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FULL LENGTH ARTICLE

The effect of stocking density on the growth and survival of improved and unimproved strains of *Oreochromis shiranus*

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Abstract Growth performance and survival rate of improved strains (F_5 and F_6) and unimproved strain of *Oreochromis shiranus* were assessed. Three stocking densities (5, 7 and 9 fish/m^3) were used to randomly allocate fingerlings, of mean weight 6 ± 0.6 g, into 27 hapas of 9 m^3 each, fixed in a pond of 700 m^2 at Bunda Fish Farm, Malawi. Stocking density significantly ($P < 0.05$) affected the growth of the 3 strains though there was no significant difference between stocking density of 5 and 7 fish/m^3. The highest final weight was noted at a stocking density of 5 fish/m^3 with an average weight of F_6 being 28.1 g, followed by the F_5 (24.9 g) and the unimproved (24.0 g) strain. The improved strains had a higher final mean weight (F_6: 23.41 g, F_5: 21.84 g) than the unimproved strain (18.70 g) but there was no significant difference between improved strains ($P > 0.05$). The apparent genetic gain due to selection between the unimproved strain and F_5, F_6 strains was estimated to be 16.8 and 25.2%, respectively. Based on this, farmers can be encouraged to use F_6 strain at a stocking density of 5 fish/m^3. The revelation that there was no difference between the improved strains has implications on the continuity of the selection program.

Introduction

Fish provides about 72% of animal protein and is a hugely important food and economic security resource for Malawians (Ecker and Qaim, 2011). High population growth and alarming levels of poverty in Malawi have, however, caused an unprecedented overexploitation of this resource in the country's lakes and rivers (Jamu et al., 2011; van Zwieten et al., 2003). Potential fish production in these natural waters is already threatened by environmental degradation and habitat loss (Jamu et al., 2011). Consequently, per capita annual fish

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consumption has decreased from 14 kg in 1988 to half that figure in 1998 and to about 5.8 kg in early 2000, with a corresponding increase in fish prices (Jamu and Chimatiro, 2004; van Zwieten et al., 2003; Weyl et al., 2010). Continued disproportionate reliance on natural fisheries does not seem to be a sustainable option and aquaculture development is now considered a top priority in this regard (Chirwa, 2009; Gondwe et al., 2011; Russell et al., 2008).

The most cultured fish species in Malawi are Tilapias, namely *Tilapia rendalli*, *Oreochromis shiranus* and *Oreochromis karongae*, which together account for about 93% of production (Chirwa, 2009; Russell et al., 2008). These herbivorous-cum-omnivorous fish have a good aquaculture potential because they are exceptionally hardy and prolific, easy to farm and thus ideal for both small farmers and industrial sized aquaculture (Chirwa, 2009; Maluwa and Gjerde, 2006). One of the major problems facing small-holder aquaculture in Malawi is the precocious breeding of these species and the resultant fish density-driven stunting, compounded by lack of capacity to control fingerling numbers in the ponds (Chirwa, 2009; Russell et al., 2008). Various efforts have been made to determine appropriate stocking densities to circumvent this limitation but until now recommended stocking densities vary considerably (Russell et al., 2008). With limited land resources and high competition with agriculture for land, there is urgent need for refining these recommendations so that appropriate stocking densities are adopted in time to minimize production deficiencies per unit space available.

Growth, survival and yield effects of stocking density on aquaculture are well known for a diversity of species (Garr et al., 2011; Khatune-Jannat et al., 2012; Mazlum, 2007; Samad et al., 2005; Zhu et al., 2011) and seem to impact production differently. Both growth performance and survival rate, for instance, tend to be higher in lower stocking densities in the African catfish, *C. gariepinus* (Hecht et al., 1996), in the Thai climbing perch, *Anabas testudineus* (Khatune-Jannat et al., 2012), in Amur sturgeon *Acipenser schrenckii* (Zhu et al., 2011), in silver catfish *Rhamdia quelen* (Pouey et al., 2011), in Crayfish *Astacus leptodactylus* (Mazlum, 2007), in *Oreochromis* spp. (Sorphea et al., 2010) and in *Macrobrachium rosenbergii* prawns (Cuvin-Aralar et al., 2007), but only survival is higher under same conditions in *Oreochromis* spp. (Ridha, 2005). In some cases such an advantage of lower stocking densities is either non-existent, as is the case in channel catfish (Southworth et al., 2009), in *Oreochromis niloticus* (Osofero et al., 2009) in *Barbus luteus* (Gokcek and Akyurt, 2007) or temporary and wanes after sometime so that generally no differences occur across different stocking densities, as in the apple snail, *Pomacea paludosa* aquaculture (Garr et al., 2011).

Although they may promote competition for food and negatively influence reproductive success via reduced fecundity and egg quality (Tave, 1986), high stocking densities may sometimes have no effect on mortality rates and may actually enhance total fish yield (Abou et al., 2007; Gokcek and Akyurt, 2007; Khatune-Jannat et al., 2012; Pouey et al., 2011; Sorphea et al., 2010) and lead to higher gross and net return at a lower cost of production (Abou et al., 2007). Where land costs, fresh water, manpower and other facilities are limiting it may be more profitable to adopt higher stocking densities (Ridha, 2005). The rearing conditions adopted for a specific aquaculture program will therefore be a compromise between biological and economical requirements of the chosen species.

Because *O. shiranus* is a very popular aquaculture species of choice among most farmers in Malawi, a selective breeding program was established in 1996 at Malawi's National Aquaculture Centre in order to improve the growth of this valuable species (Maluwa and Gjerde, 2007). The breeding program has so far progressed up to sixth (F_6) generation. In this paper, we refer to the selectively-bred strain as the improved strain while the ordinary strain left unsubjected to selective breeding is referred to as the unimproved strain. Although some research focusing on production parameters has been conducted since this breeding program was introduced, there is still a paucity of documented experimental results that specifically deal with the effect of stocking densities on the growth and survival of this improved *O. shiranus*. The main aim of this experiment was to determine whether stocking density has any effect on the growth and survival of the improved F_5 and F_6 *O. shiranus* and whether, at a given stocking density, this improved strain differs in growth performance from the local unimproved strain.

Materials and methods

Fish collection and experimental set-up

The experiment was conducted at the Bunda fish farm in 9 m^3 hapas ($3 \times 3 \times 1$ m). The area was chosen because it has ponds suitable for the layout of the hapas, security and that the offspring of the improved strains and unimproved strain of *O. shiranus* were readily available. Juveniles of the improved F_5 and F_6 *O. shiranus* strain and its unimproved strain counterpart were collected using a hand net. The juveniles of improved strains (F_5 and F_6) were collected from broodstock belonging to F_4 and F_5 respectively and were then kept in the tanks. These fingerlings were stocked at densities of 5, 7 and 9 fish/m^3 at an average weight of 6 ± 0.6 g. Prior to sampling, the fish were not fed for 24 h. A total of 1701 juveniles (567 from each treatment population) were randomly allocated to 27 experimental hapas. A completely randomized design (CRD) was used to allocate three treatments replicated three times at three levels of stocking density (5, 7 and 9 fish)/m^3.

Fish were fed formulated feed of 29% crude protein to supplement the natural feed which was boosted by application of chicken manure at the rate of 500 kg/ha/week (Kang'ombe and Brown, 2008). A majority of non-commercial aquaculture farmers in sub-Saharan Africa are economically constrained and cannot sustain on-farm availability of inorganic fertiliser and consequently use compost cribs and some animal manure, especially Chicken manure, as alternatives (Hecht, 2007). Although use of manure above some threshold of quantity may risk introduction of fish parasites (Chimatiro, 1998), many empirical works on the use of animal manure in *Tilapia* ponds show that it does not compromise water quality, has no detrimental effects on fish survival rates and may actually enhance production when combined with other low cost materials (Kaggwa et al., 2009; Kang'ombe and Brown, 2008; Maluwa et al., 1995; Mataka and Kang'ombe, 2007; Tabaro et al., 2012). The feed was formulated from a mixture of maize bran as a carbohydrate source, soybean as a protein source and wheat flour as a binder in pellet formation. The fish were hand fed twice a day at 5% body weight in line with recommendations of Lovell (Lovell, 1989) by broadcasting. Despite being cumbersome, this method is relatively cheap, allows for the feeder to observe the fish when feeding and does not have any disproportionate effects on fish survival relative to other methods (Ludoviko and Kang'ombe, 2012). A sample size of 30% of the population was used. Because no feed was given to the fish a day prior to sampling, feeding was done for thirteen consecutive days between sampling.

Data collection

Data on growth and survival of *O. shiranus* were collected every fortnight for a period of two and half months. The growth and survival of the fish were calculated as follows;

Body weight gain (BWG)

Data on the increase in body weight were analyzed in percentage using the following formula

$$\%BWG = \frac{w_2 - w_1}{w_1} \times 100$$

where W_1 = initial mean weight, W_2 = final mean weight.

Specific growth rate (SGR)

$$\%SGR = \frac{ln\ w_1 - ln\ w_0}{t_1 - t_0} \times 100$$

where W_1 = final mean weight (g) of fish, W_0 = initial mean weight (g) of fish at stocking time, t = time in days, ln = natural log.

Survival Rate

$$\text{Surival rate }(\%) = \frac{\text{Number of fish at the end of the experiment}}{\text{Number of fish at the start of the experiment}} \times 100$$

Feed efficiency

Data on the feed efficiency were also collected and analyzed by calculating the apparent food conversion ratio (AFCR) using the formula below;

$$AFCR = \text{Dry weight of feed given/Gain in weight}$$

Fish were weighed using an electrical analytical balance calibrated to 0.01 g. A stainless steel topped board calibrated to the nearest mm was used to measure total length of the fish randomly selected from each hapa.

Water quality parameters

Some water quality parameters were checked both before stocking the hapas with fish and during the experiment. Samples of water were collected, twice a day for temperature and dissolved oxygen and once a week for pH and ammonia, from each hapa at roughly the same times of day using standard water sampling protocols as outlined by APHA (1995). This was done to ensure that water quality was maintained at levels that were high enough for and could not compromise sustained rapid growth of cultured fish (Boyd, 1997).

Data analysis

Data on growth and survival were analyzed using two-way Analysis of Variance (ANOVA) at 95% level of confidence. Least Significant Difference (LSD) was used to separate the means among the fish strains and stocking densities. GenStat statistical package (12th edition) (VSN International Ltd, Hemel Hempstead, UK) was used for analysis.

Results

The initial body weights of the three fish treatments ranged from 6.6 ± 0.2 g to 6.9 ± 0.2 g (Table 1 and Figs. 1–3). There were no significant differences in initial weights (p = 0.640) and lengths (p = 0.066) among the stocked fish across the three treatments (Table 1 and Figs. 1–3). Fish survival rate, which was above 90%, was also not significantly different among the fish treatments (p = 0.63) (Table 1).

The final weight of fish differed significantly among the fish treatments (p = 0.021) (Table 1). The improved F_6 and F_5 strains did not differ in their final mean body weight (F_6: 23.41 g vs F_5: 21.84 g, p = 0.100) but both had higher final mean weight than the unimproved strain (18.70 g). The proportion of this difference which could be attributed to selection was calculated and estimated to be 16.8% gain between the unimproved strain and the F_5 and 25.2% between the unimproved strain and the F_6.

Disparities in the average total body length among the three strains were not statistically different (F_6: 92.8 mm, F_5: 90.5 mm, unimproved: 88.9 mm, p = 0.572) (Table 1). AFCR differed significantly (p = 0.044, Table 1) among the strains and was lowest for F_6, intermediate in F_5 and highest in the unimproved strain.

Table 1 Initial and final mean weights, initial mean total length (TL_1), final mean total Length (TL_2), specific growth rate (SGR), apparent feed conversion ratio (AFCR), increase in weight and survival rate of F_5, F_6 and unimproved strain.

Fish treatment				
Parameter	F_5	F_6	Unimproved	P-value
Initial mean weight (g)	6.6 ± 0.2^a	6.7 ± 0.2^a	6.9 ± 0.2^a	0.640
Final mean weight (g)	21.84 ± 0.5^a	23.41 ± 0.4^a	18.70 ± 0.6^b	0.021
Mean total length (TL_1)(mm)	75 ± 0.4^a	74 ± 0.4^a	77 ± 0.4^a	0.066
Mean total length (TL_2)(mm)	90.5 ± 0.5^a	92.8 ± 0.5^a	88.9 ± 0.5^a	0.572
SGR (%/day)	0.424 ± 0.1^a	0.558 ± 0.1^c	0.336 ± 0.1^b	0.043
AFCR	3.18 ± 0.2^a	2.42 ± 0.3^b	4.47 ± 0.2^c	0.044
Increase in weight (%)	230.9	244.93	160.87	
Survival rate (%)	92.3^a	97.0^a	98.5^a	0.63

Figures with different superscripts within a row are significantly different (P < 0.05).

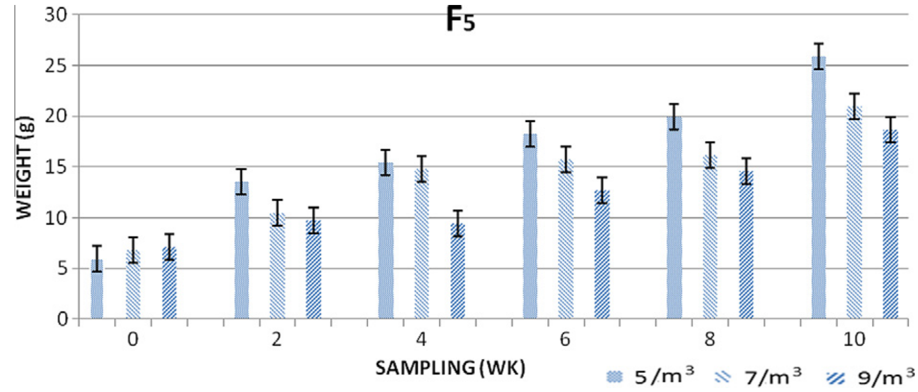

Figure 1 The mean growth of F_5 strain at three stocking densities.

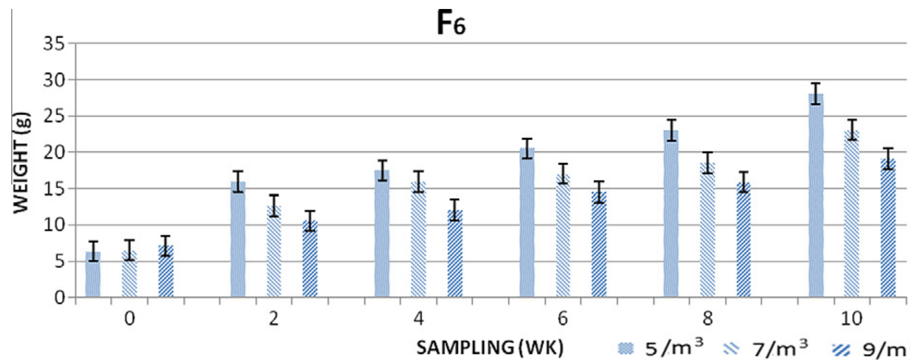

Figure 2 The mean growth of F_6 strain at three stocking densities.

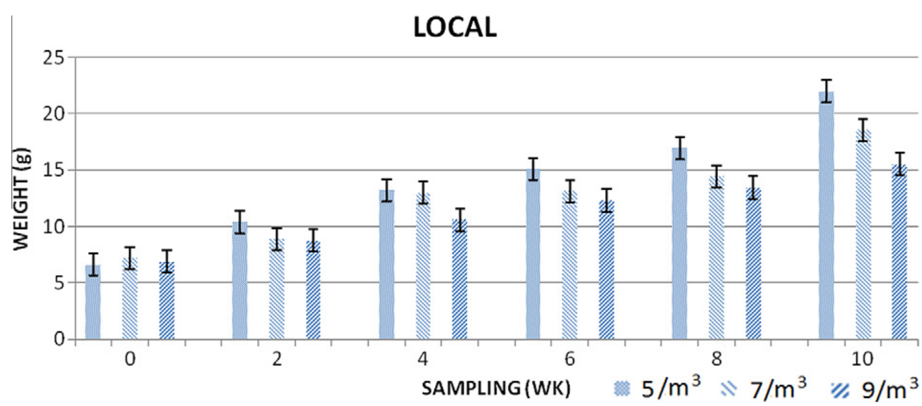

Figure 3 The mean growth of unimproved strain at three stocking densities.

Water quality parameters in the ponds were within the required ranges for the growth of *O. shiranus* during the experiment. Mean temperature ranged from 24.54 ± 1.5 °C to 29.66 ± 1.6 °C, dissolved oxygen (DO) from 4.12 ± 0.17 to 9.44 ± 0.17 mg/l, pH from 6.76 ± 0.5 to 8.01 ± 0.3 while ammonia ranged from 0.163 ± 0.02 to 0.204 ± 0.01 mg/l (Table 2). There were no differences in temperature ($p = 0.998$), dissolved oxygen ($p = 0.068$), pH ($p = 0.072$) and ammonia levels ($p = 0.057$) across the different stocking densities and fish treatments in all the hapas. Their fluctuations may not have had any differential effects on growth and survival.

Discussion

Although aquatic animals can be genetically improved for desired traits through a number of ways (e.g. hybridization, chromosome manipulation, sex control, transgenesis and selective breeding), selective breeding is the only approach that potentially allows for sustained permanent and heritable

Table 2 Water quality parameters (Mean ± SE).

Parameter	Time	1	2	3	4	5	6
Temperature (°C)	0900 h	25.04 ± 1.4	24.74 ± 2.1	25.10 ± 1.4	24.66 ± 2.2	24.54 ± 1.5	25.47 ± 1.1
	1600 h	29.06 ± 1.2	28.94 ± 2.3	29.41 ± 1.4	28.60 ± 1.6	28.30 ± 2.4	29.66 ± 1.6
Dissolved oxygen (mg/L)	0800 h	4.31 ± 0.09	4.12 ± 0.17	4.30 ± 0.02	4.40 ± 0.11	4.26 ± 0.90	4.32 ± 0.16
	1500 h	8.82 ± 0.16	9.01 ± 0.14	8.84 ± 0.08	9.21 ± 0.13	9.11 ± 0.04	9.44 ± 0.17
pH		7.04 ± 0.11	6.96 ± 0.22	7.98 ± 0.31	8.01 ± 0.3	7.12 ± 0.12	6.76 ± 0
Ammonia (mg/L)		0.163 ± 0.02	0.185 ± 0.04	0.200 ± 0.01	0.171 ± 0.02	0.204 ± 0.01	0.196 ± 0.04

genetic gain in the relevant traits (Lind et al., 2012; Ponzoni et al., 2007). Fish body weight has a genetic basis (Loukovitis et al., 2012; Nguyen Hong et al., 2010; Rutten et al., 2005) and should be expected to respond to selection (Loukovitis et al., 2012; Ponzoni et al., 2007). Tilapia fishes have been known to get genetically improved for body weight through selection (Nguyen Hong et al., 2010). This study revealed that the growth of selection-improved *O. shiranus* (F_6), in terms of the weight gain and specific growth rate (SGR), were significantly higher than that of the unimproved strain. This resonates with other findings that report selection-driven weight changes concomitant with genetic improvement in Nile tilapia, *O. niloticus*, selected for growth (Thodesen et al., 2012). The higher growth rate in the selection-improved F_6 strain may be attributed to the genetic superiority of this strain and, if so, lends support to the fact that genetic improvements on animal (and plant) species may significantly contribute to agricultural productivity and viability (Lind et al., 2012). The difference in the final mean weight between F_5 and F_6, albeit insignificant, may indicate that the selection of the brood stock for F_5 and for F_6 was rather precise and that the F_5 strain may still harbor substantial genetic variance that could allow for further phenotypic selection responses (Maluwa, personal communication).

During the experiment, one fish belonging to the unimproved strain (stocking density of 7 fish/m^3) was observed to be incubating some eggs in its mouth at a body weight of 6.3 g. This behavior occurred at a lower body weight than the 8 g previously observed for this strain (Maluwa, personal communication). All three strains had started breeding and fry were found in all the hapas by the time the last two sampling exercises were conducted. Precocious breeding is a major problem in Malawian small-holder aquaculture because it makes it hard to control fish densities and may lead to stunted growth (Chirwa, 2009; Russell et al., 2008). Our results imply that even the improved strain is prone to this problem; early breeding may therefore undercut the benefits of the selective breeding program.

Fish stocking density is one of the most important parameters affecting fish growth and health in a number of ways (Garr et al., 2011; Mazlum, 2007; Zhu et al., 2011). Growth performance and survival rate are adversely affected by high stocking densities (Pouey et al., 2011; Sorphea et al., 2010) but in some cases this effect is either temporary (Garr et al., 2011) or absent (Gokcek and Akyurt, 2007; Southworth et al., 2009). Some species can tolerate extreme crowding although competition for food will then limit their growth and lead to poor weight gain (Stickney, 1994). Such may have been the case in this study where fish stocked at higher stocking densities had poor growth. The results of this study also show that there was no significant difference in fish survival regardless of the strain and stocking density involved. Tilapias are hardy and can survive poor conditions including high stocking densities and will continue to reproduce even at very high densities (Delince, 1992).

How efficient has the selection programme from where the improved experimental strain was obtained for this study been over the 6 generations so far? This study estimated an apparent realized response to selection for *O. shiranus* growth of 25.2% at 6th generation. A realised response to selection of 101% for harvest body weight relative to the base population has been achieved within five generations of selection in *O. niloticus* (Olesen et al., 2003). Although our unimproved strain was a different species and may not fully be equated to the *O. niloticus* base population, the estimated selection response reported in this study for a comparable number of generations is relatively low. Lower than expected inter-generational genetic gains for harvest body weight in this strain have also been noted elsewhere in relation with low numbers of breeding candidates and resultant low selection intensity (Maluwa and Gjerde, 2007) although they do not seem very atypical and correspond with other results of a selection programme in *O. nilotiucs* (Thodesen et al., 2012).

Water quality plays a significant role in the biology and physiology of fish and may impact on the health and productivity of the culture system (Boyd, 1997; Landau, 1992). Throughout this experiment, water quality across all the treatments was within the favorable range required for tilapia (Boyd, 1997); the variation in fish growth in this study may not therefore be strictly attributed to the characteristics of water quality parameters.

In conclusion, the results indicate that the improved F_6 and F_5 strains have lower FCR and thus are more efficient at utilizing feed and growing better than the unimproved strain at stocking densities of 5 fish/m^3. These results support recommended Tilapia stocking densities that take into account transport and handling related mortalities (Russell et al., 2008) and should be practical for most small holder farmers in Malawi.

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