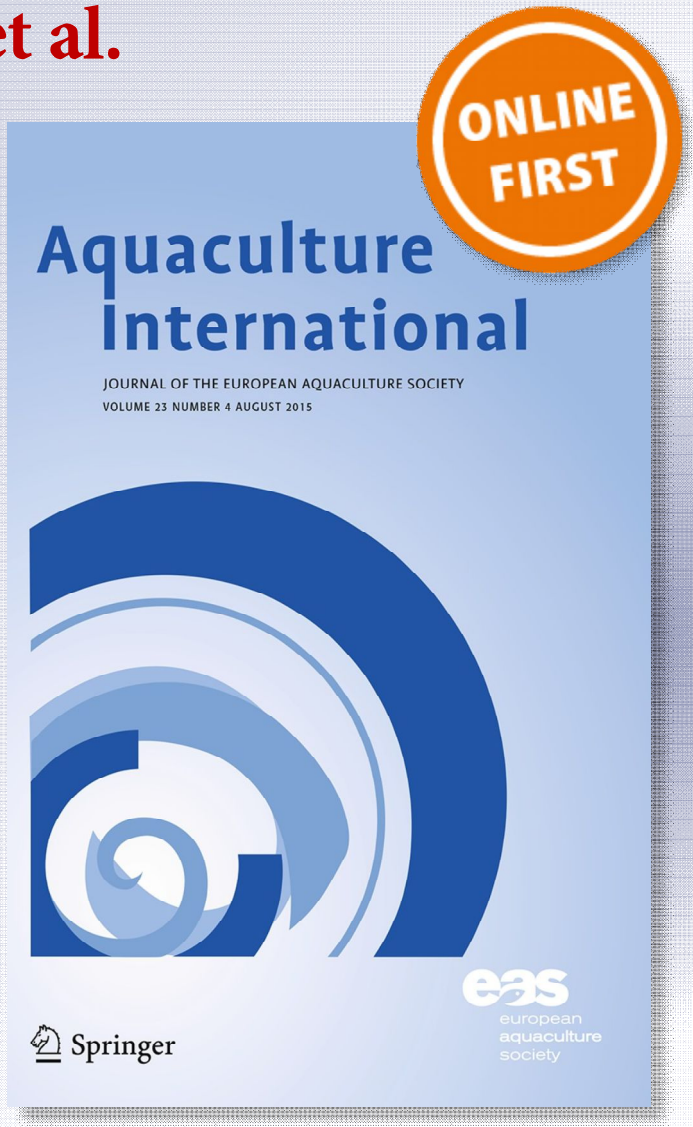
Comparative performance of mixed-sex and hormonal-sex-reversed Nile tilapia Oreochromis niloticus *and hybrids (*Oreochromis niloticus × Oreochromis urolepis hornorum*) cultured in concrete tanks*

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Comparative performance of mixed-sex and hormonal-sex-reversed Nile tilapia *Oreochromis niloticus* and hybrids (*Oreochromis niloticus* × *Oreochromis urolepis hornorum*) cultured in concrete tanks

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Abstract Uncontrolled breeding and precocious maturity in Nile tilapia, *Oreochromis niloticus* can be avoided by using all-male population. This study compared the growth performance, survival rate, condition factor and final fish tank biomass (yield) of mixed-sex and hormonal-sex-reversed *O. niloticus* and hybrids (*O. niloticus* × *O. urolepis hornorum*) cultured in tanks. Sex-reversed males were produced by feeding newly hatched fry with a diet containing 60 mg of 17α-methyltestosterone for 28 days. Fourteen *O. niloticus* with initial mean weight (±standard error) of 0.50 ± 0.04, 0.48 ± 0.06 and 0.46 ± 0.01 g for mixed-sex, sex-reversed and hybrids, respectively, were separately stocked in triplicate tanks at a density of two fish m^{-2} and fed a 300 g kg^{-1} crude protein diet for 12 weeks. Results showed that hybrids had significantly higher final mean weight (31.41 ± 0.33 g) than hormonal-sex-reversed (25.82 ± 1.51 g) and mixed-sex *O. niloticus* (19.50 ± 1.26 g; $p < 0.05$). Similarly, sex-reversed *O. niloticus* had significantly higher final mean weight than mixed-sex ($p < 0.05$). The condition factor of hybrids (1.71 ± 0.05) was significantly higher than that of sex-reversed (1.46 ± 0.01; $p = 0.001$) and mixed-sex *O. niloticus* (1.43 ± 0.01; $p = 0.001$). Survival rate was not significantly different among treatments ($p = 0.445$). The final fish tank biomass was significantly higher in hybrids (535.24 ± 31.67 g $tank^{-1}$) and hormonal-sex-reversed (486.52 ± 33.70 g $tank^{-1}$) than mixed-sex *O. niloticus* (330.05 ± 17.08 g $tank^{-1}$; $p < 0.05$). This study demonstrates that hybrids have superior growth performance and condition factor than sex-reversed and mixed-sex but have similar yields to hormonal-sex-

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reversed *O. niloticus*. These results suggest that *O. niloticus* farmers can improve growth rate and yield by rearing hybrids without affecting survival rate.

Keywords All-male tilapia · Precocious maturity · Heterosis · Androgenic effect · Anabolic effect

Introduction

Nile tilapia, *Oreochromis niloticus* (L.), is one of the most important and widely cultured fish species in the world. Its culture in many developing countries occurs mainly in earthen ponds under mixed-sex culture system. One of the main challenges of mixed-sex culture of *O. niloticus* in ponds is precocious maturity and uncontrolled reproduction leading to overcrowding, stunted growth (Omitoyin et al. 2013) and low yield at harvest (Dagne et al. 2013). These undesirable effects of *O. niloticus* mixed-sex culture can be overcome by production of all-male populations which grow at a faster rate and uniform size than females (Celik et al. 2011). This prevents recruitment, overpopulation, competition for feed and space between recruits and stocked adults, resulting in increased growth rate and yield at harvest (Budd et al. 2015).

All-male population of *O. niloticus* can be produced through hormonal sex reversal, manual sexing, hybridization and use of super males (Mair and Little 1991; Abucay and Mair 1997). Direct application of hormones using 17α-methyltestosterone (17α-MT) is one of the most common methods for commercial production of *O. niloticus* male populations (Celik et al. 2011). However, the use of 17α-MT hormone suffers limitations of availability, affordability and permitted usage in many countries due to its environmental and human health effects (Kefi et al. 2012; Mlalila et al. 2015). It has been reported that the metabolites excreted by the treated fish or leachates in culture water are more potent to induce sex reversal than dietary 17α-MT hormone (Pandian and Kirankumar 2003; Rivero-Wendt et al. 2013). Furthermore, when administered at high dose, it leads to sterility and paradoxical sex reversal and may result in growth suppression in fish (Mohamed et al. 2013). The hormone is an endocrine disrupter capable of interfering with normal functions of the reproductive systems in humans and other animals (Barbosa et al. 2013). Higher dose exposure to 17α-MT hormone can cause adverse health effects such as hepatotoxicity and fetotoxicity (Yılmaz et al. 2013). Thus, consumer acceptance of hormonal-sex-reversed fish is limited even in countries where application of hormones to food fish is an acceptable practice (Biswas et al. 2005).

To avoid negative impacts of hormonal sex reversal using 17α-MT hormone on the environment and human health, all male *O. niloticus* can be produced by hybridization techniques (Mair et al. 1997). Hybrid males can be produced from crosses involving *O. mossambicus* × *O. urolepis hornorum*, *O. niloticus* × *O. urolepis hornorum*, *O. niloticus* × *O. macrochir* and *O. niloticus* × *O. aureus* (Wohlfarth 1994). Previous studies have shown that hybridization of Nile tilapia (*O. niloticus*) and Wami tilapia (*O. urolepis hornorum*) results into predominantly male offsprings (Wohlfarth 1994) with increased growth rate (Meyer and Smitherman 1996). However, this type of hybridization has not been used widely due to limited availability of *O. urolepis hornorum* in many countries and problems of maintaining pure broodstock lines for breeding (Wohlfarth 1994). Luckily, *O. urolepis hornorum* is native and readily available in natural waters in Wami River, Tanzania (Eccles 1992). Thus, hybridization can be used as an environmental

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friendly and reliable method of producing all male hybrids in Tanzania and many other countries where the species is available or can be exported. Unfortunately, limited information exists on the growth performance and yield of hybrids produced by crossing *O. niloticus* and *O. urolepis hornorum*.

The objective of this study was therefore to compare growth rate, survival rate, condition factor and yield of juvenile mixed-sex *O. niloticus*, hormonal-sex-reversed *O. niloticus* and hybrids of *O. niloticus* and *O. urolepis hornorum*. It was hypothesized that hybrids of *O. niloticus* and *O. urolepis hornorum* will have the fastest growth rate, higher percentage survival, best condition factor and highest yield compared to mixed-sex *O. niloticus* and hormonal-sex-reversed *O. niloticus*.

Materials and methods

Experimental location and source of fish

The experiment was conducted at Magadu fish farm owned and operated by the Department of Animal Science and Production (DASP) at Sokoine University of Agriculture (SUA) in Morogoro, Tanzania. Fingerlings of *O. niloticus* with an average weight of 5.70 ± 0.45 g were collected from Lake Victoria at Nyegezi Bay in Mwanza, Tanzania, while *O. urolepis hornorum* fingerlings with an average weight of 6.10 ± 0.20 g were obtained from Wami River at Wami Dakawa in Morogoro, Tanzania. The fingerlings were raised for four months before being used as broodstock.

Production of hybrids

Before using *O. urolepis hornorum* and *O. niloticus* as broodstock, they were morphologically identified according to Eccles (1992) and Skelton (1993). All brooders were inspected in their mouth before stocking to ensure that all eggs spawned before pairing were removed. Hybrids were produced by pairing ripe *O. niloticus* females and *O. urolepis hornorum* males with an average live weight (±standard error) of 26.02 ± 0.80 and 28.00 ± 1.4 g, respectively. The chosen broodstock were stocked at a sex ratio of one male to three females in four 4.5-m^2 separate concrete tanks and allowed to spawn naturally for 40 days. The resulting F_1 hybrids were combined and acclimatized for two weeks in a 4.5-m^2 concrete tank before being used for growth performance and survival studies.

Production of hormonal-sex-reversed *O. niloticus*

A hormonal treated feed was prepared by using a diet containing 300 g kg^{-1} crude protein composed of fish meal (*Rastrineobola argentea*) (350 g kg^{-1}), whole maize meal (590 g kg^{-1}), sunflower oil (20 g kg^{-1}), wheat meal (30 g kg^{-1}) and mineral premix (10 g kg^{-1}) hereafter referred to as untreated feed. The untreated feed was mixed with 17α-MT hormone obtained from Norwegian School of Veterinary Science (NVH) in Norway to make the hormonal treated feed as follows. A standard solution was made by dissolving 60 mg of 17α-MT hormone in one litre of 95 % ethanol. After dissolving the hormone, the resulting solution was evenly sprayed over one kilogram of the diet and mixed by hand several times to ensure homogeneity. The treated feed was spread thinly

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onto a plastic sheet overnight in a large and well-ventilated room at temperature of 23.65 °C for a period of four days to allow evaporation of ethanol. The dry hormonal treated feed was packed in bags and crushed into powder by rolling a heavy glass bottle over it. The feed was then sealed in an airtight black container and stored in a refrigerator until use.

Two 4.5-m^2 concrete tanks were stocked with one adult *O. niloticus* ♂ and three *O. niloticus* ♀ for production of fry for sex reversal. After three weeks, a total of 270 healthy eyed eggs were collected from the mouth of six females from each tank. All eggs were disinfected using 10 mg L^{-1} potassium permanganate before being put in jar incubators. They were then placed in basket, counted and siphoned up and down into the jar incubator. A constant flow of water was maintained to ensure constant suspension and aeration of the eggs. After 36 h, all eggs hatched and were left in jar incubators for four days when the yolk sac was completely absorbed as indicated by inflation of the swim bladders.

After six days post hatching, a total of 152 hatchlings from jar incubators with a mean weight of 0.01 g were subjected to hormonal treatment in a 60-L aquarium at a stocking density of three fry L^{-1}. All hatchlings were fed the hormonal treated diet twice daily for 28 days at a rate of 20 % average body weight. The aquarium water was continually aerated, completely replaced and debris were siphoned on a daily basis to ensure optimum water quality parameters. The resulting sex-reversed *O. niloticus* were acclimatized in a 4.5-m^2 concrete tank for two weeks prior to start of the growth performance and survival experiment. Ninety percent (90 %) of the sex-reversed fry were confirmed to be males after a sample of 30 fry were killed for histological sex determination following procedures described by El-Greisy and El-Gamal (2012).

Mixed-sex *O. niloticus* fry

The mixed-sex *O. niloticus* fry were obtained from three pairs of crosses involving *O. niloticus* at a ratio of one female and three males reared in 4.5-m^2 concrete tank at the study site. The broodstock were allowed to spawn and breed naturally for two weeks. The mixed-sex fry produced were combined and acclimatized for two weeks in a similar concrete tank before being used for growth performance and survival studies. Histological analysis of a sample involving 30 mixed-sex *O. niloticus* fry after two weeks showed a sex ratio (M:F) of 1.00 to 0.88.

Acclimatization conditions and feeding of the experimental *O. niloticus* groups

All the experimental *O. niloticus* groups were acclimatized for two weeks, each in a separate 4.5-m^2 concrete tank prior to start of the growth and survival experiment. During acclimatization, they were hand fed on the untreated feed at 10 % average body weight twice daily. Water temperature was maintained at 23.79 ± 0.15, 22.96 ± 0.05 and 23.82 ± 0.12 °C for hybrids, sex-reversed and mixed-sex, respectively, dissolved oxygen (DO) was 8.2 ± 0.13, 8.40 ± 0.06 and 8.12 ± 0.03 mg L^{-1} in hybrids, sex-reversed and mixed-sex tanks, respectively, and water pH was 7.14 ± 0.03, 7.08 ± 0.01 and 7.08 ± 0.04 in hybrids, sex-reversed and mixed-sex tanks, respectively. Prior to stocking for growth performance and survival experiment, hybrids, sex-reversed and mixed-sex *O. niloticus* fry were graded to ensure similar initial sizes.

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Growth and survival experiment

Nine rectangular outdoor concrete tanks each with an area of seven square metre and a depth of one metre with fortnightly complete renewal of water in each tank were used for the growth and survival experiment. The experiment consisted of three treatments, i.e., mixed-sex *O. niloticus* (as a control), hormonal-sex-reversed male *O. niloticus* and hybrid males of *O. niloticus* × *O. urolepis hornorum* in three replicates. All the nine tanks were each stocked on the same day with 14 fingerlings. The initial average weight (±standard error) for mixed-sex, hormonal reversed and hybrids were 0.50 ± 0.04, 0.48 ± 0.06 and 0.46 ± 0.01 g, respectively.

All the three experimental fish groups were fed on the untreated feed used in the acclimatization process. During the first week, the fish were provided with the diet at a feeding rate of 10 % and the amount was progressively reduced as fish grow to reflect the decrease in metabolic rate to 8, 6 and 5 % of average body weight during the second, third and fourth to twelfth week of the experiment, respectively. The ration was fed twice daily between 0900 and 1000 and 1500 and 1600 hours. The amount of the diet provided was re-computed fortnightly using the average body weights obtained after each sampling.

Data collection

All fish in each tank were individually measured to determine the initial body weight and length before the start of the experiment. Body weight was recorded to the nearest 0.01 g by using a sensitive weighing balance (Boeco BBL, model 43, Hamburg, Germany). Body length was measured to the nearest 0.01 cm using a measuring ruler. Thereafter, sampling in each tank was done every 14 days by scooping out all the fish and measuring their body weights and lengths as described above. At the end of the experiment, water was completely drained out in each tank and all fish were collected and counted to determine survival rate and were weighed individually to determine mean final body weight. The following parameters were computed:

$$\mathrm{SR}\,(\%) = \frac{N_\mathrm{h}}{N_\mathrm{s}} \times 100 \tag{1}$$

where SR = percentage survival (%), N_h = total number of fish harvested and N_s = total number of fingerlings stocked

$$K = 100W/L^3, \tag{2}$$

where K = condition factor, W = weight of fish (g) and L = total length of fish (cm)

$$\text{Final fish tank biomass (yield)}\ (\mathrm{g\,tan\,k^{-1}}) = \text{Final mean weight} \times \text{Number of fish (survival)} \tag{3}$$

Determination of water quality parameters

Water quality parameters in all tanks were measured twice daily at 0830–0900 and 1630–1700 hours. Temperature and dissolved oxygen were measured by using YSI 55 dissolved oxygen meter (model 55, Yellow Spring Instrument Co. Ohio, USA). Water pH was measured using a pH meter (Mardel 5, R029B-MARDEL, USA). The water quality parameters measured were within the ranges required for optimum growth of *O. niloticus*

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and their hybrids. Temperature ranged from 23.03 ± 0.16 to 23.48 ± 0.13 °C in tanks with mixed sex *O. niloticus*, dissolved oxygen (DO) ranged from 7.72 ± 0.24 to 8.07 ± 0.19 mg L^{-1} and water pH values varied between 7.19 ± 0.05 and 7.27 ± 0.02.

Statistical analyses

Results are presented as mean ± standard error (SE) of means and data were tested for homogeneity of variance using Levene's test to guard against violation of the assumptions of parametric statistics. After confirming homogeneity of variances, analysis of variance (ANOVA) was used to test if there were any significant differences in final mean weight, condition factor, percentage survival and yield among the three treatments. When significant differences were detected, Tukey's multiple comparisons test was performed to determine specific significant differences among treatments. Percentage data were arcsine-transformed prior to ANOVA and reversed afterward for reporting purposes. Pearson's correlation was used to evaluate the relationship between final mean weight and final fish tank biomass. All statistical analyses were performed using SPSS version 20 for Windows. Significant differences were judged at a probability level of $p \leq 0.05$ for Levene's test and ANOVA and $p \leq 0.01$ for Pearson's correlation.

Results

Growth performance, condition factor and percentage survival of mixed-sex and hormonal-sex-reversed *O. niloticus* and hybrids

No reproduction was observed in all the three treatments studied. Although started at similar initial mean weights, significant final mean weight was apparent at the end of the experiment ($F = 37.449$, $p = 0.001$; Fig. 1). Hybrids had significantly higher final mean weight (31.41 ± 0.33 g) than hormonal-sex-reversed (25.82 ± 1.51 g) and mixed-sex *O. niloticus* (19.50 ± 1.26 g; $p = 0.001$). Similarly, hormonal-sex-reversed *O. niloticus* had significantly higher final mean weight than mixed-sex *O. niloticus* ($p = 0.001$).

Condition factor of *O. niloticus* differed significantly among the treatments ($F = 23.598$, $p = 0.001$). Hybrids had higher condition factor (1.71 ± 0.05) than sex-reversed (1.46 ± 0.01; $p = 0.001$) and mixed-sex *O. niloticus* (1.43 ± 0.01; $p = 0.001$). The condition factors of hormonal treated *O. niloticus* and mixed-sex were not significantly different ($p = 0.841$). Percentage survival of all the fish in the three treatments was generally high (85.7 ± 10.1–95.08 ± 2.92 %), with no significant differences among the treatments ($F = 0.929$, $p = 0.445$).

Final fish tank biomass (yield) of mixed-sex and hormonal-sex-reversed *O. niloticus* and hybrids

The final fish tank biomass (yield) of mixed-sex, hormonal-sex-reversed and hybrids differed significantly ($F = 14.190$, $p = 0.001$; Fig. 2). The final fish tank biomass for mixed-sex *O. niloticus* was significantly lower than that of hormonal-sex-reversed ($p = 0.004$) and hybrids ($p = 0.001$). However, no significant difference in final fish tank biomass was obtained between hormonal-sex-reversed and hybrids ($p = 0.465$). The final fish tank biomass of all the three treatments was positively significantly correlated with final mean weight ($r^2 = 0.904$, $p = 0.001$).

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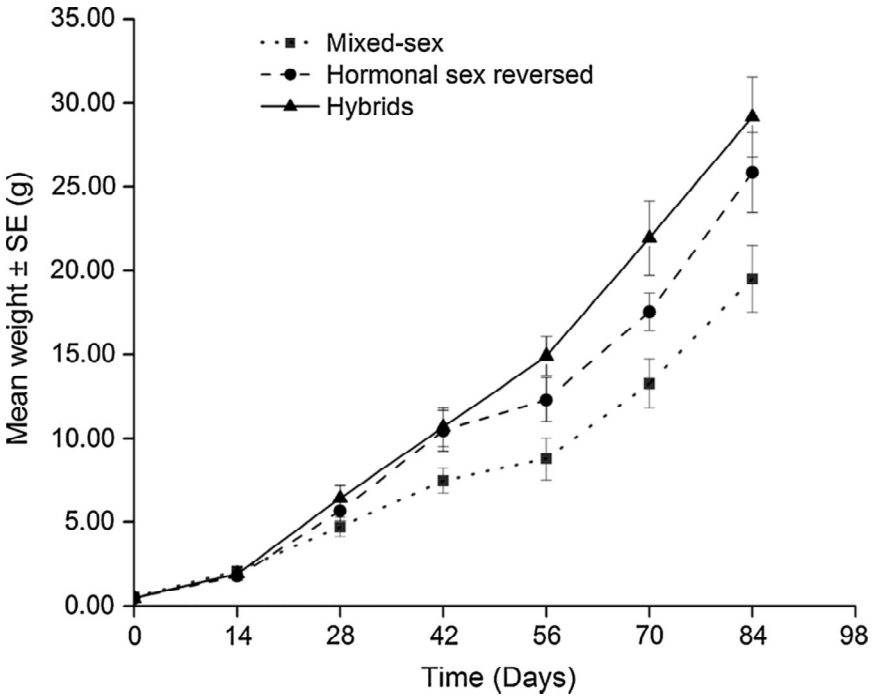


Fig. 1 Comparison of mean weight of mixed-sex, hormonal-sex-reversed *O. niloticus* and hybrids during the study period

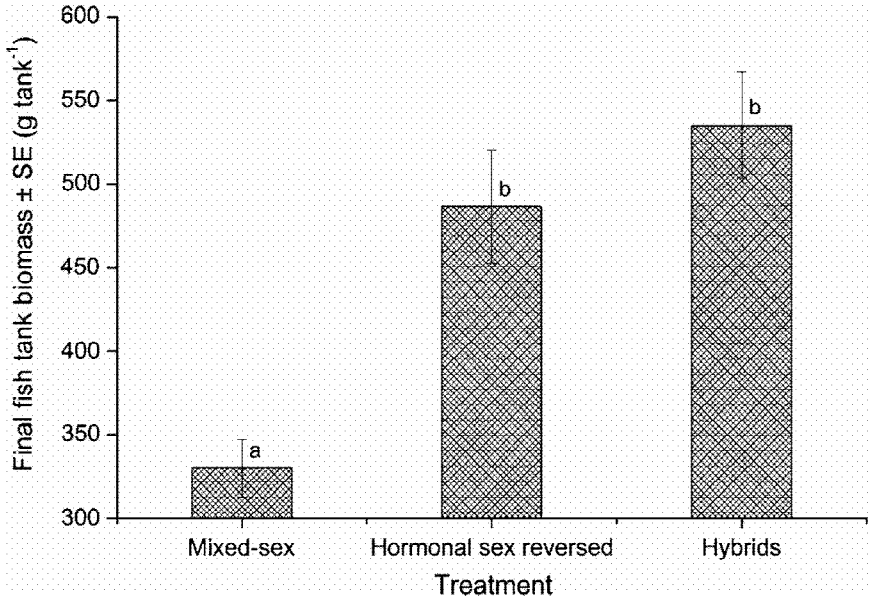


Fig. 2 Final fish tank biomass of mixed-sex, hormonal-sex-reversed and hybrids of *O. niloticus* among the three treatments. *Different letters* (*a*, *b*) above the *bars* indicate significant difference ($p < 0.05$) and *vertical bars* show standard error (SE) of the mean

Discussion

The results of the present study indicate higher growth rate for hybrid male tilapia (*O. niloticus* × *O. urolepis hornorum*) than both mixed-sex and sex-reversed *O. niloticus*. This observation is in agreement with the findings of Meyer and Smitherman (1996), Al-Hakim et al. (2012) and El-Zaeem and Salam (2013). The faster growth of hybrids in the

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present study is attributed to heterosis effect resulting from hybridization of *O. niloticus* and *O. urolepis hornorum*. Tilapia hybrids, exhibit accelerated growth and greater tolerance to unfavourable environmental conditions than pure stocks (Cai et al. 2004; Pongthana et al. 2010). Heterosis increases the capacity to grow and fitness of an individual fish. These findings suggest that farmers can hybridize *O. niloticus* and *O. urolepis hornorum* to utilize heterosis, thereby improving growth performance of their fish.

The results further showed that sex-reversed *O. niloticus* had significantly higher growth rate than mixed-sex *O. niloticus* corroborating with the results of Mateen and Ahmed (2007) and Chakraborty and Banerjee (2010). The higher growth rate of hormonal-sex-reversed *O. niloticus* is due to androgenic and anabolic effects of 17α-MT hormone (Khalil et al. 2011; El-Greisy and El-Gamal 2012). The use of 17α-MT hormone in *O. niloticus* and other species develops male sexual characters, simultaneously promoting muscle growth (Yılmaz et al. 2013). Moreover, the 17α-MT hormone acts as an appetite stimulant in fish (Mukhopadhyay et al. 1986) and functions synergistically with insulin to increase protein anabolism (Kurtsky 1973 as cited by Abdelhamid et al. 2009). In countries where 17α-MT hormone is available or can be imported and its use is permitted, *O. niloticus* farmers can use it to reverse sex and optimize fish growth rate. However, 17α-MT hormone should be used carefully to avoid its environmental and human health effects.

Condition factor (*K*) indicates the health status, fitness or well-being of fish in their habitat. Studying fish health status using condition factor assumes that fishes with higher condition factor values are in better condition (Froese 2006). In the present study, hybrids had the highest condition factor of all the experimental fish groups corroborating the results reported by Ahmad et al. (2002) and El-Hawarry (2012) for hybrids and hormonally reversed *O. niloticus,* respectively. The higher condition factor for hybrid *O. niloticus* indicates that hybrids had good health condition and were growing better than hormonal-sex-reversed and mixed-sex *O. niloticus*. This is supported by the significantly higher growth rate of the hybrids compared to other experimental groups suggesting an increased rate of muscle deposition.

The final fish tank biomass (yield) was significantly higher in hybrids and hormonal-sex-reversed than mixed-sex *O. niloticus*. These results are similar to those reported by El-Hawarry (2012) using *O. aureus* × *O. niloticus* hybrids and Mateen and Ahmed (2007) for 17α-MT hormone treated *O. niloticus*. The higher final fish tank biomass of hybrids and hormonal-sex-reversed *O. niloticus* are due to their significant higher growth rates. In the present study, hybrids and sex-reversed had higher growth rate than mixed-sex *O. niloticus*. Since final fish tank biomass was significantly correlated with final mean weight, consequently, hybrids and sex-reversed fish produced higher final fish tank biomass than mixed-sex. The higher final fish tank biomass at harvest offers an advantage to farmers for hybrids and sex-reversed than mixed-sex *O. niloticus*.

Conclusion

The current study has shown that hybrids of *O. niloticus* and *O. urolepis hornorum* have faster growth rate and higher condition factor compared to hormonal-sex-reversed and mixed-sex *O. niloticus*. Culturing hybrids and sex-reversed *O. niloticus* results in significantly higher final fish tank biomass (yield) than mixed-sex. Thus, hybridization of *O. niloticus* and *O. urolepis hornorum* has a considerable potential for improving *O. niloticus* growth, condition factor and yield in aquaculture. This study recommends that *O.*

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niloticus farmers should culture hybrids so as to improve productivity in their farms. However, the viability of hybridization will depend very much on the ability of farmers to prevent mixing of parental broodstock so that pure lines can be maintained. This problem may be solved by careful examination of the broodstock and culling for doubtful cases before breeding process.

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