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Hybrids production as a potential method to control prolific breeding in tilapia and adaptation to aquaculture climate-induced drought

Kulwa Mtaki [a], Samwel Mchele Limbu [b,c,*], Aviti John Mmochi [a], Matern S.P. Mtolera [a]

[a] Institute of Marine Sciences, P. O. Box 668, University of Dar es Salaam, Zanzibar, Tanzania
[b] Department of Aquaculture Technology, School of Aquatic Sciences and Fisheries Technology, University of Dar es Salaam, P. O. Box 60091, Dar es Salaam, Tanzania
[c] East China Normal University, Department of Biology, School of Life Sciences, Laboratory of Aquaculture Nutrition and Environmental Health, 500 Dong Chuan Road, 200241, Shanghai, China

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ABSTRACT

Establishing suitable salt tolerant all-male tilapia strains with fast growth rate is a crucial requirement for successful euryhaline farming required for controlling prolific breeding and as an adaptation to the effect of climate-induced drought. This study assessed the percentage of tilapia male hybrids, their growth performance and survival rate produced by crossing female Nile tilapia (*Oreochromis niloticus*) and male Rufiji tilapia (*Oreochromis urolepis urolepis*) at different salinities. The fingerlings were reared for 70 days in triplicate concrete tanks (1 m^3), each stocked with 15 fish weighing 1.62 ± 0.03 g, 1.69 ± 0.02 g and 1.41 ± 0.06 g exposed to 2, 15 and 35 ppt salinities, respectively. Acclimatization was done by adding seawater to freshwater at a rate of 2 ppt per day for 18 days. Fish sexing was done by visual assessment of the genital papilla followed by rearing the hybrids for six months to observe reproduction. Results showed that, the cross between *O. niloticus* (female) and *O. urolepis urolepis* (male) produced hybrids that were all-males without any reproduction within six months post hybridization. The hybrids reared at a salinity of 2 ppt had higher growth performance than those reared at 15 and 35 ppt. Similarly, hybrids cultured at 15 ppt had significantly higher growth performance than those reared at 35 ppt. All the hybrids produced had survival rates of 100%. This study indicated that, all-male tilapia produced by crossing between *O. niloticus* (female) and *O. urolepis* (male) can be used by fish farmers as alternative species for mariculture development to control prolific breeding and as an adaptation strategy to climate change.

1. Introduction

Tilapia is one of the most common farmed fish in the world, second only to carps in terms of total production (Wanatabe, Losordo, Fitzsimmons, & Hanley, 2002) and is expected to be the most important cultured fish in the near future. Despite its desirable traits as a cultured species, tilapia has a tendency to breed early and overpopulate culture systems especially earthen ponds (Kapinga et al., 2019). The overcrowding results in shortage of space and competition for food, which leads to stunted growth (Siddik, Khan, Hassan, & Hossain, 2007). One of the solutions for controlling prolific breeding is to consider mono-sex culture, preferably all-male tilapia because males grow at a faster rate and uniform size than females (Celik, Guner, & Celik, 2011).

Several techniques have been adopted for production of all-male tilapia such as manual sexing, genetic manipulation, sex reversal and hybridization (Phelps & Popma, 2000). Among these techniques, hybridization has been reported to produce offspring that perform better than both parental species (Mbiru et al., 2015) by combining valuable traits from two species into a single strain. Several tilapia species have been used to produce hybridizations (Hickling, 1960; Pruginin, 1967; Mbiru et al., 2015). Wami tilapia (*Oreochromis urolepis hornorum*) male x Nile tilapia (*O. niloticus*) female were the crosses used by Mbiru et al. (2015) and Pruginin (1967) while *O. urolepis hornorum* male x Mozambique tilapia (*O. mossambicus*) female were used by Hickling (1960) and crosses between *O. niloticus* female x *urolepis urolepis* male were done by Mapenzi and Mmochi (2016). These crosses produced 100% male hybrids.

Rufiji tilapia (*O. urolepis urolepis*) and Wami tilapia (*O. urolepis hornorum*) are currently considered as sub-species of the same species, which are genetically related although they have been treated as

* Corresponding author. Department of Aquaculture Technology, School of Aquatic Sciences and Fisheries Technology, University of Dar es Salaam, P. O. Box 60091, Dar es Salaam, Tanzania.
E-mail addresses: mchelelimbu@yahoo.com, limbu@udsm.ac.tz (S.M. Limbu).

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separate species for long period (Trewavas, 1983). In this light, *O. urolepis urolepis* can be assumed to have similar reproductive behavior as *O. urolepis hornorum* male when crossed with either *O. niloticus* or *O. mossambicus* female. However, information on the performance of the hybrids produced by crossing *O. urolepis urolepis* male and *O. niloticus* female is still limited (Mapenzi & Mmochi, 2016).

Developing brackish and seawater tilapia farming is an important strategy to tackle drought-induced by climate change coupled with shortage of freshwater due to competing demands (e.g. domestic, agriculture and industrial consumption) (El-Sayed, 2006, p. 277). Thus, identification of tilapia hybrids, which are well suited to survive and grow in brackish and seawater is an important milestone towards establishing a climate change adaptation strategy. Many authors have reported on the salinity tolerance of different tilapia hybrids such as *O. urolepis hornorum* (♂) x *O. mossambicus* (♀) (Hickling, 1960), *O. niloticus* (♀) x *O. aureus* (♂) (Hulata, Wohlfarth, & Halevy, 1988), *O. mossambicus* (♀) x *O. niloticus* (♂) (Lim, Leamaster, & Brock, 1993) and *O. niloticus* (♀) x *urolepis urolepis* (♂) (Mapenzi & Mmochi, 2016). Results from these studies showed various tolerance levels of the hybrids to salinity, suggesting their potential use as an adaptation strategy for climate-induced drought.

Studies on salinity tolerance for hybrids produced by crossing *O. niloticus* (♀) and *urolepis urolepis* (♂) is currently limited (Mapenzi & Mmochi, 2016). In a previous study, Mapenzi and Mmochi (2016) studied the effect of salinity in tilapia hybrid as an alternative to the use of hormones in sex reversal. However, information on using tilapia hybrids for promoting mariculture development and adaptation to climate-induced drought is currently unavailable. Therefore, this study assessed the efficacy of all male tilapia hybrids production resulting from *O. niloticus* (♀) x *O. urolepis urolepis* (♂), their growth performance and survival rate when reared at 2, 15 and 35 ppt salinities as a method to control prolific breeding and adaption to climate-induced drought. This information is required by fish farmers, scientists and policy makers for promoting mariculture development and adapting to climate-induced drought.

2. Materials and methods

2.1. Experimental site and source of broodstock

The experiment was carried out for 98 days at the Institute of Marine Sciences Mariculture Centre (IMS-MC) located at the Pangani River Estuary in Pangani District, Tanzania. The *O. niloticus* females with a mean weight of 63.45 ± 3.49 g (mean ± SE) were collected from the wild in Lake Victoria, Mwanza and *O. urolepis urolepis* males with mean weight of 140.52 ± 11.45 g (mean ± SE) were obtained from IMS-MC earlier collected from Rufiji River (Ulotu, 2014). The two species were morphologically identified using manual by Eccles (1992, p. 145).

2.2. Experimental facilities

The experimental design comprised two conditioning tanks each with 3.5 m diameter and 1 m height. Five tanks (1 m^3) were used for spawning brooders. Freshwater was pumped from a borehole into two 5000 L storage plastic tanks by using an electrical pump. One 10,000 L tank was used for storage of salt water pumped from Pangani River. Freshwater and seawater were supplied to the experimental tanks from respective storage tanks by gravity using water pipes.

2.3. Conditioning of broodstock and spawning

The brooders were kept in two separate round tanks with 3.5 m diameter and 1 m water depth for two weeks. Females broodstock with swelling urogenital papilla as an indication of readiness to spawn were selected for spawning. Ready to spawn males were determined by stripping out the milt. The ready to spawn brooders were removed from the conditioning tanks and placed into 1 m^3 spawning tanks (five tanks were used) at a sex ratio of 2 female: 1 male. They were allowed to breed naturally in the spawning tanks. Daily observation on the presence of fry was made in the spawning tanks. The salinity of the water in the conditioning and spawning tanks was maintained at 2 ppt. During the conditioning and spawning periods, the spawners were fed by using a pelleted feed containing 40% crude protein comprised of fishmeal (700 g kg^{-1}), maize bran (210 g kg^{-1}), herring oil (30 g kg^{-1}), cassava flour (30 g kg^{-1}) and vitamin premix (30 g kg^{-1}) two times daily at 09:30 a.m. and 04:00 p.m. at a rate of 3% of their body weight.

2.4. Saline water acclimation of tilapia hybrids

After spawning, 30 newly hatched fry were collected randomly by using a scoop net and transferred into a 1 m^3 nursery tank in triplicates at three levels of salinity and reared for 70 days. The first level was a control, with a salinity of 2 ppt and the remainders were treatments whose fry were gradually acclimated to 15 ppt and 35 ppt salinities. Salinity was raised at a rate of 2 ppt per day by adding proportionally seawater to freshwater for 8 days and 18 days for the 15 ppt and 35 ppt, respectively. The fry were allowed to acclimatize for two weeks after the required levels of salinity had been obtained.

2.5. Growth performance and survival rate of tilapia hybrids

After acclimatization, the fry were reared on the same tanks for 70 days. Twenty-four hours before the experiment, the initial body weights of 15 individual fish in each treatment were measured by using a sensitive weighing balance (BOECO BEB 61, Boeco Germany) to adjust feeds quantity. The same number of fish in each tank were sampled by using a scoop net every two weeks for consecutive measurements of their wet weight for determining growth performance and then released back into their respective tanks. Every week, 20% of the water by volume in each tank was exchanged to ensure optimum water quality while maintaining the required salinity levels throughout the experimental period.

Fish mortality was observed on daily basis. All rearing tanks during the present study were supplied continuously with aeration by using air stone aerators connected to an air pump. All fish were fed on a formulated pelleted feed used during conditioning and spawning until the end of the experiment. Fry were fed three times a day at 8.30 a.m., 12.00 p.m. and 4.00 p.m. at the rate of 10% of their body weight at the first month and thereafter reduced to 5% of their body weight to reflect decrease in metabolic rate as fish grow (Limbu et al., 2016). At the end of the experiment, all fish in each tank were fasted for 24 h and their weight measured as described previously for determination of final body weight (FBW), growth performance. The number of fish in each tank were counted for estimation of survival rate. Growth performance parameters such as specific growth rate (SGR), daily weight gain (DWG), weight gain (WG), length weight relationship and survival rate (SR) were calculated at the end of the experiment by using the formulae below:

(i) $SGR\ (\%\ day^{-1}) \frac{\ell n\ (\text{Final weight}) - \ell n\ (\text{Initial weight})}{\text{Culturing time (days)}} \times 100$

(ii) $WG\ (g) = Final\ weight\ (g) - Initial\ weight\ (g)$

(iii) $DWG\ (g\ day^{-1}) \frac{Weight\ gain\ (g)}{Culturing\ time\ (days)}$

(iv) $SR = \frac{Number\ of\ fish\ harvested}{Number\ of\ fish\ stocked} \times 100$

After the experiment, fish were reared for another one month and sexed after 98 days according to Popma and Masser (1999) to determine the percentage sexes. The fish were further monitored for six months to observe reproduction activities in each tank.

2.6. Length-weight relationship and condition factor

At the end of the experiment, individual total length (cm) and weight (g) of each experimental fish were measured by using a measuring board and a sensitive weighing balance (BOECO BEB 61, Boeco Germany), respectively. Fish were mopped by using a filter paper in order to reduce excess water from their body before weight and length measurements. The total length of each fish was measured as a distance from the snout to the tip of the caudal fin. The relationship between total length (L), and total weight (W) of hybrids (*Oreochromis niloticus* and *O. urolepis urolepis*) were then analyzed by using the equation; $W = aL^b$ (Pauly, 1993, p. 583), which was then transformed logarithmically as proposed by LeCren (1951) to obtain a formula:

$$\text{Log } W = b \log L + \log a$$

where W = weight of fish in g, L = length of fish in cm, b = weight at unit length (slope), a = describes the change of weight with length (intercept).

The value of b was obtained by using linear regression analysis of total length and weight of the experimental fish raised at different levels of salinities. The data from all the three replicates of hybrids at 2, 15 and 35 ppt were used to construct regression equations for each treatment.

The condition factor of the experimental fish was also evaluated in order to determine their degree of well-being in their culture environment by using the equation:

$$K = 100\ W/L^3$$

where K = condition factor, W = the weight of the fish in gram (g) and L = the total length of the fish in centimenters (cm).

2.7. Measurement of water quality parameters

Water quality parameters such as pH, dissolved oxygen, temperature and salinity were measured twice a day at 10:00 and 16:00 h. Water pH was measured by using pH meter (HANNA model No. HI 98128), dissolved oxygen (DO) and water temperature were determined by using Oxy-guard meter (HANNA model No. HI 98186) and salinity was measured using a hand-held refractometer (RHS-10 ATC). Water samples for analyses of ammonia and nitrite concentrations were collected on weekly basis and analyzed at the Institute of Marine Sciences (IMS) laboratory of the University of Dar es Salaam by using phenol method (Parsons, Maita, & Lalli, 1984, p. 170). All water quality parameters were within optimal ranges for the growth and survival of tilapia. Water temperature ranged from 25.65 °C to 29.05 °C, DO from 5.05 to 8.45 mg L^{-1}, pH from 7.3 to 8.4, nitrite from 0.01 to 0.06 mg L^{-1} and un-ionized ammonia NH_3 from 2.10×10^{-3} to 1.26×10^{-3} mg L^{-1}.

2.8. Data analysis

The results are presented as mean ± SE (standard error of the mean). The data were first tested for normality and homogeneity of variances by using Kolmogorov–Sminorv and Levene's tests, respectively. Differences on parameters measured among treatments were determined by using one-way analysis of variance (ANOVA). Where significant differences existed among treatments means, Post-hoc analysis was done by using Tukey's Honest Significant Difference Test (Steele & Torrie, 1980, p. 666). Multiple regression analysis was used to determine the relationship between measured parameters and salinity levels. One sample *t*-test was used to determine if b values obtained for the hybrids reared at different salinities was significant different to the isometric growth value (b = 3.00). All statistical analyses were performed by using SPSS version 20 (IBM, Armonk, NY, USA) for Windows. Results were considered statistically significant at $p < 0.05$.

3. Results

3.1. Efficacy of all-male tilapia fingerlings production

The hybrids produced in the current study were all males (100%). No fry was obtained from the hybrids six months after termination of the experiment.

3.2. Growth performance and survival rates of hybrids

Growth performance parameters of hybrids at different salinities are shown in Fig. 1. Results showed that hybrids cultured at a salinity of 2 ppt grew faster than those reared at 15 and 35 ppt. Clear separation of growth among the salinity levels became apparent during the first 14 days of culture. The final body weight of the hybrids was significantly affected by salinity ($p < 0.001$; Fig. 1). Hybrids raised at 2 ppt had higher final body weight than those reared at 15 ppt ($p = 0.018$) and 35 ppt ($p < 0.001$). The final body weight of hybrids reared at 15 ppt was significantly higher than those reared at 35 ppt ($p < 0.001$).

All the measured growth performance parameters decreased with increasing salinity (Table 1). Salinity affected significantly the WG and DWG of the hybrids ($p < 0.001$). Hybrids reared at 2 ppt recorded higher WG and DWG than those reared at 15 ppt ($p = 0.018$) and 35 ppt ($p < 0.001$). Similarly, hybrids reared at 15 ppt had statistically higher WG and DWG than those raised at 35 ppt ($p < 0.001$). The weight gain and final weight decreased negatively with increasing salinity ($p = 0.049$; $R^2 = 0.988$; Fig. 2) and ($p = 0.046$; $R^2 = 0.989$; Fig. 2), respectively.

Furthermore, salinity affected significantly the SGR of the tilapia hybrids ($p < 0.001$). The SGR of the tilapia hybrids reared at 2 ppt was higher than those reared at 15 ppt ($p = 0.025$) and 35 ppt ($p < 0.001$). Also, the SGR of tilapia hybrids reared at 15 ppt was statistically higher than those raised at 35 ppt ($p = 0.002$). The SGR decreased significantly as salinity increased ($p = 0.015$; $R^2 = 0.999$; Fig. 3).

The survival rate was 100% in all treatments throughout the experimental period.

3.3. Length weight relationship

The "b" values of the tilapia hybrids were 2.69 ± 0.03 at 2 ppt (Fig. 4A), 2.73 ± 0.03 at 15 ppt (Figs. 4B), and 2.68 ± 0.03 at 35 salinity (Fig. 4C). The b values for 2 ppt ($p = 0.007$), 15 ppt ($p = 0.016$) and 35

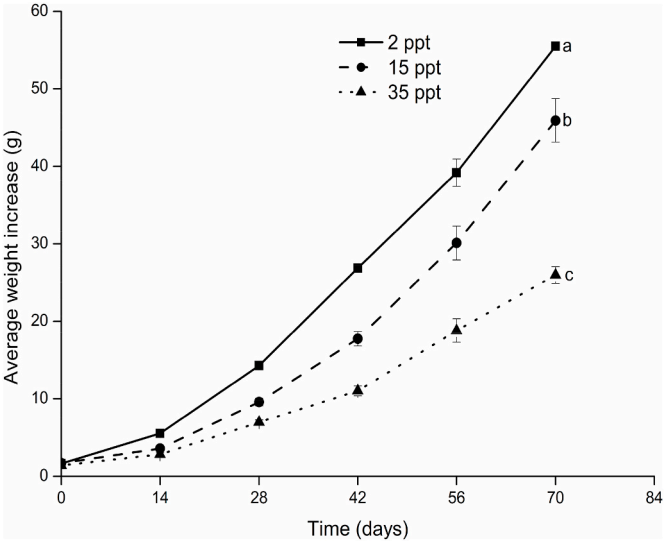


Fig. 1. Mean weight increments of tilapia hybrids reared at 2, 15 and 35 ppt salinities during the study period. Different letters (a, b and c) indicate significant differences ($p < 0.05$).

Table 1
The growth performance of tilapia hybrids reared at different salinities during the study period.

Parameters	2 ppt	Treatments 15 ppt	35 ppt
Initial mean body weight (g)	1.62 ± 0.03[a]	1.69 ± 0.02[a]	1.55 ± 0.08[a]
Final mean body weight (g)	55.50 ± 0.09[a]	45.92 ± 2.80[b]	25.96 ± 1.08[c]
Weight gain (g)	53.89 ± 0.12[a]	44.23 ± 3.45[b]	24.41 ± 1.24[c]
Specific growth rate (% day^{-1})	5.05 ± 0.03[a]	4.72 ± 0.11[b]	4.16 ± 0.02[c]
Daily weight gain (g day^{-1})	0.77 ± 0.00[a]	0.63 ± 0.04[b]	0.35 ± 0.01[c]
Survival rate (%)	100[a]	100[a]	100[a]

Values in each row with different superscripts ([a, b, c]) denote significant differences ($p < 0.05$).

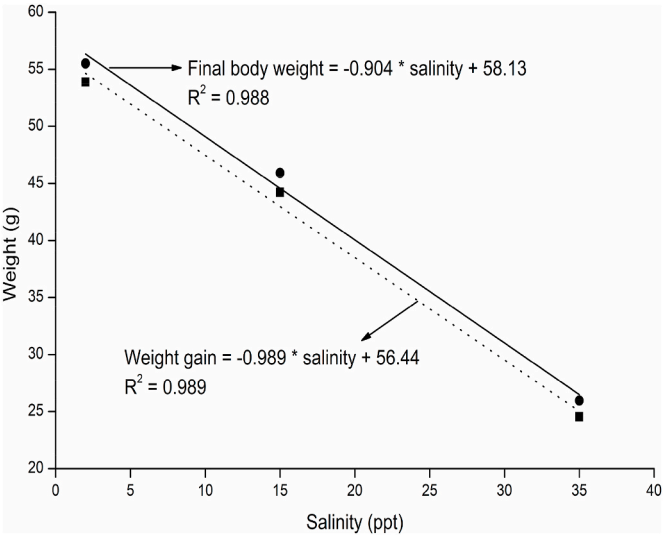


Fig. 2. The relationship between salinity (ppt) and mean weight gain (g) and final mean weight (g) of *Oreochromis niloticus* (♀) x *O. urolepis urolepis* (♂) hybrids cultured in earthen ponds for 70 days.

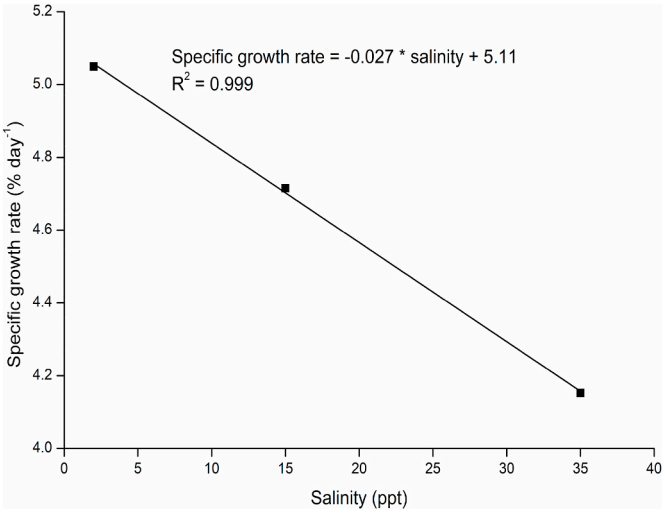


Fig. 3. The relationship between salinity (ppt) and specific growth rate (% day^{-1}) of *Oreochromis niloticus* (♀) x *O. urolepis urolepis* (♂) hybrids cultured in earthen ponds for 70 days.

ppt ($p = 0.006$) were significantly lower than the isometric growth (b = 3). Thus, experimental fish increased more in length than their individual weight, hence became slender. The condition factor of the tilapia hybrids raised at salinity 2, 15 and 35 were 4.06, 3.69 and 3.73, respectively, without any significant differences among treatments. The condition factor decreased significantly as salinity increased ($p = 0.041$, $R^2 = 0.992$; Fig. 4D).

4. Discussion

The results showed 100% male tilapia can be produced by crossing between *O. niloticus* (female) and *O. urolepis urolepis* (male). Previous studies (Fishelson, 1962; Hickling, 1960; Mapenzi & Mmochi, 2016; Mbiru et al., 2015; Pruginin, 1967) reported 100% male tilapia production through hybridization of different species. The results of the current study are consistent with these findings. This is unlike the hybridization between *O. aureus* (♂) and *O. mossambicus* (♀) or *O. niloticus* (♀), which produced 85–99% males Wohlfarth (1994) and El-Zaeem and Salam (2013) produced 77.05% of male tilapia by crossing (*O. aureus* x *O. niloticus*). Furthermore, Badawy and Rizkalla (1996) reported production of 79.8 to 84.2% male hybrids on the crosses between *O. aureus* (♂) and *O. niloticus* (♀). The production of 100% males in the present study maybe attributed to the genetic purity of the parent stocks (Fortes, 2005, pp. 492–679) among other factors. Thus, fish famers with access to the two species can hybridize them to obtain 100% males for stocking in their ponds as a method to control prolific breeding.

All growth performance parameters (SGR, DWG, FBW and WG) decreased with increasing salinities (Figs. 1–3). However, in comparison to the study conducted by Ulotu (2014, p. 84) on *Oreochromis urolepis urolepis*, the hybrids in the present study had improved growth rates at all salinities. These results are supported by Liao & Chang (1983) who reported faster growth of all-male Taiwanese red tilapia in freshwater than in seawater. Furthermore, Mena-Herrera, Sumano-Lopez, and Macias-Zamora (2002) reported decrease in hybrid red tilapia (*O. mossambicus* × *O. niloticus*) growth as salinity increased. These authors concluded that, salinity above 15 ppt impairs hybrid growth rate. Similar observation was reported by Verdegem, Hilbrands, and Boon (1997) who noted that the growth rate of hybrid red tilapia (*O. mossambicus* × *O. niloticus*) decreased as salinity increased and recommended 19 ppt as a maximum salinity for their farming.

On the contrary, some studies such as Liao & Chang (1983), Watanabe et al. (1997) and Mapenzi and Mmochi (2016) reported that tilapias grow better in brackish and seawater than freshwater. The contradicting results on growth of tilapia hybrids might be related to the differences in salt tolerance traits of the different fish species and duration of acclimatization. The higher growth rate of hybrids in salinity of 2 ppt than 15 ppt and 35 ppt in the present study could be attributed to the inherited higher freshwater growth rate character of *O. niloticus*. Fang and Chiou (1989) reported that, growth rate was 60% slower in *O. niloticus* reared in seawater than in freshwater. Villegas (1990, pp. 507–510) reported that *O. niloticus* grew better at salinities from 0 to 10 ppt and slower at 25 to 32 ppt with optimum growth at 7.5 ppt. Therefore, the results of the present study suggest that hybrids produced by crossing *O. niloticus* (female) and *O. urolepis urolepis* (male) can be cultured in both freshwater and seawater with better growth achieved in freshwater and brackish water. The culture in brackish water could be used as an adaptation to climate-induced drought in aquaculture. Studies establishing the maximum salinity level that impairs growth rate are required to provide a guidance to fish farmers.

In the present study, the survival rates of tilapia hybrids were 100% in all treatments. Similar results were reported by Jaspe, Caipang, and Corre (2007) who produced saline-tolerant tilapia hybrids by crossing *O. mossambicus* with another hybrid of *O. spilorus* × *O. niloticus* GIFT x *O. aureus*. The hybrids produced by Jaspe et al. (2007) tolerated high salinity levels of up to 50 ppt with 100% survival rates. High tolerance to salinity of the hybrid in this study might be due to the salinity tolerance character of *O. urolepis urolepis* (Ulotu, 2014). It has been shown that hybrids obtained by crossing species, which include a salt tolerant parent are highly salt tolerant (Noy, Lavie, & Nevo, 1987). Furthermore,

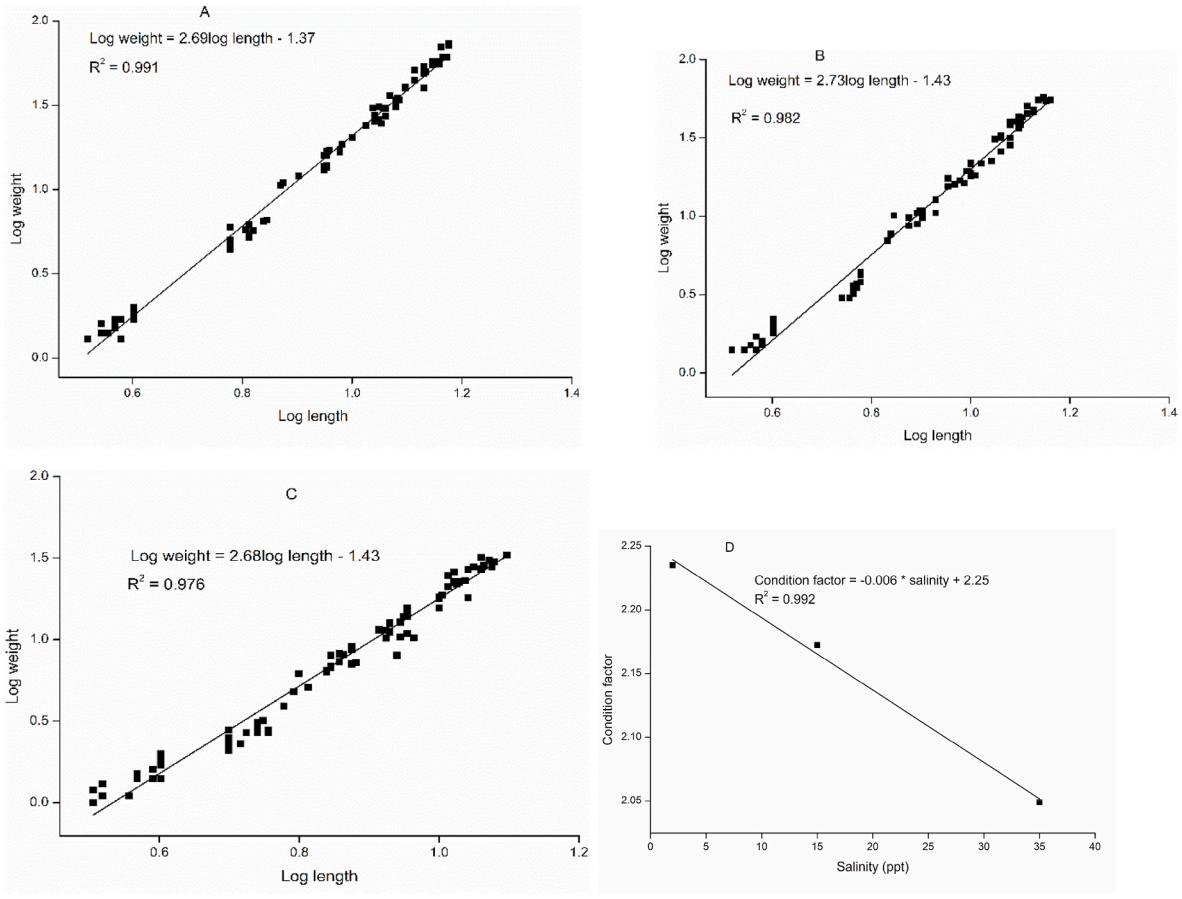


Fig. 4. Length-weight relationships of tilapia hybrids reared at three different salinities of 2 ppt (A), 15 ppt (B) and 35 ppt (C) and the relationship between condition factor and salinity (D).

male tilapias have higher tolerance to salt water than females (Watanabe, Kuo, & Huang, 1985, p. 22). Therefore, the present results suggest that the hybrids produced by crossing *O. niloticus* (female) and *O. urolepis urolepis* (male) are potential for culture in freshwater, brackish and full-strength seawater as a strategy to avoid climate-induced drought based on their survival rate.

In the present study, all hybrids underwent negative allometric growth pattern at all salinity levels. The condition factors decreased with increasing salinity levels (Fig. 4D). Similar results with 'b' value of 3 for female and 2.9 for male *O. urolepis urolepis* and *O. mosambicuss* hybrids were reported when reared in brackish water of the Salton Sea (Riedel, Caskey, & Hurlbert, 2007). In addition, Nehemia, Maganira, and Rumisha (2012) reported negative allometric growth with a 'b' value of 2.81 for *Oreochromis urolepis urolepis* in freshwater. On the contrary, the same author reported positive allometric growth pattern with "b" value of 3.46 when raised *Oreochromis urolepis urolepis* at salinity of 35 ppt. The differences in results could be attributed to salinity tolerances between the two species. The condition factors in this study were greater than 1 indicating that the experimental hybrids were in good condition. Establishing the maximum salinity level that impairs condition factor will provide clear guidance to fish farmers.

5. Conclusion

The results revealed that, the investigated cross pair gives 100% male hybrids with faster growth performance and 100% survival rate at salinity levels of 2 and 15 ppt. These results suggest that the hybrids have good potential for brackish and marine aquaculture. Thus, all-male tilapia hybrids produced by crossing *O. niloticus* (female) and *O. urolepis urolepis* (male) can be used by fish farmers as alternative species for mariculture development to control prolific breeding and as an adaptation to climate-induced drought in aquaculture. The full potential of their culture will be established after determining the maximum salinity levels at which growth rate and condition factors are impaired.

Declaration of competing interest

We have no conflicts of interest to disclose.

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