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Cover image: A seagrass meadow (*Thalassia hemprichii*) at Inhaca Island, Mozambique (© José Paula, 2017)

Effect of Stocking Density on Growth Performance of Hybrids of *Oreochromis niloticus*♀ and *Oreochromis urolepis urolepis*♂ in Saline Water

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Abstract

Fingerlings of *Oreochromis niloticus*♀ and *Oreochromis urolepis urolepis*♂ hybrids were reared at stocking densities of control, 5, 10, 15 and 20 fish/m^3 at 15 Practical Salinity Units (PSU) in 1m^3 plastic tanks for 63 days. They were kept at low, intermediate and high densities respectively. All hybrids were fed on a formulated, balanced diet consisting of 40% crude protein, at a ratio of 5% body weight, twice daily. Low stocking density showed better growth performance in terms of mean weight gain, specific growth rate (SGR), feed conversion ratio (FCR) and survival rate, than intermediate and high stocking densities. SGR, FCR and survival rates did not vary significantly between treatments ($p>0.05$). Average final weight gain was significantly different between intermediate and high densities ($p<0.05$), high and low densities, but not in low and intermediate densities. Density was found to have an effect on the growth of the juvenile hybrids, since higher growth was recorded at low stocking densities compared to the intermediate and high densities. Hybrids were 100% male when inspected. The low and intermediate densities are therefore recommended for hybrids aquaculture in brackish water with the latter being suggested due to its higher yield compared to the low stocking density.

Keywords: Hybridization, all male hybrids, stocking density, growth performance, hybrids.

Introduction

Orochromis niloticus L. is a widely cultured fish species in Tanzania. However, the species reproduces prolifically in captivity in mixed-sex culture. Its uncontrolled propagation leads to overcrowding, stunted growth, and competition for food (Mensah *et al.*, 2013) and space. The resulting early maturity and stunted growth lead to unmarketable fish size problems at harvest (Mensah *et al.*, 2013). To improve performance of *O. niloticus*, efforts have been made to produce all male (mono-sex) fish populations using various techniques. These include production of hormonally reversed males, hybridization of *O. niloticus*♀ and *O. urolepis hornorum*♂ (Mbiru *et al.*, 2016) and manual sexing (Guerrero, 1982). Males have bigger body sizes and grow faster, with more uniform size, in relation to females (Celik *et al.*, 2011). Hybrids of *O. niloticus*♀ and *O. urolepis hornorum*♂ were found to have high growth performance in freshwater (Mbiru *et al.*, 2016) but were not 100% males. Mtaki (2015) successfully produced 100% males when hybridizing *O. niloticus*♀ and *O. urolepis urolepis*♂ but did not test the influence of stocking density on such hybrids. Hybridization was aimed at producing male tilapia with good growth and salinity tolerance traits inherited from both *O. niloticus* and *O. urolepis urolepis* parents. In order to better understand their value for aquaculture, the influence of stocking densities on growth and yield needed to be determined.

Stocking density is defined as the number of fish per unit area or volume. It determines fish health (Garr *et al.*, 2011), growth, survival and yield (Khatune-Jannat *et al.*, 2012; Rahman and Rahman, 2003). Suitable stocking density varies with species, water quality and feeding regimes, and is important for sustainable aquaculture (Russell *et al.*, 2008). Higher stocking densities may increase fish yield (Khatune-Jannat *et al*., 2012), lower production costs and increase profit (Abou *et al*., 2007). Ridha (2005) recommended high stocking densities in places with limited land, fresh water and man power. However, higher stocking densities may adversely affect growth performance and survival (Sorphea *et al*., 2010; Pouey *et al*., 2011). For example, higher growth and survival rates were documented at lower stocking densities for *Oreochromis* species (Sorphea *et al*., 2010), and Ronald *et al*. (2014) observed low survival rate at high stocking density of *O. niloticus* fry in ponds. In addition, high stocking density promotes competition for food and reduces fish reproductive success.

Recommended stocking densities for tilapia culture differ extensively (Russell *et al*., 2008). Mb'alaka et al., (2012) noted that appropriate stocking density is a key to minimizing tilapia production problems, and recommended a stocking density of 5 fish/m^3 for better growth performance of improved strains of *Oreochromis shiranus*. According to Alhassan *et al*. (2012), stocking density governs the economic viability of the production system. In this regard, determining the optimum stocking density of the hybrids from natural hybridization of *O. niloticus*♀ and *O. urolepis uroelpis*♂ is crucial. Aquaculture of saline-tolerant tilapia species is vital for coastal aquaculture improvement, provision of alternative employment and a reliable protein source. The aim of this study was to produce fast growing and salinity tolerant all-male tilapia hybrids, and to rear them at different stocking densities to improve coastal aquaculture by promoting hybrid mariculture practices.

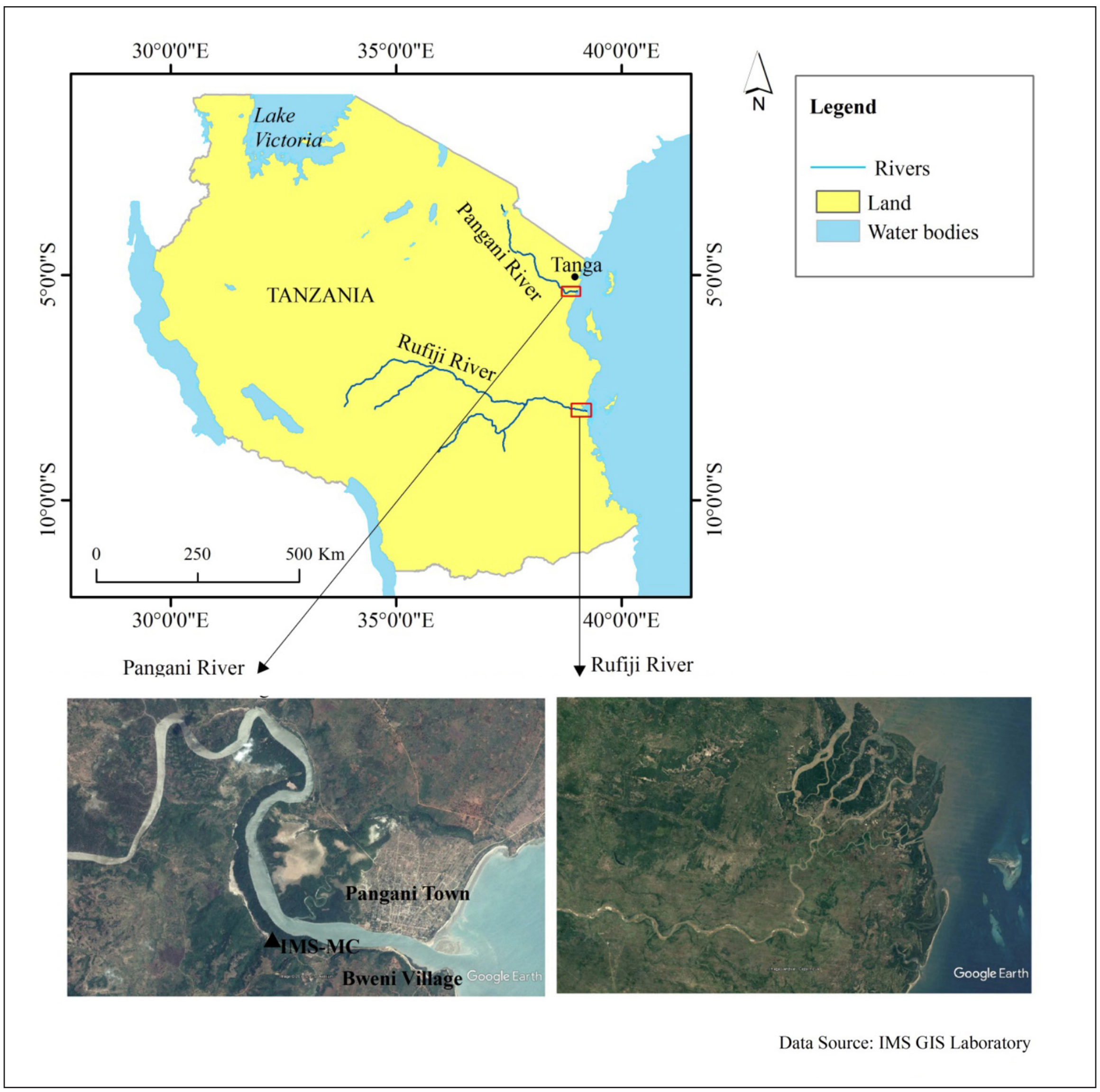

Figure 1. Map showing broodstock collection (Rufiji River) and the experimental (Bweni Village) sites.

Methodology

Study area

This study was conducted at the Institute of Marine Sciences Mariculture Centre (IMS-MC) located at Bweni Village, Pangani District in Tanga Region (05° 26´ 0˝ South, 38° 58´ 0˝ East, on the north east coast of Tanzania (Figure 1). The local communities' economic activities involve fishing, seaweed farming and subsistence terrestrial agriculture. The area is drained by the Pangani River, whose basin starts from Kilimanjaro and Meru mountains and the Pare and Usambara mountain ranges. It is well endowed with several types of terrestrial and marine ecosystems, including mangrove forests, which provide habitat for several marine and estuarine species. The Pangani River has a diversity of tilapia species which include *O. korogwe, O. pangani, O. variabilis, T.* rendalli and *T. zillii* in the lower Pangani basin (Dallas *et al*., 2006). Pangani District is characterized by an annual rainfall of above 1000 mm with temperatures varying between 22 °C and 30 °C a generally warm and humid climate. (Pamba *et al*., 2016).

Hybrid production

The *O. niloticus* and *O. urolepis urolepis* brooders were obtained from Lake Victoria, Mwanza and the Rufiji River respectively. They were raised in concrete tanks at IMS-MC. They were morphologically identified according to Eccles (1992) before being mated. Prior to stocking, the females' mouths were inspected to remove any spawned eggs before pairing with males. *O. niloticus*♀ and *O. urolepis urolepis*♂ of average weights 68.6 ± 0.2g and 75.1 ± 0.3g respectively, were naturally mated in plastic tanks. Brooders were deployed in a 2:1 female to male ratio in 10 plastic tanks, each tank having a volume of 1m^3. The brooders were fed with a 40% crude protein formulated diet. In addition, stones were placed in the tanks to serve as nests to assist with conditioning the fish to spawn.

Experimental setup

An experiment with four levels of stocking density was conducted. The experiment was replicated three times, making a total of 12 tanks. A total of 150 hybrid fingerlings were randomly distributed in the experimental tanks containing water of 15 PSU. The hybrids were batch-weighed prior to stocking. Acclimation to 15 PSU was done by gradually adding 2 PSU per day for 8 days to each tank prior to experimental start-up. Water quality parameters were measured twice a day throughout the experiment. PH and temperature were measured using a HI8424 pH meter, while salinity and dissolved oxygen (DO) were measured by a digital hand-held marine tester (DMT-10) and a dissolved oxygen meter (PDO-520), respectively. Hybrids with average weights of 0.29±0.01g, 0.29±0.01g, 0.27±0.01g and 0.28±0.01g were stocked at 5, 10, 15 and 20 fish/m^3, respectively. These were described as control, low, intermediate and high stocking densities accordingly. A 40% crude protein (CP) basal diet containing 48% maize flour, 3% cassava flour, 3% vitamins (Premix for broilers), 6% sunflower seed cake, 7% shrimp meal, and 33% sardine meal was formulated and used to feed the hybrids. The formulation aimed at synchronizing spawning, and rapid growth. Fresh sardine and shrimp for fish meal were obtained from local fisher folk. They were sun dried and milled prior to mixing with other ingredients. A meat mincer machine was used in preparing the food pellets. Fish were fed at 5% body weight, two times a day at 2.5% body weight at each feeding. Feeding was adjusted during the experiment as fish biomass per density changed with fish growth. Five individuals per tank were sampled every two weeks. The individual total weights were weighed using a digital balance. Measurements were done after removing excess water with tissue paper. The hybrids were not fed for 24 hours prior to sampling. To control water quality, 20% of the water in the tanks was replaced once a week. Siphoning was done twice a week to remove uneaten food and faeces from the bottom of tanks. Uneaten food and faeces were dried, weighed and subtracted from the amount fed, to obtain the FCR. The experiment was conducted for 63 days from 2nd October to 5th December, 2015. The hybrids were confirmed to be 100% males by external visual examination and internally through dissection and gonadal inspection. The hybrids were then raised for nine months without showing any breeding signs.

Determination of Growth Performance

Growth performance in the studied hybrids was determined using the following indices:

$$\text{Specific Growth Rate (\% day}^{-1}) = \left[\frac{\ln Wf - \ln Wi}{T}\right] \times 100 \quad \ldots\ldots 1$$

$$\text{Food Conversion Ratio (FCR)} = \frac{\textit{Total feed intake by fish (g)}}{\textit{Total weight gain by fish (g)}} \quad \ldots\ldots 2$$

$$\text{Weight Gain (g)} = W_f - W_i \quad \ldots\ldots 3$$

$$\text{Survival Rate (\%)} = \left[\frac{Nf}{Ni}\right] \times 100 \quad \ldots\ldots 4$$

Where, T is the number of days of the experiment, and N_f, N_i are the numbers of harvested and stocked fish, W_i and W_f are the initial and final mean body weights, respectively.

The coefficient of condition $\frac{W}{L^3}$ 5

Where W = Weight of individual fish (g), L = Total length of individual fish, and K = condition factor

Length weight relationship was calculated as $W = a L^b$ ...6 which was transformed into common logarithm as

log W=log a+b.log L 7

Where W = Weight of fish in gram (g), L = Total length of fish in centimeters (cm), a = proportionality constant, b = the value obtained from the length-weight equation/coefficient of regression.

Statistical Analysis

Results are presented as mean ± standard error of the means (SE). The within-treatment replicas were tested for significant differences, and were found to be insignificant at the 95% confidence level. Therefore, the within-treatment growth data were pooled for each treatment and tested for normality using Kolmogornov–Smirnov. The data were tested for homoscedasticity with Levene's test in Statistica 10 software. The data were found to be parametric and behaved homoscedastically. Therefore, analysis was done following one way analysis of variance (ANOVA). In the cases where a statistical difference between means of stocking densities was found, a Tukey test was used to determine which particular means differed significantly from each other.

Results

Water quality parameters

Average water temperature ranged from 27-28.5°C, dissolved oxygen from 5.8-6.5mg/L and pH from 7.0-7.9 units. All parameters revealed no significant difference between density treatments ($p > 0.05$, Table 1). The parameters were within the acceptable range for tilapia culture and fairly stable throughout the experimental period. The parameters were measured twice a day in the morning and evening.

Stocking Densities

The 10 fish/m³ stocking density provided marginally higher SGR, weight gain and survival rate than other density treatments, including the 5 fish/m³ control density. FCR was lower in the 10 fish/m³ than in the 15 and 20 fish/m³ densities. However, all these parameters did not vary significantly between treatments ($p > 0.05$, Table 2). On the other hand, the final mean weight of the fish did show a significant difference between treatments ($p < 0.05$, Table 3), with the 10 fish/m³ density treatment being higher than the control, 15 and 20 fish/m³ densities (Figure 2). The average initial weights of fish, though it varied slightly, did not differ significantly among treatments ($p < 0.05$, Table 2). Survival rate was managed by lowering the tanks' water and counting all fish at sampling. No replacement was done to maintain the fish density in tanks.

Length-weight Relationship (LWR) and Condition Factor (K)

Studies of length-weight relationship indicate fish wellbeing and the effects of the environment on fish (Mansor *et al*. 2012). Where the regression coefficient "b" value in a simple regression equation is greater, less than, or equal to 3, the fish undergoes positive or negative allometric growth, and isometric growth respectively (Mansor *et al*. 2010). The condition factor "K" reflects fish health and environmental suitability (Mansor *et al*. 2012).

In the present study the LWR "b" values were approximately 2.5, indicating negative allometric growth (Table 3). The recorded "R^2" values were 0.98, 0.97, 0.98 and 0.94 for control, low, intermediate and high stocking densities, respectively, which are similar. The correlation between length and weight was also significant ($p < 0.05$) in all densities, including the control. Moreover, the mean "K" value was about the same for all treatments (Table, 3), and did not differ significantly among treatments ($p > 0.05$).

Discussion

Stocking density is very important for aquaculture as it can influence fish yield and growth. In this study positive growth occurred in all treatments. However,

Table 1: One way ANOVA results for water quality parameters between hybrid density treatments in tanks. ($p > 0.05$)

Parameter	Density treatments				
	5 fish/m³(control)	10 fish/m³	15 fish/m³	20 fish/m³	p
Temperature	27.32±0.42	27.65±0.12	27.6 ±0.2	27.86 ±0.15	0.51
Dissolved oxygen	6.33 ±0.11	6.31 ±0.07	6.26 ±0.04	6.28 ±0.08	0.6
p H	7.69 ±0.07	7.68 ±0.09	7.75 ±0.09	7.77 ±0.08	0.84

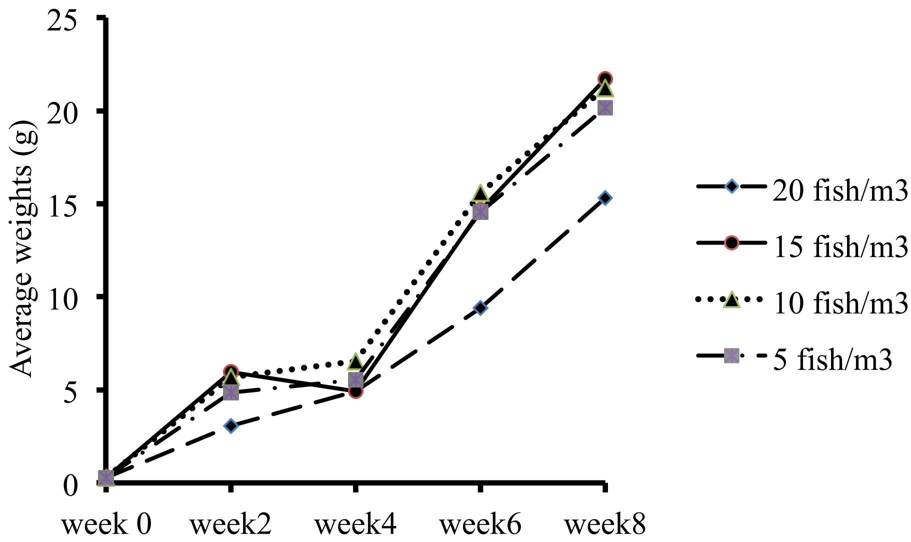

Figure 2. Increase in weight of the hybrids with time at different stocking densities, where week 0 refers to the initial average weights measured.

the observed overlaps in growth may be due to management anomalies (Alhassan *et al*., 2012). For instance, the 15 fish/m³ treatment showed a slight decline in growth in week four. The low stocking density (10 fish/m³) exhibited better growth in terms of weight gain, SGR, FCR and survival rate, than intermediate (15 fish/m³), high (20 fish/m³), and control stocking densities, respectively. However, none of these measures were significantly different at the 95% confidence level. The results are similar to Alhassan *et al*. (2012), and Chakraborty and Banerjee (2010) who observed reduced growth rates of *O. niloticus* at high stocking densities in concrete tanks and hapas in Ghana, and in all-male *O. niloticus* reared in ponds in the Gangetic plains of West Bengal, India, respectively. Reduced growth performance of hybrids at high stocking density may be due to extra energy expenditure on competition for food and space. High stocking density also reduces water quality, resulting in poor fish health. Improved growth at low stocking density may therefore be attributed to favourable water quality factors (Osofero *et al*., 2009) and better feed quality. In this study, a balanced diet for hybrids was prepared fortnightly using locally available feeds to ensure quality. All treatments had better growth rates than the control. According to Yan *et al*. (2002), low growth performances at very low densities are due to strong aggressive behaviour. Mbiru *et al*. (2016) described high growth rates in hybrids of *O. niloticus*♀ and *O. urolepis hornorum*♂. The authors stated that the high growth rates found were due to the effect of hybrid heterosis inherited from parents.

The survival rates were greater in low and intermediate stocking densities than high and control stocking

Table 2. One way ANOVA results for growth performance indices of hybrids. Means without common superscripts in a row are significantly different ($p < 0.05$,Tukey test).

Parameter	5 fish/m³(control)	10 fish/m³	15fish/m³	20 fish/m³	p
Initial mean weight(g)	0.29±0.5[a]	0.29±0.5[a]	0.27±0.5[a]	0.28±0.5[a]	0.31
Final mean weight (g)	20.14±6.66[a]	22.22±9.05[ab]	21.09±11.2[ab]	15.31±8.59[ac]	0.03
Yield (g)	362.52	1562.28	2642.4	4627.2	-
Weight gain	19.85±7.38[a]	21.93±7.47[a]	20.83±7.91[a]	15.03±5.45[b]	0.84
Survival rate (%)	90±12.2[a]	95±14.2[a]	92±12.37[a]	88±13.1[a]	0.25
FCR	1.35±2.34[a]	1.01±1.99[a]	2.30±5.06[a]	2.85±4.18[a]	0.2
SGR (%/day)	6.84±0.50[a]	8.33±0.60[a]	8.31±0.49[a]	7.8±0.60[a]	0.21

Table 3. Parameters for hybrids length-weight relationship at different density treatments.

Density (fish/m³)	L-W Relationship			
	a	b	R^2	Mean K
Control	-1.14	2.58	0.98	2.73
10	-1.15	2.51	0.97	2.83
15	-1.12	2.46	0.98	3.06
20	-1.11	2.48	0.94	3.11

densities, but not statistically significant. The results agree with Chakraborty and Banerjee (2010), who documented an inverse relationship between high stocking density and fish survival. However, in this study the maximum survival rate of 90% was above the 84.5% found by Chakraborty and Banerjee (2010), and the 86.78% found by Alhassan *et al*. (2012). In addition, acclimation, sampling method and tank cleaning mechanisms can account for lower survival rates. In adapting fish to live in saline conditions, some were unable to withstand the harsh salty conditions and water mixing procedures and died. Scoop nets that were locally made of iron materials were noted to cause injuries to fish during the sampling process and could lead to injury and later mortality. Similarly, siphoning using a plastic pipe may have caused deaths in the tanks if fish were injured by the pipe, or by swimming into the tank walls in an attempt to evade the pipe. According to Ronald *et al.* (2014), high stocking density leads to high mortalities because of poor water quality at densities above the carrying capacity of the culture unit.

The ability of hybrids to convert food into biomass (FCR) was not significantly affected by stocking density. The best FCR was observed at the low stocking density, implying better food utilization at low density. Current findings correspond with those of other authors who documented non-significant FCR from monosex male *O. niloticus* fry reared in hapas in ponds, and hybrids of *O. mossambicus* and *O. niloticus*, respectively, due to water quality factors, stress levels of fish and feed sources (Ferdous *et al*., 2014; Alhassan *et al*., 2012; Guimaraes *et al*., 2008, Yan *et al*., 2002). In this study hybrids were fed with the same basal feed and raised in water coming from the same source. However, the FCR increased with stocking density, similar to the findings of Ferdous *et al*. (2014) and Ronald *et al*. (2014). Common reasons for poor FCR are stress, and high crude fibre content in diets (El-Sherif and El-Feky, 2009; Ali and Al-Asgall, 2001). El-Sherif and El-Feky (2009) reported that increased stress caused increased cortisol production resulting in inappropriate dietary energy utilization due to physiological alterations, leading to poor growth. The 1.01-2.85 FCR in this study was less than the 3.4-4.0 range recommended for *O. niloticus* (Liti *et al*., 2006). However, the results fall within the recommended FCR for *O. niloticus,* a faster grower.

SGR values in this study are higher than the 0.77-1.49 values reported for red tilapia by Iluyemi *et al*. (2010). The SGR values of 8.33, 8.3, and 7.8 are also higher than the 4.72 and 4.16 reported by Mtaki (2015) for hybrids reared in concrete tanks. Besides the quality of the feed used, heterosis for growth performance inherited from both *O. niloticus* and *O. urolepis urolepis* parents may account for higher SGR in this study. Weight gain and final mean weight were higher in the low stocking density, corresponding to the results of Ferdous *et al.* (2014). However, final mean weight varied significantly between intermediate and high stocking density.

Condition factor describes fish health and fitness in the environment (Mbiru *et al*., 2016). The mean "K" values in this study were higher than the 1.02 and 1.12 reported for *O. niloticus* (Migiro *et al*., 2014), 0.53 for *O. urolepis urolepis* (Nehemia *et al*., 2011) and 1.71 for *O. niloticus*♀ and *O. urolepis hornorum*♂ hybrids (Mbiru *et al*., 2016). The current findings concur with Mahomoud *et al.* (2011) who described higher "K" values in tilapia males; which indicates good fish health (Froese, 2006). The present mean "K" values suggest an increase in hybrid weight relative to length which indicates suitability of the applied stocking densities for fish growth in tanks.

The hybrids of *O. niloticus*♀ and *O. urolepis urolepis*♂ can be used as an alternative to hormonally reversed males for aquaculture, as hybridization produced 100% males. In addition, this study shows that farmers can potentially utilize the hybrids to obtain fish of market size earlier, because of their faster growth rates. The potential shown by this hybrid for aquaculture needs to be investigated further in Tanzania.

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