional, biotechnical, and economic factors (Hecht, 2006). Rural fish farming in Kenya dates to the 1940s and was popularized in the 1960's by the Kenya Government through the "Eat More Fish Campaign". The number of small-scale farmers increased and peaked at about 20000 in 1985, with annual production of slightly over 1000 tonnes (Aloo and Ngugi, 2005).

In 2010, the Government of Kenya launched an Economic Stimulus Program (ESP) to boost economic growth of the country (Musa et al., 2012). One of the targeted areas by ESP was improving fish farming through the renovation of government fish rearing facilities, establishment of research programs to determine best practices for pond culture, initiating intensive training programs for fisheries extension workers and construction of fish ponds to farmers (Musa et al., 2012). Kenyan aquaculture revolves around monoculture of Nile Tilapia (*Oreochromis niloticus*) or mixed culture with African Catfish (*Clarius gariepinus*) (Omasaki, 2017).

Kenya Vision 2030, which is a development programme in Kenya launched by the government covering the period from 2008 to 2030, has identified agriculture (crops, fisheries and livestock) as one of the key sectors to deliver the 10 percent annual economic growth rate envisaged under the economic pillar (GoK-ASCU, 2013). In recognition of its potential contribution to the economy, aquaculture has been designated as one of the flagship projects of Vision 2030. The overall objective is to increase the total fish production by 10 percent annually from the current 150000 tonnes to 450000 tonnes by the year 2030 (Ngugi et al., 2017).

Fish farming in Kenya has focused on inland aquaculture of freshwater fish, which accounts for nearly its entire aquaculture production since the 2000's (Ngugi et al., 2017),

contributing to 98 % of the total aquaculture (Opiyo et al., 2020). Land occupied by inland freshwater aquaculture is a negligible area generally found in crop-growing areas where aquaculture competes with other farming activities for resources (Ngugi et al., 2017). In a bid to alleviate poverty through increased food production and minimization of environment degradation, a major priority development need of the government has been low-cost aquaculture, which promises to increase availability of quality protein food to communities in the short term (FAO, 2004). Because of this policy drive, earthen ponds and dam aquaculture have been the dominant and preferred culture systems due to their cost-effectiveness (Ngugi et al., 2017).

Common culture systems used by small-scale farmers is the earthen pond system. The economic viability of pond tilapia culture is further enhanced by the warm year-round climate, suitable land, and availability of relatively large quantities of water in most areas. A major drawback of pond tilapia culture is the high risk of uncontrolled reproduction when effective measures are not in place (Ngugi et al., 2017). Most small-scale fishponds in Kenya are earthen ponds constructed manually. The common pond size is 300 m^2 with the depth varying from 45 cm to 110 cm. In places where temperature is suitable for tilapia farming, yet land resources are limited, farmers may construct ponds in soils with poor water retention capacity and use pond liners to prevent leaking. Most tilapia farmers in Kenya grow monoculture tilapia as the targeted species (Ngugi et al., 2017).

Tilapia farming in Kenya depends primarily on farm-made feeds since pelleted feeds are too expensive for most small-scale farmers (Ngugi et al., 2017) as is the general case for sub-Saharan Africa (El-Sayed, 2013). Feed's ingredients include oilseed cakes (cotton, soybean or sunflower), freshwater shrimp and or fishmeal as protein sources; energy sources include rice and wheat bran, corn, kitchen wastes and or vegetables. The ingredients are mixed at predetermined ratios by mechanical mixers (Munguti et al., 2014). Feed prices are about US $ 0.95 (KSh 80) per kilogram of pellet feed (30 – 32 % crude protein (CP)) and US $ 0.60 (KSh 50) per kilogram of powder feed (28 – 30 % CP) (Ngugi et al., 2017).

A shortage of good quality seeds has been a bottleneck for development of tilapia farming in Kenya (Ngugi et al., 2017). Tilapia farmers, mainly small-scale farmers, restock self-grown fingerlings which tend to be of low-quality seed. In 2011, there were 129 accredited fish hatcheries through the ESP programme where the government also provided capacity building to

improve on seed quality. Seed availability and quality has since improved with still challenges faced on stocking of self-produced fingerlings. Plans to curb this were put in place by offering quality seeds at an affordable price to the small-scale farmers (Ngugi et al., 2017).

At the Kenyan coast, most aquaculture activities are conducted in brackish water environments (Mirera, 2007) mainly conducted by communities organized into small scale groups (Mirera, 2007). The small-scale mariculture farmers have been doing the practice in earthen ponds, in open areas behind the mangrove systems, in intertidal areas (Mirera, 2019; Mwaluma, 2003). This is in practice to conserve the mangrove ecosystem while gaining economically from the mariculture practice (Mirera, 2019). The Kenyan mariculture has seen a stalling development since its inception three decades ago (Mirera, 2019; Troell et al., 2011). The initiative has not grown to fully exploit its ecological, economic and cultural value (Troell et al., 2011). With the activity mostly carried out by local communities (Mirera, 2007). Unsuccessful culture of the species has been seen in milkfish, crabs, oyster and mullets (Mirera, 2016; Mirera and Ngugi, 2009). With key of the challenge impairing its development identified as lack of hatcheries for marine seed production (Mirera, 2011).

2.2 Salinity Tolerance and Growth of Tilapiine Species

For many years it has been recognized that the culture of euryhaline fish species in brackish water or marine systems could potentially provide animal protein in areas where freshwater resources are limited (Loya and Fishelson, 1969) and land that is marginal for agriculture. In recent times, the rapidly increasing competition for freshwater for urbanization, industrialization and agricultural activities has limited the scope of freshwater aquaculture, especially in arid regions (Fridman et al., 2012). Due to the increasing lack of freshwater in the world, it is beneficial to culture tilapia stocks in brackish or saline rearing environments to ensure a source of cheap and high-quality animal protein into the future (Mateen, 2007). In general, it is well established that salinity conditions during incubation and rearing are highly relevant for embryonic development, affecting variables such as hatching rate, and even later causing a lower survival rate and deformities in larvae (Takuma et al., 2007), or affecting larval size, particularly when salinity is above the species tolerance, producing smaller fish when reared at higher salt concentrations (Fielder et al., 2005). Also, there is an increasing commercial interest in tilapia species or hybrids that can tolerate salinity and still exhibit acceptable growth

(Armas-Rosales, 2006). To date, the consumption of saltwater tilapia fish has increased because of their tasty flesh and not too strong fishy taste rather than freshwater tilapia (Hassan et al., 2013).

Tilapias are popular cultured species because of their high environmental tolerant characteristics (Sallam et al., 2017). The ability to grow under sub-optimal nutritional conditions, and high fecundity, make them well suited for aquaculture (Lawson and Anetekhai, 2011). Tilapia is one of the important fish species which has several good qualities and can face wide range of salinity and other environmental conditions and can grow well in water salinities ranging from 11 parts per million (ppm) to 29000 ppm, tolerate temperatures between 8 °C to 42 °C and can survive in low dissolved oxygen (DO) levels (0.1 ppm) (Pullin and Lowe-McComell, 1982). *Oreochromis mossambicus* and its hybrids, including red tilapia are the major representatives of these euryhaline cichlids in aquaculture (Tayamen et al., 2002).

Numerous reviews of salinity tolerance for various cultured tilapias have been published such as Balerin and Hatton (1979), Chervinski (1982), El-Sayed (2006), Prunet and Bournancin, (1989), Stickney (1986) and Suresh and Lin (1992). The Nile tilapia (*O. niloticus*), which has now extended well beyond its natural range, dominates tilapia aquaculture because of its adaptability and fast growth rate (Macintosh and Little, 1995; Shelton, 2002). Although *O. niloticus* is not considered to be amongst the most tolerant of the cultured tilapia species, it still offers considerable potential for culture in low-salinity water (Stickney, 1986; Suresh and Lin, 1992). Feasibility studies for rearing tilapiine species have been conducted with rearing red hybrid tilapia in brackish and seawater first studied by Liao and Chang (1983) who reported good growth of Taiwanese red tilapia *(O. mossambicus* and *O. niloticus)* at salinities of 17 parts per thousand (ppt) and 37 ppt, although fish appeared susceptible to handling stress. Seawater-rearing studies of Taiwanese red tilapia in Kuwait (Hopkins et al., 1985) showed that survival at 38 - 41 ppt was impaired at water temperatures below 24 °C. Following a preliminary study which showed higher growth and feed conversion of juvenile, monosex males in brackish and seawater than in freshwater (Watanabe et al., 1988), detailed studies on culture methodology were initiated (Watanabe et al., 1989a).

Limited information on the effect of salinity on growth of tilapia is available (Watanabe et al., 1989a). In general, optimal ranges of salinities for growth have been inferred from natural distribution data or fragmented experimental evidence. According to Watanabe et al. (1988),

when they investigated growth of Florida red tilapia strain, they found out that the strain had faster growth rates in brackish and sea water as compared in freshwater with modifications in stocking density. At intermediate densities, 15 fish per tank, there was increased growth with salinity due to increased feed consumption and declining feed conversion ratios with salinity (Watanabe et al., 1988). Their findings are in line with previous studies that there is faster growth in brackish and seawater in certain tilapias including *O. mossambicus* (Canagaratnam, 1966; Jurss et al., 1984) and Taiwanese red tilapia hybrids *(O. mossambicus* and *O. niloticus)* (Liao and Chang, 1983).

Based on the growth performance in saltwater, *O. mossambicus* and red tilapia are competent strains for breeding tilapia in saltwater (Tayamen et al., 2002). While the suitability of the Florida red tilapia strain for seawater grow-out has been demonstrated by high growth rates and feed conversion efficiencies, the hatchery phase of production remains restricted to water of lower salinities. The need for low-salinity water for maintaining brood stock and fry, thus affecting the ability of farmers to obtain fingerlings, restricts the establishment of future hatcheries in low-salinity water areas. Methods for seawater adaptation have been developed that minimize reliance on low-salinity water during the hatchery phase of production and that maximize survival and growth following transfer to seawater (Watanabe et al., 1989a). Other tilapias are generally less euryhaline and can tolerate water salinities ranging from about 20 to 35 ‰. Most of these tilapias grow, survive, and reproduce at 0-29 ‰, depending on the species and acclimation period (Sallam et al., 2017).

2.3 Tilapia Culture in Marine Water

Whilst the overall proportion of aquaculture production taking place in brackish water has decreased over the past decade, there has been a significant increase in the production of tilapia in brackish water reflecting a paucity of finfish species well suited to this environment (Kamal and Mair, 2005). FAO (2002) report tilapia production in brackish water rose from just 65,989 metric tonnes in 1996 to 190,176 metric tonnes in 2001, increasing from 8.1 % to 13.7 % of total global tilapia production. This increase in brackish water production of tilapia is in large part due to the production of tilapia in abandoned shrimp ponds or more recently in polyculture with shrimp (Fitzsimmons, 2001). The introduction of tilapia to shrimp production systems has shown considerable potential for improving the overall productivity of brackish water systems

and has been shown to bring about an increase in shrimp yields in some cases (Anggawa, 1999; Fitzsimmons, 2001). As a result, there is a growing interest in the identification of tilapia strains well suited to brackish water salinities (Kamal and Mair, 2005).

The potential of tilapia culture in saline environments is significant (Celik, 2012). There is an increasing interest (Balcazaar et al., 2002) in many coastal areas of the world, particularly arid regions, to culture tilapia in marine habitats (Cnaani et al., 2011). Therefore, tilapia producers must obtain and develop a tilapia hybrid capable of surviving and growing well in seawater culture facilities (Baroiller et al., 2000) and to utilize deserted marine shrimp farms (Ostrensky et al., 2000). Most commercially cultured tilapia species are suitable for production in fresh and low-salinity waters only, as they vary considerably with respect to salinity tolerance (Suresh and Lin, 1992; Avella et al., 1993). Although tilapias are well known examples of fresh water, some strains are euryhaline and able to tolerate high salinity values (Celik, 2002). However, there are some serious limitations for commercial tilapia production in saline waters. For instance, *Oreochromis spilirus* has been reported to have low fecundity (Al-Ahmed, 2001). In addition, *O. niloticus* x *O. mossambicus* hybrid has failed to adapt at 35 ‰ (Alfredo and Hector, 2002). The Nile tilapia, *O. niloticus*, has lower salinity tolerance than other tilapia species such as *O. mossambicus* (Villegas, 1990) and does not tolerate salinities above 20 g L^{-1}. Some tilapia species are euryhaline and tolerant to a wide range of salinities and can grow and reproduce in full- strength seawater after proper acclimation. Among the tilapia species known to tolerate and thrive successfully in full-strength seawater (salinities 38 g L^{-1}) are *Tilapia zilli* (Chervinski and Hering, 1973), the biparental tilapia, *Sarotherodon melanotheron*, which can survive salinities up to 120 g L^{-1} (Baroiller et al., 2000), and the maternal mouth brooders such as *O. mossambicus* (Villegas, 1990) and *O. spilurus* (Hopkins et al., 1989; Cruz and Ridha, 1990, 1994; Deguara and Agius, 1997; Jonassen et al., 1997).

2.4 Sex reversal and Fry Immersion Technique

Nile Tilapia is a prolific breeder attaining sexual maturity within 60 days (Arriesgado, 2011). This gives them the ability to breed frequently resulting in inbreeding, stiffer competition for food and space thus resulting in smaller fish size (Arriesgado, 2011), lower yields of harvestable fish (Omasaki, 2017). This has prompted for all-male sex production. To achieve all-male Nile Tilapia, several techniques have been employed such as manual sexing, hybridization,

genetic manipulation and hormonal sex reversal (Singh et al., 2018). All-male tilapia production offers advantages of enhanced growth (Abdelhamid et al., 2009), better food conversion ratio, relatively high survival (Tran-Duy et al., 2008; Angienda et al., 2010) and prevention of unwanted reproduction in aquaculture (Fitzpatrick et al., 1999). It is well known that anabolic steroids may produce fish with increased weight gains and muscle deposition (Sambhu and Jayaprakas, 1997). Since it increased the feed digestion and absorption rate causing increase in body weight (Yamazaki, 1976). It increased the proteolytic activity of the gut leading to increase the growth rate (Lone and Matty, 1981). It may also promote the release of growth hormone (Higgs et al., 1976). Little et al. (2003) came to the same conclusion, where sex-reversed tilapia grew better and economic than the non-sex-reversed fish. El-Saidy (2005) observed that the growth in weight and length was higher significantly in mono-sex male compared with mono-sex female and normal mixed sex Nile tilapia.

Several androgens have been applied and proven to masculinize various tilapia species including methyltestosterone (MT) (Pandian and Varadaraj, 1990 for *O. mossambicus*); mibolerone (Torrans et al., 1988 with *O. aureus*); fluoxymesterone (Phelps et al., 1992 with *O. niloticus*); norethisterone acetate (Varadaraj, 1990 with *O. mossambicus*); 17-α-ethynyltestosterone (Shelton et al., 1981 with *O. aureus*); 17a-methylandrostendiol (Varadaraj and Pandian, 1987 with *O. mossambicus*), and trenbolone acetate (TBA) (Galvez et al., 1996 with *O. niloticus*). MT is widely used in the production of all-male population in aquaculture, especially *Oreochromis* spp due to their precocious sexual maturity and high reproductive efficacy (Singh et al., 2018).

Use of steroid hormones as sex reversal agents has a long history through feed incorporation. Tilapia fry are normally fed for 21 - 28 days. Successful sex inversion is observed in this method; however, several inefficiencies raise a concern (Gale et al., 1999). The dosage per fish varies due to differences in body sizes and social status of the fish which results to partial or incomplete sex reversal which compels the culturist to increase the dosage to achieve 100 % inversion (Gale et al., 1999). The long period of treatment with the hormone in feeding increases the higher human chances interaction given the tumorigenic and teratogenic effects of anabolic androgenic steroids (Lewis and Sweet, 1993). Proper handling measures could mitigate the risk which is not the case in developing countries. Torrans et al. (1988) described an immersion technique for masculinization of blue tilapia, using synthetic androgen mibolerone

(MB), the method still had 5 days of exposure. The technique of fry immersion is well developed in salmonid aquaculture as described by Piferrer and Donaldson (1989) and Feist et al. (1995). Gale et al. (1995, 1999) demonstrated that fry immersion in three hours in 17-α-methyldihydrotestosterone (MDHT) in two days resulted in masculinization of Nile Tilapia.

The fry immersion is gaining momentum since shorter exposure periods by workers and the steroid will be contained for controlled filtration or biodegradation thus reducing environmental and health risks (Phelps et al., 1999). For *O. aureus*, immersion of fry in mibolerone at 0.6 mg L^{-1} for five weeks resulted in populations that were 82% male (the remaining fish were intersexual), and a 0.3 mg L^{-1} mibolerone immersion for five weeks resulted in less than 1 % functional females (Torrans et al., 1988). Immersion of *O. mossambicus* in 17-α-methylandrostendiol at 5 mg L^{-1} for 11 days beginning at seven- or ten-days post hatching caused 100% masculinization (Varadaraj and Pandian, 1987). Fitzpatrick et al. (1998) were able to produce greater than 90% male populations of *O. niloticus* when trenbolone acetate was administered as a 2-hour bath on days 11 and 13 post-fertilization. Singh et al. (2018) was able to demonstrate highest percentage of male populations following fry immersion in 300 µg L^{-1} MT solution for 12 hours thus concluding a significant increase in male population with increasing dose. According to Srisakultiew and Kamonrat (2013), MT immersion significantly increased the percentage of male ($P<0.05$) at 500 µg L^{-1} while Gale et al. (1995) achieved a 93 % male population after fry immersion at 500 µg L^{-1} MT.

3.0 METHODOLOGY

3.1 Study Site

The study was carried out in Kilifi County (Field set up) and Mombasa County (Laboratory set up) in Kenya during the period from November 2020 to January 2021. The marine water tilapia field set up was conducted in a fish farm belonging to one of the community groups (Kibokoni community) established as small-scale mariculture farm, practising culture of tilapia under brackish water environment in Kibokoni, Kilifi County. The marine water setup was to be undertaken as it had earlier been planned, however due to technical hitches, it was partly conducted (sex reversal part) in a laboratory setup. Freshwater field set up was conducted in a private fish farm (Bamba Water Company), Mtwapa, Kilifi County. The laboratory set up for fresh and marine waters was carried out at Kenya Marine and Fisheries Research Institute (KMFRI), Mombasa (Figure 1).

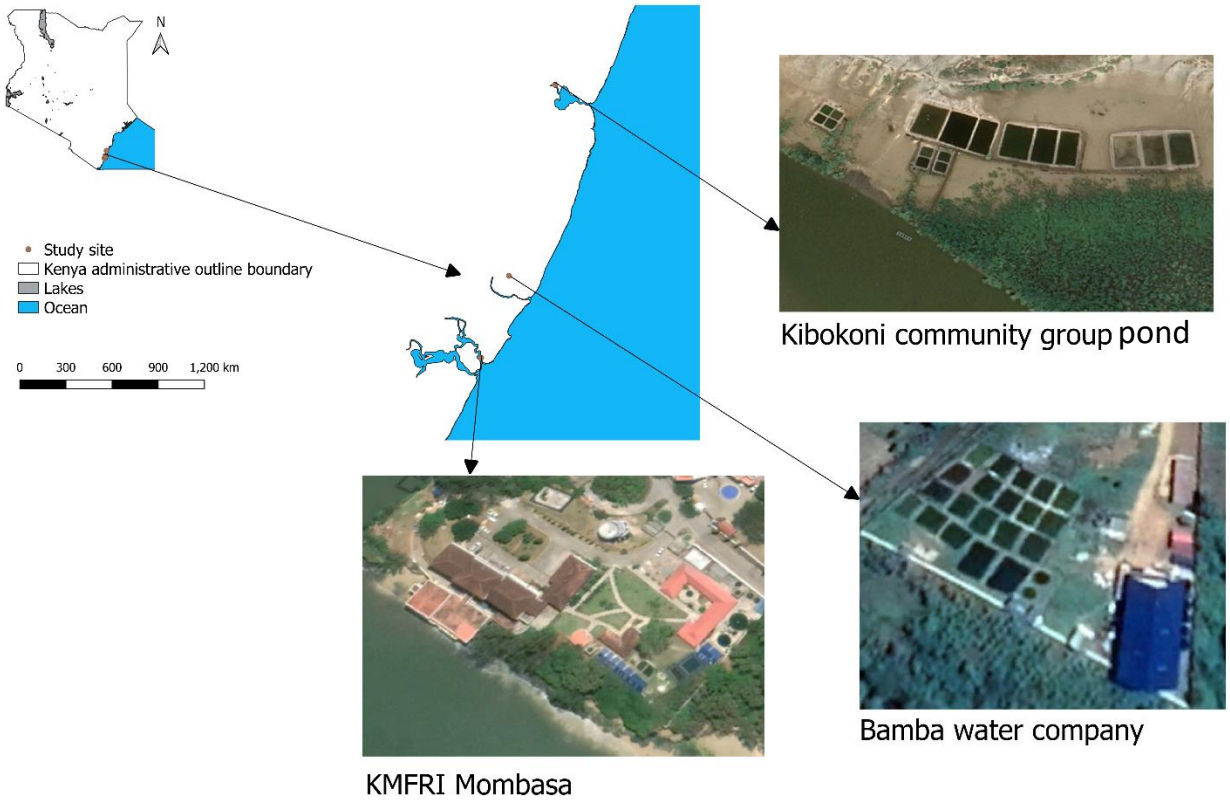

Figure 1: Map showing study sites

3.2 Laboratory set up

3.2.1 Breeding

Nile tilapia brood stock for marine water setup were selected from grow out farm at Kibokoni. Freshwater Nile tilapia brood stock were sourced from Bamba water company. The brood stocks were bred in hapa nets (1 m^3) at a ratio of three females to one male. Monitoring was conducted at KMFRI for egg production and hatching.

3.2.2 Fry immersion technique

Sex reversal was conducted through the fry immersion technique. A solution of 17-α-methyltestosterone (MT) at different concentrations (0, 100 and 400 µg L^{-1}) was used. The different concentrations served as treatments; C-Control, 0 µg L^{-1} (immersed in water), T1-Treatment one, 100 µg L^{-1} and T2-Treatment Two, 400 µg L^{-1} of MT (Figure 2). 0.5 g and 2 g of MT for T1 and T2 respectively were dissolved in water (5 L) and aerated for 30 minutes. The fry were immersed in the MT solution for 3 hours on the first and third day after egg yolk absorption. The fry were then cleaned with fresh clean water (freshwater and marine water), put in aquaria, and held on a standard water volume of 15 L in quintuplicate of each treatment. The fry were fed on *Artemia* and supplemented on *omena* dust. 1 ml of formalin was added to the solution after immersion for disposal.

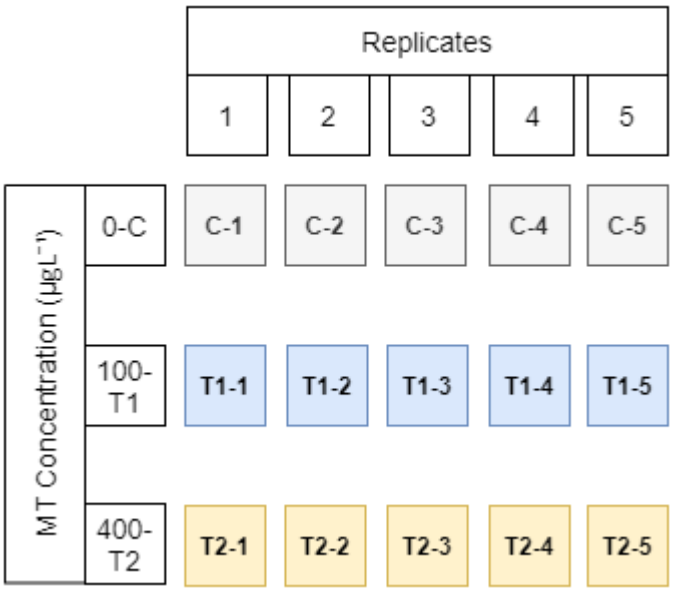

Figure 2: Experimental design for sex reversal of fry by immersion at different MT concentration

3.3 Field set up

On a field set up, a liner pond of 14 m by 8 m was used for freshwater culture and an earthen pond of 15 m by 10 m was to be used for the marine water culture. 15 hapa nets of 1 m^3 were randomly installed (Figure 3) to facilitate feeding, sampling, and monitoring of the culture species. 40 fingerlings of total weight 20 g were grown randomly per m^3 and monitored as described in section 3.3.2 - 3.3.4.

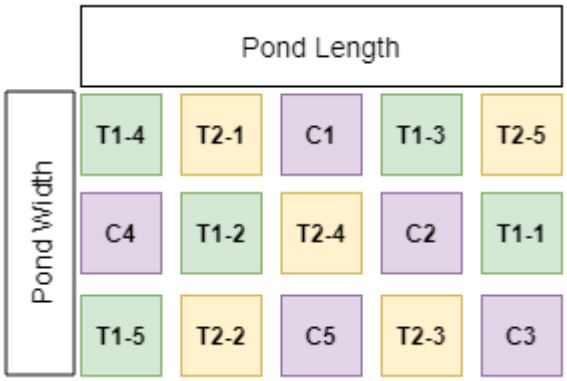

Figure 3: Randomized field hapa nets installation

3.3.1 Feeding

Feeding was done three times a day (10 am, 12 pm and 4 pm) on the total weight 20 g fingerlings at 5 % body weight with weekly weight adjustments. They were fed on commercial feeds obtained from Unga feeds with a crude protein content of 35 %.

3.3.2 Growth Parameters

Fish biological parameters that were under investigations during the culture period include;

a. Length – weight measurements

Sampling for fish length (cm) and weight (g) was conducted biweekly for the laboratory setup while for the field setup, it was conducted on a weekly basis. The obtained measurements were then used for determination of growth parameters with the below given formulas.

b. Weight gain

Weight gain (WG) was determined as follows,

$$WG = W_f - W_i$$

Where WG = weight gained (g), W_f = mean final fish weight (g), W_i = mean initial fish weight (g).

c. Survival Rate

Survival rate was determined as percentage using the following formula (Khater et al., 2017):

$$SR = \frac{Number\ of\ fish\ at\ the\ end\ of\ experiment}{Total\ number\ of\ fish\ stocked} \times 100$$

Where SR = Survival rate as a percentage

d. Specific Growth Rate

Specific growth rate (SGR) was determined with the following formula (Khater et al., 2017),

$$SGR = \frac{\ln W_f - \ln W_i}{t} \times 100$$

Where SGR = specific growth rate (% or g day^{-1}), ln = natural logarithm, W_f = mean final fish weight (g), W_i = mean initial fish weight (g), and t = time (days).

e. Feed conversion ratio

Feed conversion ratio (FCR) was determined through the following formula (Khater et al., 2017),

$$FCR = \frac{FI}{WG.Nt}$$

Where FCR = feed conversion ratio (g feed g^{-1} fish weight), FI = feed intake (g), WG = weight gained (g), and Nt = final number of fish in hapas.

f. Length-weight relationship

Length-weight relationship was determined using the formula (Le Cren, 1951),

$$W = a * TL^b$$

Where W = fish body weight (g), TL = total length (cm), a = the regression intercept, b =slope of the regression line

g. Relative condition factor

Relative condition factor (Kn) was determined according to Le Cren (1951) as follows,

$$Kn = \frac{W}{a * TL^b}$$

Where *Kn* = relative condition factor, W = weight of fish (g), TL = total length of fish (cm) *a* (intercept) and *b* (slope) are constants of regression equation.

3.3.3 Sex Determination

Towards the end of the feeding period, the fish from each treatment were individually examined for sex through genital papilla examination. The percentage male was determined through dividing the number of male fish by the total stocked fish and multiplied by 100.

Figure 4: Female sex genital papilla

Figure 5: Male sex genital papilla

3.3.4 Water quality monitoring

Water parameters monitored during the study include salinity, water temperature, pH, dissolved oxygen, and ammonia to ascertain the limnology of the culture unit. Sampling for water quality was done weekly in the morning and afternoon hours.

3.4 Data Analysis and Presentation

Survival rate, specific growth rate, weight gain, feed conversion rate, relative condition factor and length-weight relationship were determined based on the formulas given and results presented in tables and graphically. The results are presented as means and standard errors of the mean.

3.5 Statistical Analysis

Means of growth parameters, feed utilization parameters, survival rate and sex reversal were treated using One-way Anova (Analysis of variance) and Tukey's HSD test at 95 % confidence interval and at a significance level of $P<0.05$. Control, treatments and setups were treated as independent (grouping) variables, while the different parameters taken as dependent variables. Two-way Anova was used to compare the performance of the setups during the laboratory phase. IBM Statistical software for social sciences version 26 and R statistical software version 4.0.3 was used for the analysis.

4.0 RESULTS

4.1 Summary of descriptive statistics

The laboratory phase was conducted for 28 days for both the freshwater and marine setup. The marine experiment was faced with challenges of huge mortalities and limited production of fries before conducting the immersion technique. This restricted the number of replications to three but maintained the sampling size (n = 40). Thus, in the analysis, randomly selected three replications from the freshwater setup were used for comparison with the marine setup. Freshwater setup had a high mean length of 2.103 ± 0.055 cm in T2 while the marine setup had 2.160 ± 0.031 cm in T2. Highest mean weight was recorded in T1 (0.172 ± 0.001 g) and T2 (0.189 ± 0.002) in freshwater and marine setup respectively. SGR was highest in T2 in both the freshwater setup (1.953 ± 0.079 % day^{-1}) and marine setup (1.630 ± 0.069 % day^{-1}). FCR was lowest in T2 in both the freshwater (0.198 ± 0.010) and marine setup (0.234 ± 0.010). Percentage survivals were similar in the freshwater setup (100 ± 0.000 %), while T1 in the marine setup showed the highest survival rate (5.833 ± 2.2.05 %) (Table 1).

The field phase was only conducted for the freshwater setup since the marine setup broke down due to technical problems encountered during the laboratory phase. The freshwater was run for 49 days with an initial stocking mean weight for the treatments and control as follows; C (0.536 ± 0.040 g), T1 (0.669 ± 0.039 g) and T2 (0.542 ± 0.021 g). At the end of 49 days of the experiment, the mean weight was as follows; C (7.936 ± 0.249 g), T1 (8.452 ± 0.171 g) and T2 (8.635 ± 0.258 g), mean length; C (9.032 ± 0.063 cm), T1 (9.428 ± 0.160 cm) and T2 (9.560 ± 0.235 cm) (Table 2). Percentage survival was uniform across C and both treatments at 100.00 ± 0.00 % (Table 2).

Table 1: Laboratory phase, Descriptive statistics of mean growth parameters and their standard error of the mean for the freshwater and marine setup (28 days)

Setup	Treatment	Length	Weight	WG	SGR	FCR	% S
Marine	C	1.980 ± 0.042	0.160 ± 0.000	0.046 ± 0.000	1.184 ± 0.007	0.320 ± 0.002	0.000 ± 0.000
	T1	2.120 ± 0.031	0.178 ± 0.002	0.062 ± 0.004	1.542 ± 0.109	0.249 ± 0.018	5.833 ± 2.205
	T2	2.160 ± 0.031	0.189 ± 0.003	0.069 ± 0.003	1.630 ± 0.069	0.234 ± 0.010	3.333 ± 2.205
Freshwater	C	2.093 ± 0.029	0.158 ± 0.000	0.056 ± 0.002	1.558 ± 0.062	0.245 ± 0.001	100 ± 0.000
	T1	2.100 ± 0.012	0.172 ± 0.001	0.067 ± 0.004	1.748 ± 0.118	0.220 ± 0.014	100 ± 0.000
	T2	2.103 ± 0.055	0.161 ± 0.002	0.068 ± 0.002	1.953 ± 0.079	0.198 ± 0.008	100 ± 0.000

Table 2: Field Phase, Descriptive statistics of mean growth parameters and their standard error of the mean for the freshwater setup (49 days)

Treatment	Length	Weight	Kn	WG	SGR	FCR	% S	%_Male
C	9.032 ± 0.063	7.936 ± 0.249	1.005 ± 0.002	7.399 ± 0.228	5.517 ± 0.126	3.273 ± 0.090	100.00 ± 0.00	58.5 ± 5.280
T1	9.428 ± 0.160	8.452 ± 0.171	1.006 ± 0.001	7.782 ± 0.170	5.188 ± 0.123	3.277 ± 0.074	100.00 ± 0.00	87.0 ± 2.0
T2	9.560 ± 0.235	8.635 ± 0.258	1.005 ± 0.001	8.093 ± 0.257	5.651 ± 0.101	3.039 ± 0.098	100.00 ± 0.00	91.5 ± 1.275

4.2 Survival Rate

During the laboratory phase, freshwater setup had a survival rate of 100 % across its control and treated groups (Table 1). For the marine water setup, survival rate was highest in T1 (5.833 ± 2.205 %) and lowest in T2 (3.333 ± 2.205 %), there were no survivals in C (0.000 ±

0.000 %) (Table 1). Survival rate showed no significant differences between C, T1 and T2 (ANOVA, $F = 2.643$, $df = 2,6$, $p = 0.150$) (Figure 6).

The field phase for the freshwater setup had a survival rate of 100 % (Table 2).

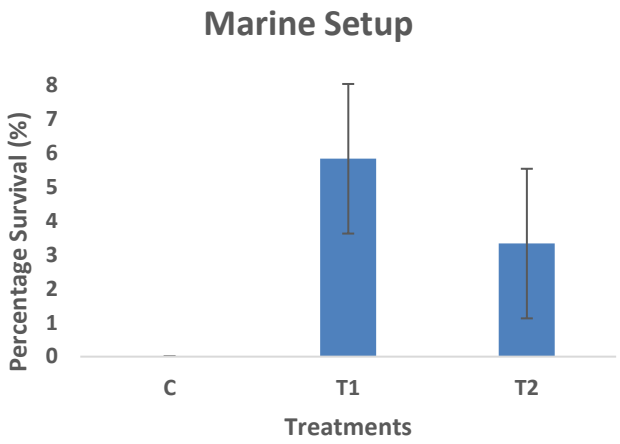

Figure 6: Laboratory phase, Percentage survival for marine setup

4.3 Sex Inversion

During the freshwater field phase, T2 had the highest percentage of sex inversion (91.5 ± 1.275 % male) with C having the lowest inversion (58.5 ± 5.28 % male). T1 had an inversion of 87 ± 2 % male (Table 1). The sex inversion was significant between the C and the treatments (ANOVA, F (2,12) = 28.679, $df = 14$, p< 0.005). After conducting a post hoc test, Tukey's HSD, mean for C was significantly different with both T1 and T2 ($p < 0.005$). There were no significant variations in the means of T1 and T2 ($p = 0.619$) (Figure 7).

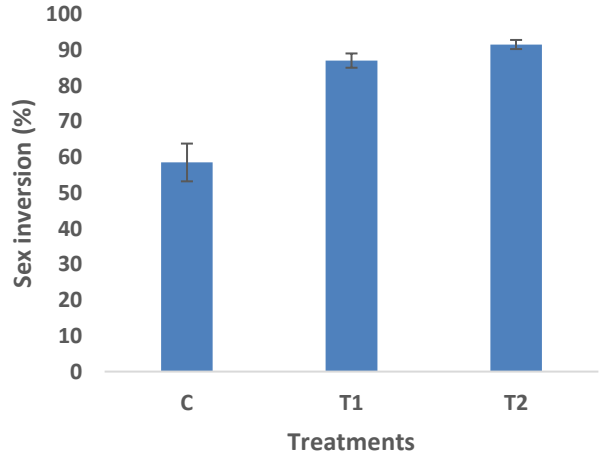

Figure 7: Field phase, Sex inversion for freshwater setup

4.4 Growth parameters

4.4.1 Water quality

Water quality parameters monitored during the culture period are presented in table 4.

Table 3: Mean water quality values with standard error during field phase of freshwater setup (49 days)

	pH	Temperature (° C)	DO (mgL^{-1})	NH_3 ($mg\ L^{-1}$)	Conductivity ($\mu S\ cm^{-1}$)
Morning	7.6 ± 0.044	29.1 ± 0.073	7.368 ± 0.096	0.010 ± 0.000	957.429 ± 1.937
Evening	7.8 ± 0.049	32.4 ± 0.207	8.381 ± 0.113	0.067 ± 0.011	984.095 ± 2.137

4.4.2 Length - Weight Relationship

In the freshwater setup, C, *b* values ranged from 1.804 – 2.353 and R^2 values were in the range of 0.472 – 0.618. Three of the replicas in C had R^2 values above 50 %. In T1, b values ranged from 1.448 – 2.726 and R^2 values in the range of 0.497 – 0.766. Four of the replicas in T1 had R^2 values above 50 %. In T2, b values ranged from 1.924 – 3.007 and R^2 ranged from 0.631 – 0.840 with all the replicas above 60 % (Table 4).

Table 4: Length-weight relationship co-efficient for the field phase of the freshwater setup (49 days)

Treatment	Replica	a	b	R^2
C	C-1	0.066	2.353	0.576
	C-2	0.070	2.330	0.618
	C-3	0.110	2.091	0.517
	C-4	0.117	2.091	0.382
	C-5	0.204	1.804	0.472
T1	T1-1	0.035	2.636	0.766
	T1-2	0.121	2.068	0.542
	T1-3	0.207	1.804	0.563
	T1-4	0.027	2.726	0.497
	T1-5	0.473	1.448	0.731
T2	T2-1	0.151	1.924	0.688
	T2-2	0.068	2.342	0.631
	T2-3	0.046	2.553	0.823
	T2-4	0.076	2.300	0.638
	T2-5	0.018	3.007	0.840

4.4.3 Weight gain

During the laboratory phase, the freshwater setup had a high mean weight gain of 0.068 ± 0.002 g in T2 and low in C, 0.056 ± 0.002 g. The means weight gain was distributed around the median with some minor variances in T1 and T2 (Figure 8a). There were significant differences in the mean weight (ANOVA, F = 5.466, df = 2,6, p = 0.045). After conducting a post hoc test (Tukey's HSD), C and T2 mean weights varied significantly (p = 0.056) (Figure 8a). In the marine setup, mean weight gain was highest in T2 (0.069 ± 0.003 g) and lowest in C (0.046 ± 0.000 g). Distributions of the means were concentrated in the lower quartile with minimal variations (Figure 8b). There were significant differences in the means of weight gain (ANOVA, F = 16.424, df = 2,6, p = 0.004). Post hoc test (Tukey's HSD) was run on the means to determine variations. C was different with T1 (p = 0.018) and T2 (p = 0.003) (Figure 8b).

For the field phase, T2 had the highest weight gain (8.093 ± 0.257 g) and C had the lowest weight gain (7.399 ± 0.228 g). C and T2 had their mean weight gain distributed in the upper quantile while T1 had its means concentrated in the lower quantile range (Figure 9). There were no significant differences between the C and the treatments (ANOVA, F (2,12) = 2.461, df = 2,12, p = 0.127) (Figure 9).

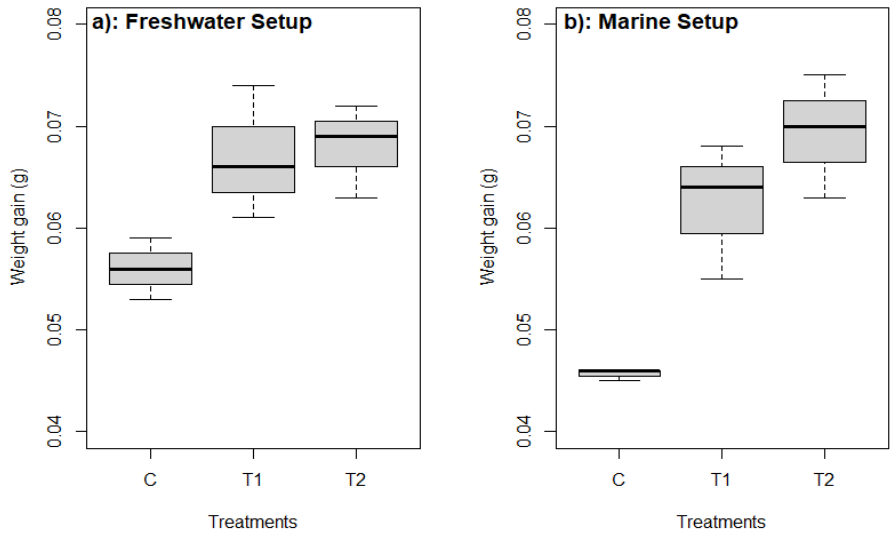

Figure 8: Laboratory phase, Weight gain for freshwater and marine setup (28 days)

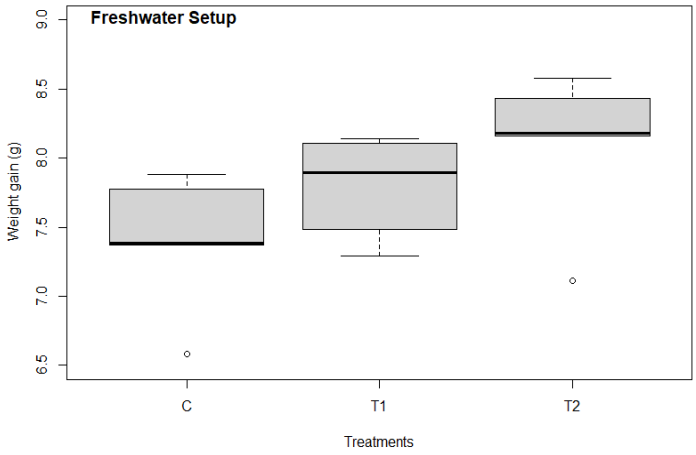

Figure 9: Field phase, Weight gain for freshwater setup (49 days)

4.4.4 Specific Growth Rate

During the laboratory phase, freshwater had a higher SGR in T2 (1.953 ± 0.008 % day^{-1}) and lowest in C (1.558 ± 0.062 % day^{-1}). The mean SGR were distributed in the upper quartile in both T1 and T2 as compared to the C (Figure 10a). Significant differences were found (ANOVA, F = 4.879, df = 2,6, p = 0.055). Tukey's HSD showed significant differences between C and T2 (p = 0.047) (Figure 10a). For the marine water setup, mean SGR was highest in T2 (1.630 ± 0.069 % day^{-1}) and lowest in C (1.184 ± 0.007 % day^{-1}). T2 had slight distributions in the upper quartile while C had uniform distribution of the SGR means whereas T1 in the lower quantile (Figure 10b). SGR showed significant differences in the marine setup (ANOVA, F = 10.056, df = 2,6, p = 0.012). After conducting a Tukey's HSD test, the means of C with T1 (p = 0.033) and T2 (p = 0.013) varied (Figure 10b).

In the freshwater setup, T2 had the highest SGR (5.651 ± 0.101 % day^{-1}) with T1 having the lowest SGR (5.188 ± 0.123 % day^{-1}), C had an SGR of 5.517 ± 0.126 % day^{-1}. C had uniform distribution while T1 had its SGR means concentrated in the upper quantile and T2 in the lower quantile (Figure 11). There were significant differences among C and the treatments (ANOVA, F = 4.136, df = 2,12, p = 0.043). Tukey's HSD was conducted to determine variations in the means. T1 and T2 means were varying (p = 0.040) however, there were no significant differences between C and means of T1 (p = 0.159) and T2 (p = 0.703) (Figure 11).

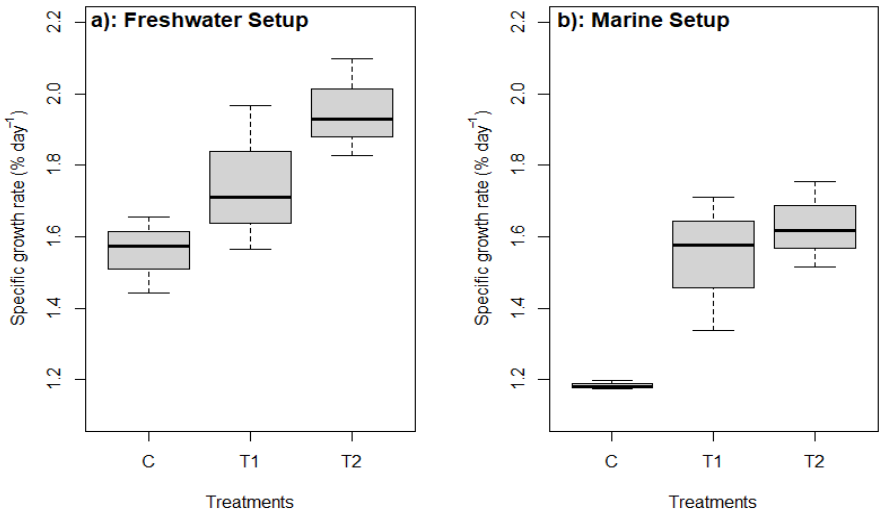

Figure 10: Laboratory phase, Specific growth rate for freshwater setup and marine setup (28 days)

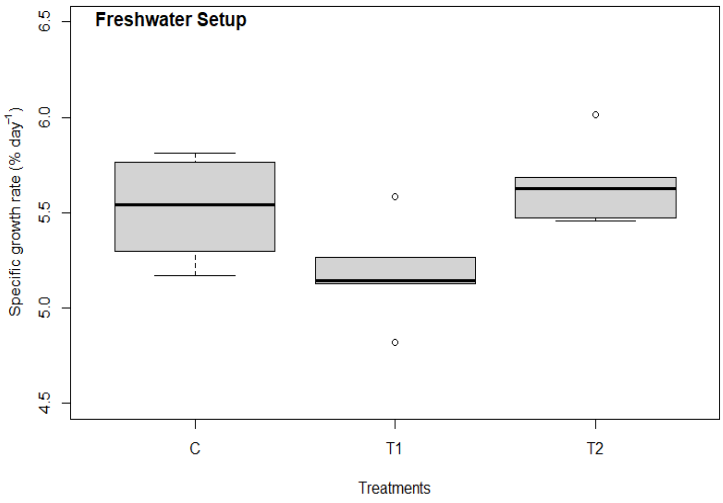

Figure 11: Field phase, Specific growth rate for freshwater setup (49 days)

4.4.5 Feed Conversion Ratio

In the laboratory phase, freshwater had best FCR in T2 (0.198 ± 0.008). For T1 and T2, their FCR values were distributed in the lower quartile as compared to C (Figure 12a) Significant differences were found (ANOVA, F = 2.649, df = 2,6, p = 0.055) between C and treated groups. Tukey's HSD showed significant differences between C and T2 (p = 0.047) (Figure 12a). For the marine water setup, mean FCR values were best in T2 (0.234 ± 0.010). T2 had uniform distribution of the FCR mean values while T1 had much concentrations in the upper quantile and C in the lower quantile (Figure 12b). FCR was significantly different between C, T1 and T2 (ANOVA, F = 15.103, df = 2,6, p = 0.005). Post hoc Tukey's HSD test showed mean variations of C with T1 (p = 0.013) and T2 (p = 0.005) (Figure 12b).

In the freshwater setup, T1 had the highest FCR (3.277 ± 0.074) followed by C (3.273 ± 0.09), and T2 had the lowest FCR (3.039 ± 0.098). C and T1 had their means of FCR distributed in the lower quantile while T2 was distributed on the upper quantile (Figure 13). There were no significant differences among the C and the treatments (ANOVA, F = 2.649, df = 2,12, p = 0.111) (Figure 13).

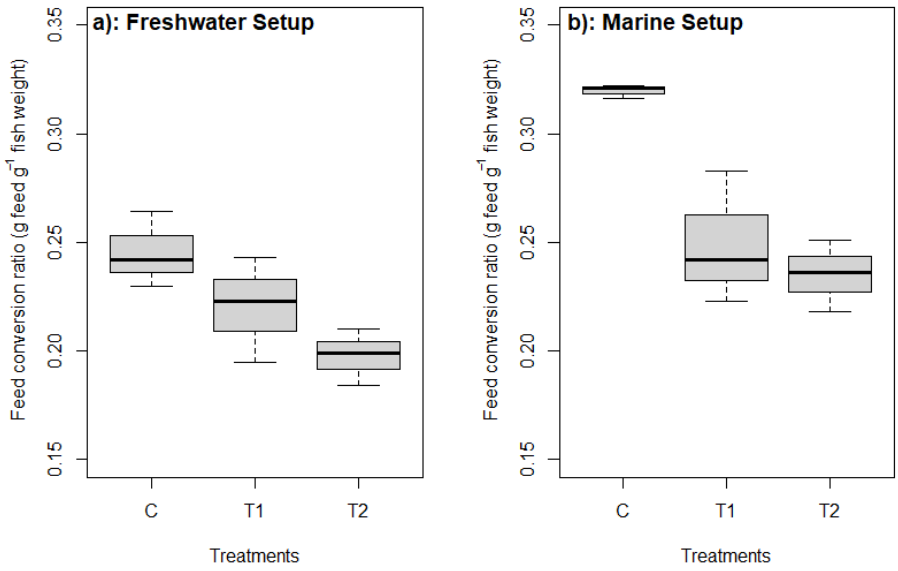

Figure 12: Laboratory phase, Feed conversion ratio for freshwater setup and marine setup (28 days)

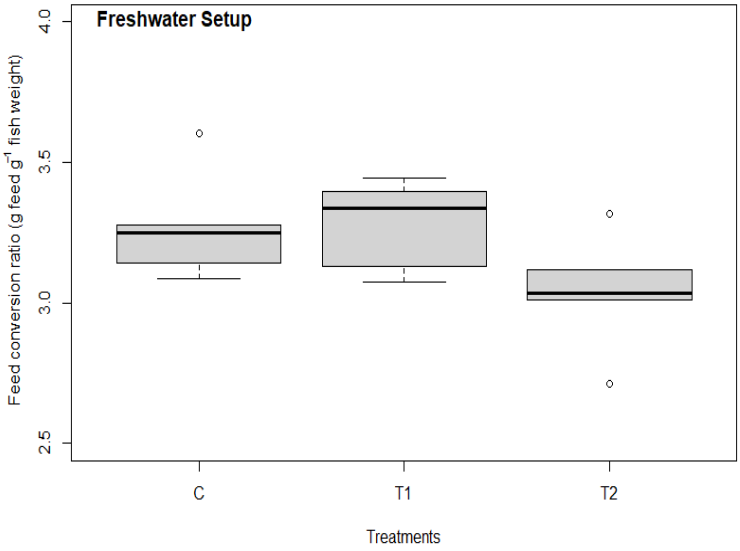

Figure 13: Field phase, Feed conversion ratio for freshwater setup (49 days)

4.5 Setups Performance

2-way ANOVA was used to compare the two setups during the laboratory phase at a setup and treatment level with the former and latter serving as independent factors. The growth parameters served as dependent variables. The results of the test are presented as; interaction effect (between setup and treatments), setup and treatments.

4.5.1 Interaction effect

Interaction effects between the two setups (freshwater and marine) and the treatments were conducted in a 2-way ANOVA on the growth parameters. Only weight showed significant interaction effect (ANOVA, $F = 29.110$, $df = 2$, $P<0.05$) (Figure 14).

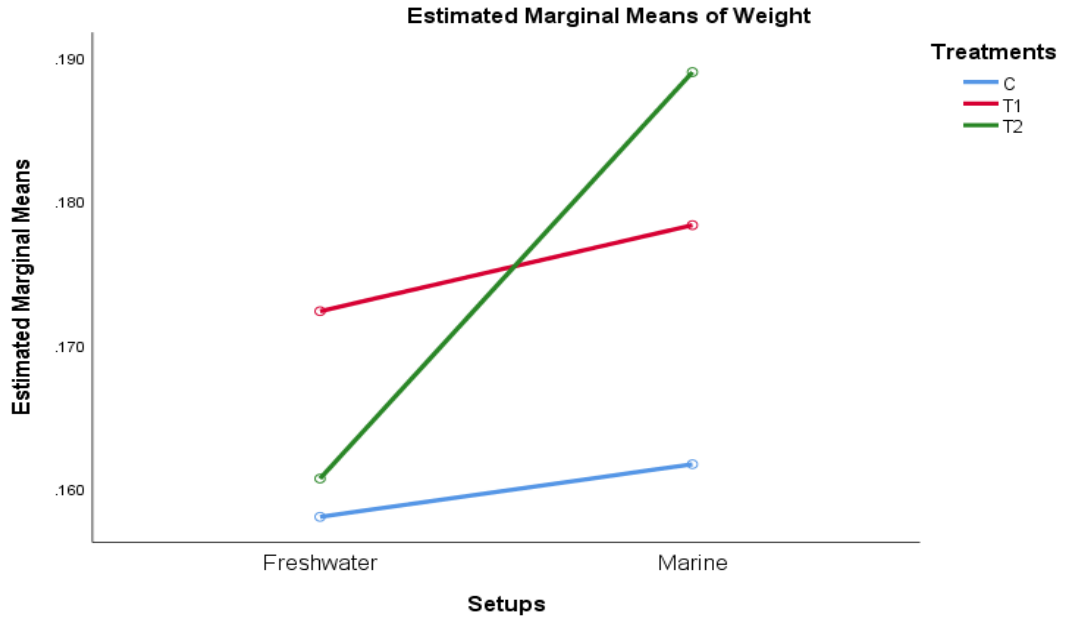

Figure 14: Interaction effect of setups and treatments

4.5.2 Setup

The two setups were compared in 2-way ANOVA and all growth parameters were significant except for length ($p = 0.681$) and weight gain ($p = 0.081$) as presented in the following table (Table 5).

Table 5: Test between setups

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Setups	Length	0.001	1.000	0.001	0.177	0.681
	Weight	0.001	1.000	0.001	75.558	0.000*
	WG	0.000	1.000	0.000	3.639	0.081
	SGR	0.407	1.000	0.407	20.006	0.001*
	FCR	0.010	1.000	0.010	26.120	0.000*
	SR	42292.014	1.000	42292.014	8700.071	0.000*

4.5.3 Treatments

C, T1 and T2 were run through 2-way ANOVA, all growth parameters were statistically significant except for survival rate (p = 0.112) (Table 6).

Table 6: Test between treatments

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Treatments	Length	0.030	2	0.015	3.919	0.049*
	Weight	0.001	2	0.000	48.715	0.000*
	WG	0.001	2	0.001	20.470	0.000*
	SGR	0.547	2	0.274	13.459	0.001*
	FCR	0.014	2	0.007	18.460	0.000*
	SR	25.694	2	12.847	2.643	0.112

5.0 DISCUSSION

5.1 Survival Rate

Survival rate in this study was at 100 % for the freshwater setup. This is in support of El-Sherif and El-Feky (2009) who reported that higher (100 %) survival rates could be associated to favorable ecological conditions. Lower survival rates were found in *Oreochromis jipe* by Ogada et al. (2018) where they attributed it to its low resilience to handling stress. Olufeagba et al. (2017) observed similar survival rates (75 %) in both mixed-sex and all male fish indicating that hormone treatment had no adverse effect on fish health. This is in agreement with the report of Cruz and Mair (1994) that 17-α-methyltestosterone treatment had no effect on survival of tilapia. Studies by Workagegn and Gjoen (2012), Ridha (2006) and El-Sayed (2002) reported a survival rate of 96 % to 100 % with Komba et al. (2020) reporting a survival rate of 89.47 %. The higher survival rate could possibly be attributed to better culture conditions throughout the experimental period, particularly the suitable average water temperature, dissolved oxygen, pH and conductivity which were in the optimal range for survival of Nile tilapia (Bhatnagar and Devi, 2013; Saber et al., 2004).

The marine setup showed low survival rates of fry at 0 - 5.833 %, this was lower than most of the studies conducted. The mortalities occurred before and after the sex reversal process, with huge mortalities experienced before the process. The mean survival rates reported by Ridha (2004) in the three tilapia strains was higher than the 67 % and 50 % reported by Al-Ahmed et al. (1985) and by Ridha and Lone (1990), respectively, for *O. spilurus* fry reared in a salinity of 2 - 5 mg. Nugon (2003) reported that *O. aureus, O. niloticus* and Florida red tilapia exhibited survival rates of approximately 81 % in salinity regimes of up to 20 ‰, and lower survival rates for *O. aureus* (54 %) and Florida red tilapia (33 %) at 35 ‰ salinity.

5.2 Sex reversal

The freshwater setup observed masculinization of 58.5 - 91.5 %. These were lower when compared with what was reported by other studies, however, they were quite interesting considering the aspect that there were no significant differences among the hormone treated groups. When compared to the incorporation of hormone into feeds, in terms of its performance with regards to exposure time, human and environmental hazards and the efficiency of fish feeding as explained by Gale et al. (1999) and Lewis and Sweet (1993), the fry immersion

technique is better, with minimal exposure and low chances of environmental hazards in its application (Gale et al., 1999). Fitzpatrick et al. (1998) were able to produce greater than 90 % male populations of *O. niloticus* when trenbolone acetate was administered as a 2-hour bath on days 11 and 13 post-fertilization. Singh et al. (2018) was able to demonstrate highest percentage of male populations following fry immersion in 300 µg L^{-1} MT solution for 12 hours, showing a significant increase in male population with increasing MT dose. According to Srisakultiew and Kamonrat (2013), MT immersion significantly increased the percentage of male at 500 µg L^{-1} while Gale et al. (1995) achieved a 93 % male population after fry immersion at 500 µg L^{-1} MT.

With the marine setup, despite the experiment coming to a stop indefinitely, the method of sex determination (hand sexing) applied for the freshwater setup could not be applied due to the size limitations of fry. However, other methods, such as genetic determination could have been made possibly but due to limitations within the locality, the method could not be applied. Hence, the sex reversal for the marine setup could not be determined coupled with the untimely mortalities and shutdown of the experiment. Thus, a wider time frame is needed for the marine sex reversal setup.

5.3 Growth parameters

5.3.1 Water quality

Water quality is key in the production of Nile tilapia (*O. niloticus*) and should be constantly monitored (Lemos et al., 2018). However, due to the tendency of farmers to realize increased production (Telli et al., 2014), overstocking takes place affecting water quality (Lemos et al., 2018). This study observed DO values of 7.3 – 8.3 mg L^{-1} which are in line with Lemos et al. (2018). Nile tilapia performs best in the upper end of the optimal temperature range of 27 – 32 °C (Mengistu et al., 2020), similar results as observed in this study (29.1 - 32.4 °C). According to El-Sherif and El-Feky (2009), the pH range of 7.0 – 8.0 is ideal for Nile tilapia production, which is in line with the results obtained in this study. Ammonium values were higher in the afternoon due to increased physiological activities of the fish. Ahmad et al. (2013) reported that pH, temperature and dissolved oxygen are very important parameters and have great influence on fish growth (Kasozi et al., 2014).

5.3.2 Length – Weight relationship

Length-Weight relationship is an important tool in fish management (Sarkar et al., 2008) where several parameters can be derived (Abbasi et al., 2019). Such of uttermost importance is the condition factor, which assesses the well-being of fish under different environmental and physiological conditions (Mouludi-Saleh et al., 2021). In this study, the freshwater setup *b*-values were lower with only one replica in treatment 2 with values of 3.007, highest in treatment 1 being 2.726 and in control 2.353. *b*-values higher and lower than 3 indicate positive and negative allometric respectively (Mouludi-Saleh et al., 2021). Froose (2006) got *b*-values ranging 2.5 - 3.5 while Tesch (1971) obtained *b*-values of 2 - 4. Generally, the *b*-value depends on the species, sexuality, age, sexual maturity, season, nutrition, geographical location, environmental conditions and time of samples in terms of gut fullness or parasitic contamination (King, 2013). According to Opiyo et al. (2020), they exhibited an isometric growth on genetic and sex reversed Nile tilapia which was an indication of ideal growth as recommended by Froese (2006). The R^2 values were lower than 0.9 and it has been reported that R^2 value less than 0.8 are associated with either low numbers of individuals or a limited size range (Purrafee Dizaj et al., 2020).

Condition factor is paramount to variations based on the nutritional quality, aquatic system and season (Mouludi-Saleh and Eagderi, 2019). *Kn* values in this study ranged from 0.666 - 1.387. Lower *Kn* values is attributed to poor conditions of habitats which may be due to unavailability of proper food and lower habitat's environmental conditions (Blackwell et al., 2000). A $Kn > 1$ indicates suitability of a specific water body and environmental condition for growth of fish (Mouludi-Saleh and Eagderi, 2019). As reported by Ogada et al. (2018), condition factor higher than one indicates an isometric growth and suggests good fish health condition, also as stated by Opiyo et al. (2020).

5.3.3 Weight Gain

In this study, both freshwater and marine setup hormone treated groups attained good weight compared to control group. This result was similar while culturing monosex tilapia by Sarbajna et al. (2006), Guerrero and Guerreo (1975), Shelton et al. (1978), Hanson et al. (1983), Pandian and Varadaraj (1988). They also came to the findings of high weight gains in the treated group compared to the control throughout the culture period. Faster growth of monosex tilapia has been related to the lack of energy expenditure in egg production and mouth brooding by

females and lower energy expenditure on courtship by males (Dan and Little 2000; Green et al., 1997). Islam et al. (2015) reported that hormone treated monosex tilapia achieved greater mean individual weight and length than mixed-sex fish. Olufeagba et al. (2017); Chakraborty et al. (2011); Little et al. (2003); Dan and Little (2000), Mair et al. (1995) and Abella et al. (1990) reported higher values of weight gain for all-male Nile tilapia. The increased growth performance is justified by the hypothesis that 17-α-methyltestosterone has growth-promoting actions on tilapia (Shepherd et al., 1997). This is further supported by Bhasin et al. (2001) who reported inducement of muscle hypertrophy by the testosterone hormone through increased muscle protein synthesis. Olufeagba et al. (2017), through interlinkages with growth hormone metabolism and higher insulin-like growth factors, affirmed the hypothesis.

Growth increase in androgen treated fish was also reported in *Oreochromis mossambicus* (Kuwaye et al., 1993), *Oncorhynchus kisutch* and *Cyprinus carpio* (Pandian and Sheela, 1995). Chakraborty et al., 2011 reported that the better growth of fish in pond culture system in their study could have been facilitated by the additional availability of relatively energy-rich natural food materials that may confer an energetic advantage for increased growth (El-Sayed, 2002; Bwanika et al., 2007). This might have resulted in less consumption of supplemented feed, leading to comparatively poor feed utilization efficiency, but achieving better growth in their culture systems. High feeding and growth rates may also have been influenced by salinity, as growth and feed consumption have been found to increase, and feed conversion ratios to decrease, with increasing salinity (up to 36 ppt) for juvenile, sex-reversed male Florida red tilapia (Watanabe et al., 1988, 1989b). Growth rates in this study were also comparable to those reported for Taiwanese red tilapia (*O. mossambicus* x *O. niloticus)* under intensive freshwater culture (Liao and Chen, 1983).

5.3.4 Specific growth rate

In this study, freshwater setup observed SGR values of 5.19 - 5.61 % day^{-1}. These values are in line with Singh et al. (2017) of 5.77 and 5.14 while comparing for both mono-sex and mixed sex groups respectively showing a better growth of monosex. Haq et al. (2017) obtained SGR values of 2.49 for monosex tilapia while Chakraborty et al. (2011) obtained 5.25 % day^{-1}. According to a study by Ogunji et al. (2007), they reported SGR values of 3.39 % day^{-1}, Siddiqui et al. (1991) reported higher values of 3.7 - 4.9 % day^{-1}. The growth performance of tilapia is

generally influenced by genetics, quality and quantity of feed, brood stock management and environmental conditions (Mair et al., 1997). The higher SGR values may also be due to the high amount of energy content in the feed. Guimaraes et al. (2008) states that efficient utilization of diets may vary within a single species because of the environmental conditions, which was not the case in the current study.

For the marine setup, SGR values observed were 1.184 - 1.630 % day^{-1}. These results are in line with Emre et al. (2003) (1.19 - 1.89 % day^{-1}), but comparable with those reported by Kapute et al. (2016) 1.8 % day^{-1} for *Tilapia rendalli* reared in 200 m^2 brakishwater ponds, Malik et al. (2018) observed SGR of 0.9 - 3.3 % day^{-1}, Ogunji and Wirth (2001) (1.11 - 3.46 % day^{-1}) and Abdel-Warith et al. (2001) (2.83 - 3.68 % day^{-1}). Yidirim et al. (2009) found that *T. zillii* attained a daily SGR ranging from 2.12 - 2.98 % day^{-1} in brackish water. The SGRs recorded for the marine experimental treatments in the present study are higher than those reported by El-Sayed (1989) of 0.54 - 0.87 % day^{-1} for *T. zilli*, while Abdel-Tawwab (2008) recorded 0.10 - 0.82 % day^{-1}, Ridha (2006) (0.83 - 1.18 % day^{-1}), Sallam et al. (2017), red tilapia showed good growth (0.56 – 0.85 % day^{-1}) at salinity ranging from 9 ‰ – 36 ‰. In addition, Abbas and Siddiqui (2009) reported SGR (0.9 - 2.2 % day^{-1}) of mangrove red snapper (*Lutjanus argentimaculatus*) cultured at salinity level of 35 ‰. Similar results were also reported by Solomon and Okomoda (2012) and Daudpota et al. (2016), for *O. niloticus* at 25 - 30 ‰ salinity level.

5.3.5 Feed conversion ratio

Feed conversion ratio (FCR) is an important economic indicator of the quality of the fish feed (Mugo-Bundi, 2013), how efficiently fish utilizes feed thereby reducing wastage (Opiyo et al., 2020) and efficient extraction of nutrients and conversion into flesh (Yakubu et al., 2013). In this study, freshwater setup had FCR values of 3.039 - 3.277. These values are within the range of Opiyo et al. (2020) (2.33 - 3.26), and lower compared to Liti et al. (2006) (3.4 - 4.0). Different studies obtained varying FCR values. Githukia et al. (2015) results coincided with those of Opiyo et al. (2014) in the given ranges of 1.43 – 2.30. Olufeagba et al. (2017) found that all male tilapia had better feed utilization and absorption which was in line with the results of Pechsiri and Yakupitiyage (2005). Haq et al. (2017) got FCR values of 1.39 for monosex Nile tilapia. Lower FCR's have been previously recorded in monosex tilapia by Islam et al. (2015) and

Toguyeni et al. (1997) confirming the trend of monosex tilapia exhibiting better FCR than mixed-sex tilapia. Poor FCR values can be attributed to variation in feed utilization efficiency within a single species and environmental factors (Ellis et al., 2002).

The marine setup FCR values observed in this study ranged 0.234 - 0.320. These values are lower, however close to those reported by Malik et al. (2018) where they observed FCR values similar at 0.55 across all salinity levels. The findings of Malik et al. (2018) are in agreement with those reported in other studies, in terms of culture of tilapia at different salinity levels (Daudpota et al., 2014; Rahim et al., 2017a, b). Other studies to have reported higher FCR values include Mapenzi and Mmochi (2016) of 1.01 - 2.85, Daudpota et al. (2016) of 0.84 of red tilapia in concrete tanks. While assessing different strains, Ridha (2004) observed that, FCR values in Experiment 1 in the non-improved strain group was better (0.89) than the lowest FCR (1.14) reported by Ellis and Watanabe (1993) for red tilapia fry. The variations of FCR's among different studies might be explained by the different experimental conditions (feed formulation and diet content, stocking density, age and sex of fish) applied (Jauncey, 1982). As argued by Ridha (2004), the impact of FCR is more significant during the grow out stages as compared to the fry stages due to the increase in food consumption.

6.0 CONCLUSION AND RECOMMENDATION

Survival rates were similar in the freshwater setup; however, mortalities were experienced in the marine setup even before and after the immersion process. The brood stock used for the reproduction of the fry were a generation of already acclimatized and natural growing brood stock in the marine environment. However, since the brood stock were sourced from a point of uncontrolled reproduction, higher chances of selecting stock of low quality due to inbreeding resulting to reduced capacity to reproduce in cultured environments, could have impacted on the survival rates of fry. This is in agreement with several authors who were assessing the condition of the brood stock in relation to survival rates of fry. Therefore, more efforts in the development and control of tilapia production in the marine environment should be considered.

Sex reversal was highest in the treatment with the highest MT concentrations in the freshwater setup. However, among the replicas of treatment one, some occurrences of up to 90 % masculinization were observed. This opens up more possibilities into the effects of exposure time, concentration levels, age of fry and activity, stock density and the interaction effects during immersion. The marine setup could not be assessed for reversal effects bearing in mind the challenges encountered during the setup. Hence, effort and resources in terms of time, infrastructure and personnel in the development and advancement of the technique.

Growth rates were higher in the hormone-treated groups with instances of good growth in the low hormone concentration group. Some authors have reported on the best growth rates under higher MT concentrations; however, this should not be overlooked and is an open question as to whether the same higher growth rates reported by different authors while using higher MT concentrations can be achieved while using lower concentrations as it has been observed in this study.

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