

Comparative Growth Performance of Genetically Male, Sex-Reversed, and Mixed-Sex Nile Tilapia (*Oreochromis niloticus*) Reared in Earthen Ponds in Sagana, Kenya

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Abstract

Nile tilapia (*Oreochromis niloticus*) culture has been faced by challenges of prolific breeding and early maturity of mixed-sex tilapia that can be solved by the culture of all-male tilapia. A study was conducted to compare the growth performance, survival and condition factor of genetically male tilapia (GMT), sex-reversed tilapia (SRT) and mixed-sex tilapia (MST) cultured in earthen ponds. Fingerlings of initial weights ranging from 0.41 to 0.91 g for GMT, SRT and MST were stocked in 150 m² earthen ponds in triplicates at 3 fish m⁻² and fed on a 35% crude protein diet. After 180 days culture period, the final body weights of SRT (202.82±7.30 g) was significantly higher than GMT (174.34±3.71 g) and MST (148.06±4.60 g) (P<0.05). The survival of MST (74.11±4.73%) was higher than GMT (67.22±9.72%) and SRT (65.22±2.94%) but no significant differences were observed (P>0.05). The condition factor of all the fish was above 1 and was significantly different between the groups (P<0.05). This study demonstrates that SRT has superior growth performance than GMT and MST hence sex reversal should be used for improved Nile tilapia production.

Introduction

Aquaculture contributes approximately 50% of fish production globally and is considered one of the fastest-growing agri-food production sectors worldwide (FAO, 2018). In the developing countries, aquaculture has a great potential in enhancing food and nutrition security and bridging the food fish demand-supply gap (Allison, 2011; Béné *et al.*, 2016). Capture fisheries has stagnated over the recent past hence the need to refocus on aquaculture production (Ansah *et al.*, 2014). The aquaculture sector has grown rapidly in Kenya over the last decade and plays an increasingly important role in national food fish supply. Freshwater fish account for approximately 98% of Kenya's reported aquaculture production (KMFRI, 2017). The high freshwater fish production is attributed to the government intervention between the year 2009 to 2012 through the inter-

sectoral economic stimulus program (Musa *et al.*, 2012). The fisheries component; Fish Farming Enterprise Productivity Program (FFEPP) enhanced aquaculture production from 4,218 metric tons (MT) in 2006 to a peak of 24,096 MT in 2014 (Opiyo *et al.*, 2018). However, aquaculture production registered depressed performance in the last three years, with total production dropping from 24,096 MT in 2014 to 18,542 MT in 2019 (KNBS, 2020). The outcome arising from the government interventions has remained marginal, due to high tax on inputs, little efforts in improving the enabling environment for aquaculture investment and infrastructure to enhance inputs accessibility, extension services, marketing and most importantly promotion of best management practices that involve genetic improvement in the existing hatcheries (Chu *et al.*, 2018). Inadequate monitoring and evaluation of hatcheries in Kenya to ensure quality and adherence to

set guidelines has resulted to poor quality seeds supplied to fish farmers (Nyonje *et al.*, 2018).

Tilapias are among the most important warm-water fishes cultured globally (Charo-Karisa *et al.*, 2006). Among the wide variety of tilapias, Nile tilapia (*Oreochromis niloticus*) is the most cultured fish species in Kenya contributing 80% of aquaculture production (Mbugua, 2008; Opiyo *et al.*, 2018). *O. niloticus* has favourable aquaculture characteristics which include high tolerance to poor water quality, market acceptance, breeding in captivity, efficient utilization of plant-based diets and resistance to disease (Fitzsimmons, 2000; Liti *et al.*, 2005; El-Greisy & El-Gamal, 2012). Other indigenous tilapia species, though not popular include Singida tilapia (*Oreochromis esculentus*, Graham 1928); Victoria tilapia (*Oreochromis variabilis*, Boulenger 1906); and, Redbelly tilapia (*Coptodon zillii*, Gervais 1848). Africa's and Kenya's contribution to tilapia production worldwide is still minimal despite the existing potential and resources that can promote aquaculture production including; favourable climate, good soil and sufficient water (FAO, 2018; Opiyo *et al.*, 2018). In Kenya, the culture of Nile tilapia has been faced by significant setbacks from inbreeding, uncontrolled reproduction resulting in stunted growth and low market value (Ngugi *et al.*, 2007). These challenges can be addressed through production of all male tilapia and genetic improvement initiatives.

Monosex tilapia production through sex reversal has been the most commonly used method embraced by farmers globally (Mair & Little, 1991; Celik *et al.*, 2011). Monosex male tilapia culture enhances the production of faster-growing males and control challenges emanating from unwanted reproduction, overcrowding and harvest of undersized fish due to feeding competition (Kamaruzzaman *et al.*, 2009; Singh *et al.*, 2017). In this technology, the metabolic energy in males is directed towards growth and masculinization (El-Greisy & El-Gamal, 2012; Amoussou *et al.*, 2019). In females, however, the metabolic energy is usually channeled towards reproduction leading to slow growth (El-Greisy & El-Gamal, 2012). The most preferred production method of all-male tilapia involves oral administration of diet impregnated with the male hormone, 17 α -methyltestosterone (17- α -MT) to tilapia fry at day one after yolk absorption when they are still sexually undifferentiated for 28–30 days (Phelps & Popma, 2000).

As a result of unpredictable seed quality and environmental concerns over the use of hormones for sex reversal, genetic research led to the production of genetically male tilapia (GMT), where YY "super males" are crossed with genetically female tilapia (XX females) to produce all-male (XY males) progeny and have been undertaken for years for commercial applications (Mair *et al.*, 1997; Chen *et al.*, 2018). The GMTs are all-male and are supposed to have higher growth, delayed maturity and higher yield than other male tilapias.

Production of male tilapia through YY technology is considered environmentally friendly and reliable. However, limited information exists in Kenya on the growth performance and yield of genetically male tilapia produced from the super YY males. Although improved tilapia strains have significantly increased the productivity, farm output and profit levels associated with tilapia farming is still very low in Kenya and other Sub-Saharan countries compared to China and other Asian countries (Chen *et al.*, 2018), leading to low production (Kumar & Engle, 2016). In commercial aquaculture facilities, the growth performance of fish is the most important influencing factor concerning economic benefit (Lugert, *et al.*, 2016). Therefore, determination of optimal environmental conditions for fish growth and achievement of the desirable market size is essential for optimizing aquaculture production (Azaza *et al.*, 2008). This study was designed to compare the growth performance of genetically male tilapia (GMT), hormonally sex-reversed male tilapia (SRT) and mixed-sex tilapia (MST) reared in earthen pond culture system in Kenya.

Materials and Methods

Study Area and Experimental Fish

The study was conducted at the Kenya Marine and Fisheries Research Institute (KMFRI) - Sagana Centre (located at 0°39'S, 37°20'E and 1230 m above the sea level). The culture units were nine earthen ponds of 150 m² each stocked with 450 Nile tilapia fingerlings at the rate of 3 fish m⁻². The genetically male tilapia from YY "super males" and genetically females (GMT) were purchased from Athi Fish Farm and Hatchery located in Athi River town, Kenya. Both the YY males and genetically female tilapias were initially sourced from Til Aqua International, the Netherlands by Jambo Fish Western Ltd. The YY male and genetically female broodstock were expected to produce genetically all-male tilapia (GMT) with a male ratio >99% without use of hormones. Sex reversed tilapia fingerlings (SRT) were produced at KMFRI Sagana hatchery through hormonal sex reversal using 17 α -methyltestosterone treated diet (60 mg kg⁻¹ feed) prepared by the alcohol evaporation technique (Shelton *et al.*, 1978). Fry were fed on hormone-laced feed for 28 days and reared in a nursery for 2 weeks before the experiment. Mixed-sex Nile tilapia (MST) fingerlings were sourced from KMFRI Sagana hatchery.

Experimental Design

The experiment was conducted for 180 days (August 2018 to January 2019). During these months, August was the coldest month, with average water temperatures of 22 °C while November was the hottest month with an average water temperature of 26 °C. Before the start of the experiment, fish were transferred

into different hapa nets installed in an earthen pond where they were fed three times a day with a standard 35% crude protein (CP) commercial diet (Aller Aqua feeds) for 2 weeks as an acclimatization period before introduction into the grow-out earthen ponds. The ponds were limed and treated to remove any unwanted fish. The GMT, SRT and MST fingerlings of average body weight of 0.91 ± 0.06 g; 0.41 ± 0.02 g and 1.42 ± 0.08 g respectively, were stocked in 150m^2 grow-out earthen ponds at a stocking density of 3 fish m^{-2} . Fish were fed twice daily on commercial tilapia diets (35% CP Aller Aqua feeds) at 5% body weight and reduced to 3% body weight in the fourth month. The feed was administered to fish using pre-weighed feed containers depending on the biomass in the pond.

Water Quality Monitoring

Water quality parameters including Dissolved oxygen (DO) concentrations, temperature, and pH were measured in situ weekly using a multi-parameter water quality meter, model H19828 (Hanna Instruments Ltd., Chicago, and IL., USA). Total Ammonia Nitrogen (TAN), Total nitrogen (TN), Nitrates (NO_2), and Nitrites (NO_3), were measured weekly using standard laboratory water quality analysis methods by Boyd & Tucker (1998).

Fish Sampling

Fish were sampled monthly using a seine net. A random sample of 30 fish was collected from each pond for individual weight and length measurements. The fish were weighed using an electronic balance (readability 0.01 g) model KERN 572-33, Germany, and total length (cm) measured using a measuring board to the nearest 0.1 cm and returned to their respective ponds. At the end of the experimental period, fish were deprived of feed for 24 hours, all the experimental ponds were drained and all the fish were harvested, counted, and weighed individually. Fish growth performances were

evaluated in terms of final total length (cm), weight (g), daily weight gain (DWG), specific growth rate (SGR), survival (%), feed conversion ratio (FCR) and condition factor (K-Factor). The following formula were used for the calculations;

$$\text{SGR (\%)} = 100 (\ln W_t - \ln W_0) / t$$

Where:-(ln=natural logarithm, W_0 =initial weight (g), W_t =final weight (g) and t =time in days from stocking to harvesting.

$$\text{Daily Weight gain (g/day)} = 100(W_t - W_0) / t$$

$$\text{Condition factor (K)} = 100W / L^3$$

Where K is the condition factor, L is the total length of fish in cm while W is the weight of fish in grams.

$$\text{FCR} = \text{feed given (g)} / \text{body weight gain (g)}$$

$$\text{Survival} = \frac{\text{Number of fish harvested}}{\text{Number of fish stocked}} \times 100$$

Data Analysis

Statistical data analysis was done for growth rate, SGR, FCR, and survival rates, using the Statistical Product and Service Solutions (SPSS Inc. version 20.0). Data were subjected to one-way analysis of variance (ANOVA) followed by Duncan multiple test for multiple comparisons. A statistical significance of $P < 0.05$ was considered for all analyses conducted.

Results

Growth Performance

The growth trends of the different groups of *O. niloticus* are presented in Figure 1. Sex reversed tilapia

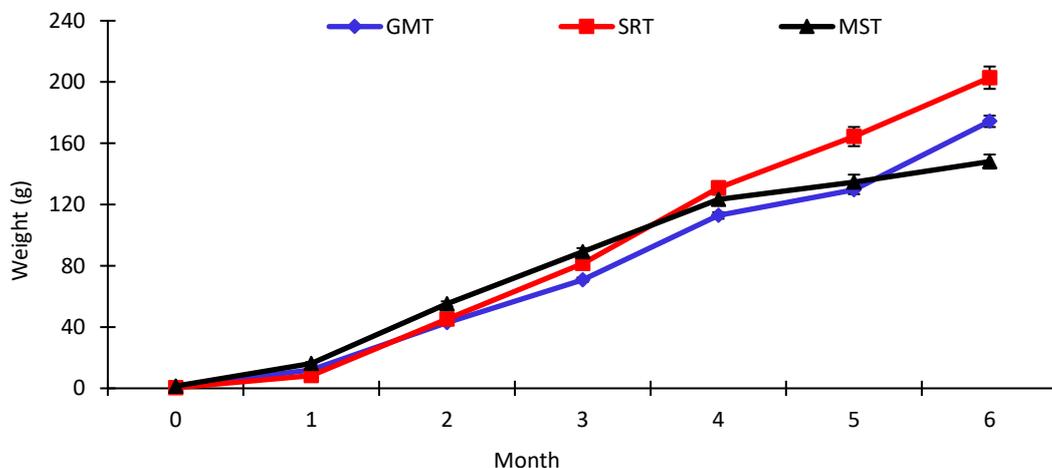


Figure 1. Growth curve of genetically male tilapia (GMT), sex reversed tilapia (SRT) and mixed sex tilapia (MST) reared in earthen ponds.

(SRT) had significantly higher growth compared to the genetically male tilapia (GMT) and the mixed sex tilapia (MST). The sex-reversed tilapia achieved a final average weight of 202.82 ± 7.30 g. The lowest final mean body weight (148.06 ± 4.60 g) was recorded in MST (Table 1). Generally, the low growth of the fish in all the treatments could be attributed to the low temperatures exhibited in August and September (temperature of an average of 22°C). The growth performance of the different groups of *O. niloticus* under the study is shown in Table 1. Highest SGR and DWG were recorded in the SRT followed by the GMT. There was a significant difference in the final body weight, SGR, FCR, and DWG among the treatment ($P < 0.001$). The FCR was lower in SRT but was not significantly different from GMT while MST had significantly higher FCR ($P < 0.001$).

Fulton's condition factors were 1.74 ± 0.01 , 1.81 ± 0.02 , 1.78 ± 0.01 for GMT, SRT and MST respectively. This demonstrates that the condition factors of all the fish were above 1 and were significantly different between the treatments with SRT having the highest condition factor ($P < 0.05$). Length Weight Relationships (LWR) analysis indicated that the regression slope b of the different fish were 3.11 for GMT, 3.11 for SRT and 3.06 for MST and were ≥ 3 with an R^2 value of 1.00, 0.99 and 0.98; for GMT, SRT and MST respectively (Figure 2). Compared to the GMT and the MST, the proportion of males to females was significantly higher in SRT ($P < 0.05$). MST had the lowest male to female ratio value of 56.66%. The percent survival of fish in all the groups was between 67.22 to 77.11%. The highest survival was recorded in MST (74.11%) followed by GMT (67.22%) while the lowest survival was exhibited in the SRT group (65.22%). However, there was no significant difference on survival between the different groups ($P > 0.05$).

Water Quality Parameters

The water quality parameters during the experimental period are shown in Table 2. Dissolved oxygen (DO) ranged from 4.31-4.36 mg L^{-1} ; temperature,

24.76 - 25.08°C ; pH, 7.70-7.74; Total Ammonia nitrogen (TAN), 0.14 - 0.16 mg L^{-1} ; Total nitrogen (TN), 1.58 - 1.62 mg L^{-1} ; Nitrates (NO_2), 0.01 - 0.03 mg L^{-1} and Nitrites (NO_3), 0.01 - 0.02 mg L^{-1} . The recorded water quality parameters did not show any significant differences among the treatments ($P > 0.05$).

Discussion

The main objective of this study was to evaluate the growth performance of genetically male tilapia (GMT) to hormonally sex-reversed male tilapia (SRT) and mixed-sex tilapia (MST) reared in earthen ponds in Sagana, Kenya. At the end of 180 days of the experiment, all the tilapia groups had different final mean weight (FMW), specific growth rate (SGR), and daily weight gain (DWG). The SRT group exhibited higher final body weight, SGR, and DWG than the GMT and MST groups. These results contradict those of Islam *et al.* (2015) and Mair *et al.* (1995) in which the GMT had the highest mean average weight at the end of 105 days of the experiment. Mixed sex tilapia recorded a lower growth performance because of a higher proportion of females than the other monosex groups. This is attributed to the fact that female tilapia spend more energy in egg production and mouth-brooding hence exhibiting a lower growth rate compared to the males as posited by Chakraborty *et al.* (2011).

The results of the present study are similar to studies of Mbiru *et al.* (2016) who reported that sex-reversed tilapia attained greater final weight compared to mixed-sex tilapia in Tanzania. Although many scientists have recorded the lack of a significant difference in growth between sex-reversed males and genetic male tilapia (Phelps & Popma, 2000), the present study indicates that SRT had a significantly higher growth rate compared to MST confirming the findings of Mateen & Ahmed (2007) and Chakraborty & Banerjee (2012). The higher growth rate of SRT is a result of anabolic and androgenic impacts of 17α -methyltestosterone hormone which advances male sexual traits in *O. niloticus* while simultaneously

Table 1. Growth parameters for genetically male tilapia (GMT), sex reversed tilapia (SRT) and mixed sex tilapia (MST) reared in earthen ponds

Parameter	Treatments			F- value	p- value
	GMT	SRT	MST		
Initial length (cm fish ⁻¹)	3.75 ± 0.09^a	3.08 ± 0.04^b	4.50 ± 0.10^c	72.78	<0.001
Initial weight (g fish ⁻¹)	0.91 ± 0.06^a	0.41 ± 0.02^b	1.42 ± 0.08^c	70.83	<0.001
Final length (cm fish ⁻¹)	21.44 ± 0.16^a	21.88 ± 0.30^a	20.60 ± 0.23^b	7.41	0.01
Final weight (g fish ⁻¹)	174.34 ± 3.71^a	202.82 ± 7.30^b	148.06 ± 4.60^c	25.51	<0.001
SGR (% day ⁻¹)	2.62 ± 0.03^a	2.96 ± 0.02^b	2.27 ± 0.02^c	174.09	<0.001
DWG	0.83 ± 0.02^a	0.96 ± 0.03^b	0.70 ± 0.02^c	26.76	<0.001
FCR	2.44 ± 0.06^a	2.33 ± 0.10^a	3.26 ± 0.10^c	31.80	<0.001
Condition Factor (K)	1.74 ± 0.01^a	1.81 ± 0.02^b	1.78 ± 0.01^b	6.09	0.002
% Survival	67.22 ± 9.72^a	65.22 ± 2.94^a	74.11 ± 4.73^a	5.05	0.627
% Males	81.94 ± 1.10^a	94.45 ± 1.36^b	56.66 ± 4.41^c	62.99	<0.001

*Values are Mean \pm S.E of three replicates. Means having the same letter in the same row are not significantly different at $P < 0.05$

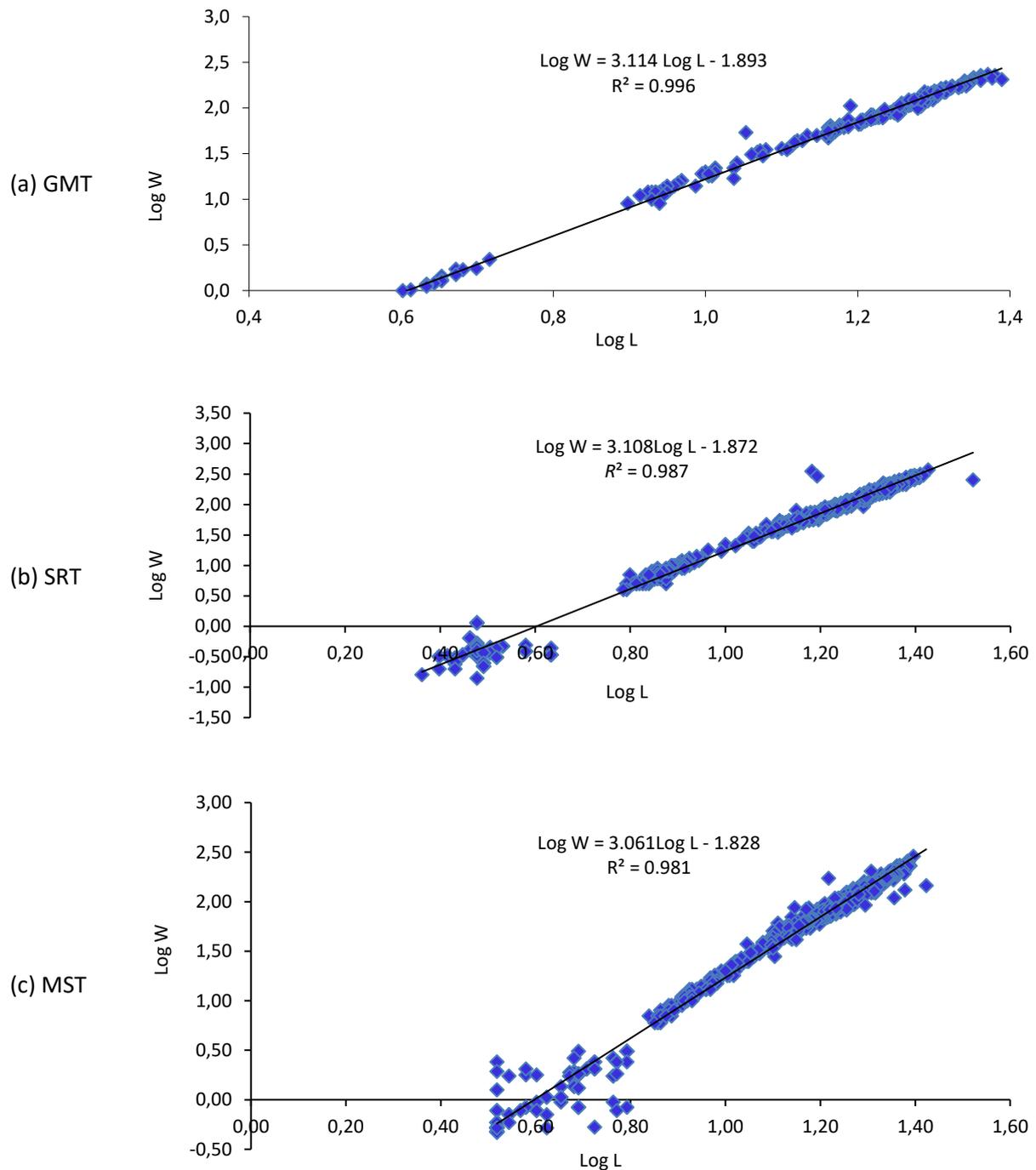


Figure 2. Length Weight relationship of (a) genetically male tilapia (GMT), (b) sex reversed tilapia (SRT), and (c) mixed sex tilapia (MST) reared in earthen ponds.

Table 2. Water quality parameters for genetically male tilapia (GMT), sex reversed tilapia (SRT) and mixed sex tilapia (MST) reared in earthen ponds

Parameter	Treatments		
	GMT	SRT	MST
Dissolved oxygen (mg L^{-1})	4.36 ± 0.24^a	4.35 ± 0.23^a	4.31 ± 0.22^a
Temperature ($^{\circ}\text{C}$)	25.08 ± 0.38^a	24.80 ± 0.25^a	24.76 ± 0.34^a
pH	7.74 ± 0.07^a	7.66 ± 0.08^a	7.70 ± 0.60^a
TAN (mg L^{-1})	0.16 ± 0.02^a	0.14 ± 0.02^a	0.15 ± 0.02^a
TN (mg L^{-1})	1.62 ± 0.19^a	1.62 ± 0.17^a	1.58 ± 0.17^a
TP (mg L^{-1})	0.39 ± 0.01^a	0.37 ± 0.11^a	0.36 ± 0.11^a
Nitrates (mg L^{-1})	0.03 ± 0.00^a	0.02 ± 0.00^a	0.01 ± 0.00^a
Nitrites (mg L^{-1})	0.01 ± 0.00^a	0.01 ± 0.00^a	0.02 ± 0.00^a

*Values are Mean \pm S.E of three replicates. Means having the same letter in the same row are not significantly different at $P < 0.05$

promoting the growth of muscles (Khalil *et al.*, 2011; El-Greisy & El-Gamal, 2012). SRT achieved greater mean individual weight and length than the other groups and could be attributed to low energy expended during courtship by males (Adamneh, 2013; Githukia *et al.*, 2015). Moreover, several studies indicated that testosterone produces muscle hypertrophy by increasing muscle protein synthesis (Bhasin *et al.*, 2001).

Feed conversion ratio (FCR) is an important economic indicator of how efficiently fish utilizes feed thereby reducing wastage. The lowest FCR (2.33 ± 0.1 and 2.44 ± 0.6) was observed in the MST and GMT respectively. The FCR observed in all-male groups was significantly lower than the MST group (3.26 ± 0.10). This is explained by the effect of sexual maturity where the female stops feeding during mouth brooding leaving uneaten feeds hence the suppressed growth in MST which has a higher proportion of females (Mair *et al.*, 1995). Lower FCRs 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. The FCR in the current study was directly related to the proportion of males in the experimental groups. However, the low temperature recorded during the study could also have contributed to low feed intake hence the high FCR in the fish in all the experimental groups.

In the present study, the survival rate of *O. niloticus* was comparatively low (below 75%) during the study period. There was no significant difference amongst the experimental groups. However, the MST treatment exhibited the highest survival and could be attributed to the sizes at stocking. The MST was stocked at a bigger size compared to the SRT and GMT thereby giving them the advantage of surviving during the early stages of grow-out (Sogard, 1997). In previous studies by Mamun *et al.* (2016) and Islam *et al.* (2015), the survival rates were above (90%) which was higher compared to the present study perhaps due to limited control of the environmental conditions in the earthen ponds compared to indoor tanks. The fish were affected by the low temperatures recorded throughout the culture period affecting the survival. Survival rates higher than 85% were also recorded for SRT and GMT reared in tanks by Mbiru *et al.* (2016).

The Length Weight Relationship (LWR) during the culture period indicates that GMT and SRT had isometric growth, which is the ideal growth as recommended by Froese (2006). Condition factor is an indicator of the health condition and well-being of fish in their natural environment. According to Froese (2006), studying fish health condition using condition factor presumes that fish with higher condition factor values are in a better state. SRT had the highest condition factor 1.81 ± 0.02 followed by MST 1.78 ± 0.01 and finally GMT 1.74 ± 0.01 . The condition factor in all groups was higher than the ones recorded in SRT (1.46) and GMT (1.43) by Mbiru *et al.* (2016). Fish condition factor is a crude measure of the level of energy reserve (i.e. nutritional status) in fish.

Reduction in condition factor is a sign of the poor condition of the fish. In this study, there was no significant difference in the condition factor among the treatments but lower condition factor in MST could indicate that the fish were not having a healthy nutritional status due to early maturity, which led to depressed growth.

The proportion of males in SRT was significantly higher than that of GMT. This was contrary to the expectation that the latter would produce a higher proportion of males. The results from this study contradict earlier studies by Beardmore *et al.* (2001) which indicated that genetically male tilapia has a sex rate of 99.58% males compared to 71.6% males in MST, and Kamaruzzaman *et al.* (2009) which had 94.3% of GMT and 71.3% of MST. This could be attributed to a possible inefficiency in the YY male production procedure, which could have led to the unsuccessful YY male production in a given batch, used in the production of the GMT fingerlings.

Water temperature affects the behaviour, growth, food intake, and food conversion of fish hence the determination of optimal temperature is essential in tilapia culture (El-Sayed *et al.*, 1996). The growth of the different groups of fish was not significantly affected by the water quality parameters. However, overall water temperature recorded during the culture period was lower than the optimum range of 27-30°C recommended for Nile tilapia in ponds by Boyd & Tucker (1998). The most preferred temperature range for optimal growth of tilapia is 27 to 30°C, while the ideal pH ranges between 6 and 9 (Rakocy, 1989). From this study, the low water temperature could have led to the low growth observed in fish in all the experimental groups.

Conclusion and Recommendation

The sex-reversed Nile tilapia grew better than the other tilapia groups and had a low FCR, which indicates that the sex-reversed tilapia is more viable for pond aquaculture compared to the genetically male tilapia and the mixed-sex tilapia. The sex-reversed tilapia used less feed to grow and attained the market size faster than the other tilapia groups in this study. The results of this study indicate that sex reversal is a highly promising technology for application in the production of monosex Nile tilapia in Kenya and can result in substantial yields since it is highly adaptable to different agro-ecological zones. It is recommended that the performance of the genetically male tilapia from YY technology be further evaluated in other on-farm environments and culture systems to determine its performance.

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