

On-farm Assessment of Different Fingerling Sizes of Nile Tilapia (*Oreochromis niloticus*) on Growth Performance, Survival and Yield

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How to Cite

Wainaina, M., Opiyo, M.A., Charo-Karisa, H., Orina, P., Nyonje, B. (2023). On-Farm Assessment of Different Fingerling Sizes of Nile Tilapia (*Oreochromis niloticus*) on Growth Performance, Survival and Yield. *Aquaculture Studies*, 23(2), AQUAST900. <http://doi.org/10.4194/AQUAST900>

Article History

Received 17 February 2022

Accepted 19 September 2022

First Online 06 October 2022

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Keywords

Aquaculture

Oreochromis niloticus

Fingerlings

Growth

Yield

Abstract

Rapidly increasing hatchery-raised Nile tilapia (*Oreochromis niloticus*) in most developing countries lack informed recommended fingerling weight for stocking in semi-intensive grow-out ponds. The current study assessed the growth performance, survival and productivity of all-male Nile tilapia fingerlings of 0.2 g, 1 g, and 5 g in an on-farm experiment. The final mean weight of fingerlings stocked at size 5 g was significantly higher (113.80 ± 4.21 g) ($P < 0.05$) compared to the 0.2 g (36.99 ± 1.14 g) and 1g (91.93 ± 5.59 g) fingerling sizes. The mean daily weight gain was highest in 5 g stocked fingerlings (0.91 ± 0.04 g day⁻¹) resulting in significant differences in the final mean weight. The coefficient of correlation between fish body length and weight was high and positive ranging ($R = 0.95-0.98$). Significantly low percent survival was recorded in 0.2 g stocked fingerlings ($64.4 \pm 1.93\%$). The net annual fish yield (NAY) and profit index (PI) were significantly lower at the 0.2 g. The highest NAY and PI were recorded in the 5 g stocked fingerlings (8.59 ± 0.09 tons ha⁻¹ year⁻¹ and 6.6 ± 2.08), respectively. Thus, with appropriate Nile tilapia fingerling weight at stocking, fish farmers can maximize fish growth, yield and profits.

Introduction

Among the wide variety of tilapias, Nile tilapia (*Oreochromis niloticus*) is the most important farmed fish species in the world after carps and its culture is being undertaken in most of the tropical, subtropical and temperate regions throughout the world except Antarctica (Fitzsimmons, 2003; Brummett *et al.* 2000; Neira *et al.* 2009; Darko *et al.* 2016). It is a preferred species for aquaculture in Sub-Saharan Africa countries due to its ability to tolerate a wide range of

environmental conditions, successful reproductive strategies and ability to feed at different trophic levels. In developing countries, tilapia fingerlings are usually reared in either extensive or semi-intensive static ponds or hapas (Charo-Karisa *et al.* 2006; Abdel-Fattah and Kawana, 2004; Opiyo *et al.* 2017; Shoko *et al.* 2014). Nile tilapia farming has expanded in these countries due to its ability to be cultured under very basic conditions and so is ideal for rural small-scale farmers. This is seconded to the warmer weather conditions throughout the year combined with high demand in Sub-Saharan Africa

(Brummett *et al.* 2008). Increased interest in tilapia farming has resulted in mushrooming of hatchery-produced Nile tilapia fingerlings in most Sub-Saharan Africa countries (Matthew *et al.* 2015; Nyonje *et al.* 2018; Kajungiro *et al.* 2019). Hatchery-produced seeds are now accessible, and supply is reliable as opposed to the past where small-scale farmers were obtaining fingerlings from the wild where quality and supply were not guaranteed. In the hatchery, Nile tilapia fingerlings are produced in two forms depending on the demand; all-male tilapia through sex reversal using methyltestosterone or produced as mixed-sex fingerlings. In the latter fry are left to attain their inherent sex orientation without sex manipulation. In most developing countries, standards guiding hatchery operations and production for Nile tilapia fingerlings have largely been based on broodstock quality, production protocols, hatchery facilities (equipment and ponds) and water quality assessment. However, no attention has been focused on appropriate fingerlings weight sizes suited for grow-out ponds to provide better growth performance.

The high demand for Nile tilapia fingerlings has been occasioned by subsidies introduced by governments in many developing countries in Sub-Saharan Africa in efforts to develop the aquaculture sector (Charo-Karisa and Gichuri, 2010; Kaminski *et al.* 2018; Jacobi, 2013; Obiero *et al.* 2019). In response to the demand, hatcheries have only focused on numbers of fingerlings for supply and have been detached from farmers' production performance. Stocked Nile tilapia fingerlings have, therefore, been largely dictated by what is supplied by tilapia hatcheries. Nile tilapia fingerlings supplied are normally defined by age (days old) rather than the attained weight. Defining fingerlings solely on age disregards challenges occasioned by poor larviculture e.g., stunted growth arising from lack of sufficient quality feeds or overstocking during the hatchery phase, passing the problem to grow-out farmers. Fish fingerlings are among the variables related to a farmer's costs of production thus, fingerlings ought to be in a state that can exert a positive influence on productivity when other factors (feeds, feeding and water quality) remain unaffected. The current study aimed to assess the effect of three Nile tilapia fingerling weight sizes on growth performance and productivity in liner ponds to help farmers determine the right size for stocking.

Materials and Methods

Nine farmer's ponds of 300 m² and a mean depth of 1.25 m were used in this study located on North Coast of Kenya (3°11'S, 40°04'E), Kilifi County. Three different sizes of tilapia fingerlings of 0.2 g, 1 g, and 5 g were stocked in triplicates. The different sizes of fingerlings were sourced from Kenya Marine & Fisheries Research Institute, National Aquaculture Research Development and Training Center, Sagana, each pond was stocked at

3 fish m⁻². The fish were fed at a rate of 3% average body weight by a feed bought from a local industrial producer of tilapia feed whose proximate composition is as prescribed by the producer: 28 % crude protein, 10% fat, 6% Crude Fiber and 9% ash. The feed calculated ratio was divided into two meals and one ratio was fed in the morning and the other in the evening (09:00 and 16:00hrs) for 120 days.

Monthly sampling was conducted using a seine net. A representative sample of thirty percent of the total stocked fish were taken from each experimental pond for weight measurements. The total length (cm) was measured using a measuring board to the nearest 0.1 cm and weight measurements were done using an electronic weighing balance (readability of 0.01 g), fish were returned to their ponds. Throughout the study period, physicochemical parameters of the pond water including dissolved oxygen (mg L⁻¹), temperature (°C), pH, total suspended solids (mg L⁻¹) and salinity (ppt) were determined before using multiparameter water quality meter (Hanna Instruments HI 9829). Fish wet weight data were used to evaluate fish growth performance by calculating average daily growth (ADG) and specific growth rate (SGR). Feed conversion ratio (FCR) was evaluated from fish weight and feed data. At the end of the study period, a complete harvest of all ponds was done for survival and productivity analysis. The following formulae were used to calculate the above parameters:

$$\text{Average daily growth (ADG)} = \frac{\text{Mean weight gained}}{\text{period of the experiment (days)}}$$

$$\text{Specific growth rate} = (\ln W_t - \ln W_0) / t \times 100$$

Where W_0 = initial fish weight, (W_t) = fish weight at time t , t = time (culture period in days).

$$\text{Food Conversion Ratio} = \frac{\text{feed given (g)}}{\text{weight gain (g)}}$$

$$\text{Survival Rate (\%)} = \frac{\text{Number of Fish at the end of the experiment}}{\text{(Initial number of fish stocked)}} \times 100$$

$$\text{Fulton's condition factor (K)} = \left(\frac{g}{L \text{ cm}^3} \right) \times 100$$

Where W = body weight and L = total length.

The length-weight relationship ($LWR = W = aL^b$) was determined by linear regression analysis Where, the W = Weight of fish (g), L in total length (cm), a and b are the regression slope and intercept (regression coefficient), respectively (Froese, 2006).

$$\text{Net annual yield (NAY, tons ha}^{-1}\text{year}^{-1}) = \frac{(W_{\text{harvest}} - W_{\text{start}}) \times 365}{\text{Area} \times \text{culture days}} \text{ (Shoko } et al. 2019)$$

$$\text{Profit index} = \frac{\text{Value of fish}}{\text{Cost of fish production}} \text{ (El-Dakar } et al. 2007)$$

Statistical analyses were carried out using Statistical Product and Service Solutions (SPSS version 26.0). The Levene test on the homogeneity of variances was used to test the normality of the data (Shapiro and Wilks, 1965). Results are presented as means \pm standard error (SE). Mean data on SGR, ADG, FCR, Fulton condition factor, survival rate, NAY were subjected to one-way analysis of variance (ANOVA) followed by Tukey post hoc analysis for multiple comparisons of means between fingerling sizes. The significance level was set at $P < 0.05$.

Results

Growth Performance and Survival Rate

Changes in mean fish body weights during the experimental period are presented in Figure 1. The fingerling size significantly affected the final mean weight of Nile tilapia ($P < 0.05$). The fish's mean body weights increased with increasing culture days for all the treatments. Nile tilapia fingerlings stocked at size 5 g exhibited significantly higher final mean weight (113.80 ± 4.21 g) compared to the other fingerling sizes ($P < 0.05$).

The average daily growth (ADG) of Nile tilapia fingerlings was significantly affected by fingerling size ($P < 0.05$). The highest ADG of 0.91 ± 0.04 g day⁻¹ was

recorded in fingerlings stocked at 5 g while the lowest ADG was recorded in fingerlings stocked at 0.2 g at 0.31 ± 0.01 (Table 1). Similarly, % SGR was significantly affected by the size of the stocked fingerlings. Nile tilapia stocked at 0.2 g fingerling size had significantly higher SGR (4.30 ± 0.03 % day⁻¹) ($P < 0.05$) compared to the other treatments. There were no significant differences ($P < 0.05$) in FCR amongst the fingerling size treatments, the FCR was around 2 (Table 1). There was a significant difference ($P < 0.05$) in survival among fingerling sizes. Significantly lower survival rates ($P < 0.05$) were recorded in 0.2 g fingerling size ($64.4 \pm 1.93\%$) compared to those stocked at sizes 1 g and 5 g ($95.6 \pm 2.19\%$ and $97.82 \pm 2.44\%$ respectively). The mean indices for the Fulton condition factor of the Nile tilapia fingerlings were generally higher than $K=1$ in all treatments (Table 1).

Length-Weight Relationship (LWR)

The overall LWR analysis of the pooled data was described by a regression coefficient ("b") and coefficient of correlation (R) of 2.8 and 0.94 respectively. The coefficient of correlation within treatments was high and positive ($R=0.95, 0.98$ and 0.97) for treatments 1, 2 and 3 respectively. Further, LWR analysis showed the Nile tilapia fingerlings in treatment 2 exhibited a near positive allometric growth with an allometric "b"

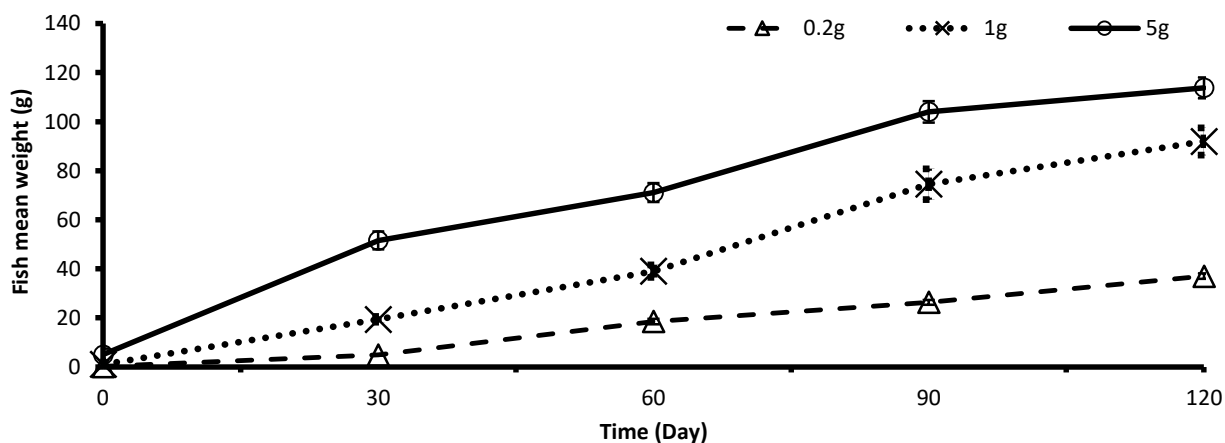


Figure 1. Mean growth of body weight \pm s.e of Nile tilapia, *O. niloticus* stocked at different weights at 0.2 g, 1.0 g and 5.0 g reared in ponds

Table 1: Growth performance parameters of Nile tilapia *Oreochromis* stocked at weight at 0.2 g, 1 g and 5 g reared in ponds for 120 days

Parameter	Stocking weight		
	0.2 g	1.0 g	5.0 g
Final mean weight (g)	36.99 \pm 1.14 ^a	91.93 \pm 5.59 ^b	113.80 \pm 4.21 ^c
Total weight gain (g)	36.79 \pm 1.14 ^a	90.93 \pm 5.59 ^b	108.80 \pm 4.2 ^c
Daily weight gain (g day ⁻¹)	0.31 \pm 0.01 ^a	0.76 \pm 0.05 ^b	0.91 \pm 0.04 ^c
Specific growth rate (% day ⁻¹)	4.30 \pm 0.03 ^a	3.71 \pm 0.06 ^b	2.59 \pm 0.04 ^c
Food conversion ratio	2.38 \pm 0.36 ^a	2.08 \pm 0.17 ^b	2.17 \pm 0.13 ^b
Survival (%)	64.43 \pm 1.93 ^a	95.6 \pm 2.19 ^b	97.82 \pm 2.44 ^b
Condition factor (K)	1.97 \pm 0.02 ^a	1.95 \pm 0.02 ^a	1.94 \pm 0.05 ^a

* Means in the same row with the same superscript are not significantly different ($P > 0.05$).

value of 2.99 while treatments 1 and 3 recorded 2.90, 2.88, respectively (Figure 2).

Water-Quality Parameters

The results of water quality parameters are listed in Table 2. Dissolved Oxygen was approximately 6 mg L⁻¹, pond water temperature ranged from 31.29 to 32.56°C, pH ranged between 6.87±0.13 and 6.93±0.18 total suspended solids ranged from 255.67 to 255.67 mg L⁻¹ and salinity ranged between 1.33 to 1.43 ppt. There were no significant differences in physicochemical water quality parameters among the three groups of experimental ponds (P>0.05).

Yield and Profitability Parameters

The size of stocked Nile tilapia fingerlings affected the final yield of the experimental fish (P<0.05). The net annual yield (NAY) was highest (8.59±0.09 tons ha⁻¹ year⁻¹) in the 5 g stocked fingerlings (Figure 3). The profit index ranged from 1.2 to 6.6 with the highest achieved in treatment 3 (5 g) fingerlings. The net annual yield (NAY) and Profit index (PI) were significantly lower in the 0.2 g fingerling size compared to the other treatments (Figure 4). However, NAY and PI were not significantly different between Nile tilapia fingerlings stocked at 1 g and 5 g (P>0.05).

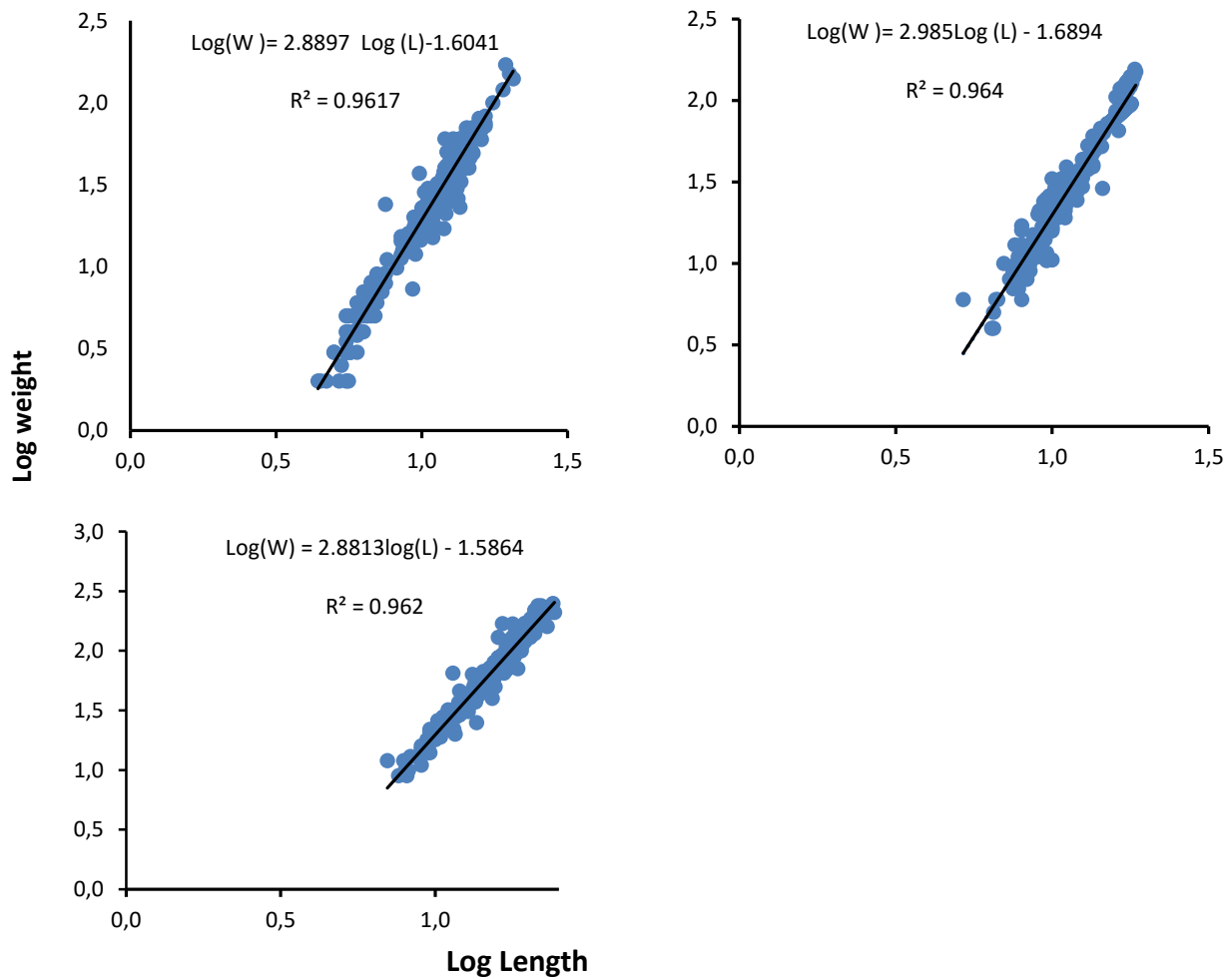


Figure 2. Logarithmic Relationship between length and weight with regression equation of Nile tilapia, *O. niloticus* fingerlings stocked at 0.2 g, 1 g and 5 g reared in ponds for 120 days

Table 2: Physico-chemical water quality parameters of Nile tilapia *Oreochromis niloticus* liner ponds stocked at weight 0.2 g, 1.0 g and 5.0 g for 120 days. Values represent the mean ± SE

Physico-chemical parameters	Stocking weight			P - value
	0.2 g	1.0 g	5.0 g	
Dissolved oxygen mgL ⁻¹	6.24 ± 0.75	6.33 ± 0.29	6.33 ± 0.25	0.075
Temperature (°C)	32.56 ± 0.46	31.51 ± 0.57	31.29 ± 0.72	0.079
pH	6.87 ± 0.13	6.89 ± 0.13	6.93 ± 0.18	0.085
Total suspended solids (mg L ⁻¹)	278.38 ± 7.44	255.67 ± 8.61	265.68 ± 9.32	0.062
Salinity (ppt)	1.43 ± 0.30	1.33 ± 0.40	1.35 ± 0.22	0.892

All measured parameters were not significantly different between the stocking weight sizes (P>0.05).

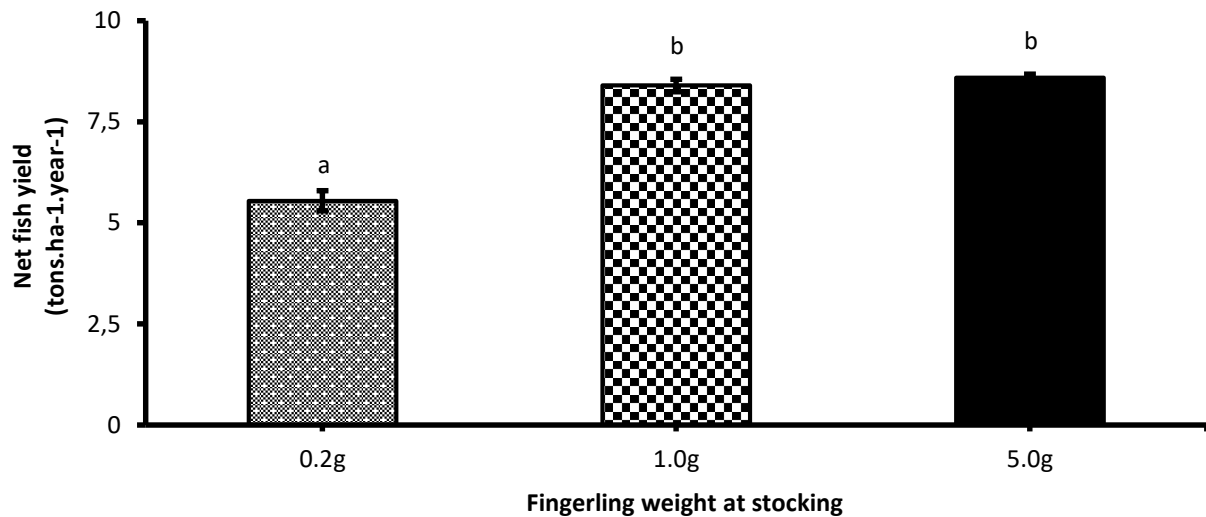


Figure 3. Mean annual net fish yield (tons ha⁻¹ year⁻¹) of Nile tilapia, *O. niloticus* stocked at different weight at 0.2 g, 1.0 g and 5.0 g. Different letters denote significant differences ($P<0.05$).

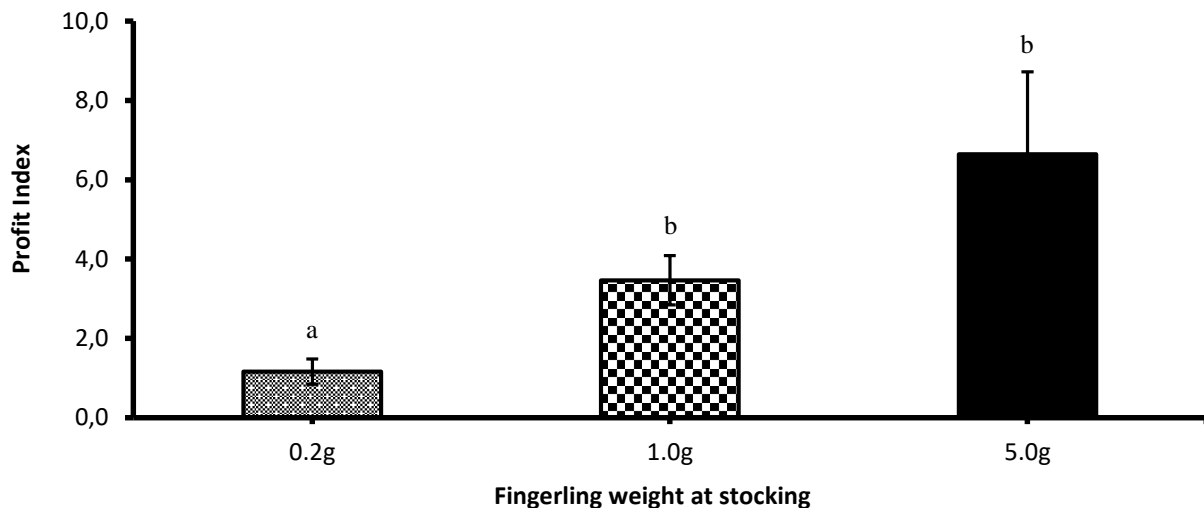


Figure 4. Profit index of Nile tilapia, *Oreochromis niloticus* stocked at different weight of 0.2 g, 1.0 g and 5.0 g. Different letters denote significant differences ($P<0.05$).

Discussion

This study provides information on the growth rates of different fingerling size groups of Nile tilapia. While many studies have looked at the growth performance of Nile tilapia fingerlings based on other factors such as stocking density, photoperiod, protein feed replacement among others, the present study addresses the information gap existing on appropriate Nile tilapia fingerling size suitable for stocking in semi-intensive ponds to ensure better growth. By the end of this study, the growth variation was evident with the significant differences in final mean weight ($P<0.05$), where 1 g and 5 g groups of fingerlings differed in growth from the 0.2 g fingerlings. Further, average daily growth (ADG) and specific growth rate (SGR) were able to depict clear variation in the growth of the different sizes of Nile tilapia fingerlings.

The developmental stage of the fish plays a critical role in the growth trajectory where high SGR and ADG of reared fish denote fast growth. As the experimental period progressed a general declining mean SGR was observed indicative of fish larvae transiting into post fingerling stage. A study by Kapinga *et al.* (2014) on the growth performance of Nile tilapia fingerlings of 2 g reared at low density for five months attained about 86 g in 120 days culture period. On the other hand, Abdel-Hakim *et al.* (2008) and Bolivar *et al.* (2004) showed that fingerlings of 1.3 g attained about 100 g by the end of 120 days in ponds. Kapinga *et al.* (2014) in her study recorded an average daily gain (ADG) of 0.75 ± 0.07 g day⁻¹ and a specific growth rate (SGR) of $3.47\pm 0.03\%$ day⁻¹ close to the current study as well as Bahnasawy *et al.* (2003) who recorded an ADG of 0.9 g day⁻¹ and SGR of 1.55% day⁻¹ for 6 g fingerlings reared in ponds. Further, the findings of the present study corroborate the results

by El-Sayed and Kawanna (2004) while rearing Nile tilapia fingerling of 0.02 g and 2 g in a normal photoperiod experiment and reported highest mean SGR of $6.42 \pm 0.18\%$ day⁻¹ in small-sized Nile tilapia fingerlings compared to $3.33 \pm 0.05\%$ day⁻¹ in 2 g sized fingerlings. In the present study, the highest average ADG was recorded in 5 g sized fingerlings (0.91 ± 0.04 g day⁻¹).

The mean FCR attained in this current study is comparable with earlier research work reported by Mensah *et al.* (2013) who recorded mean FCR in the range of 2.34 ± 0.312 while conducting a stocking density experiment on Nile tilapia fry. Kapinga *et al.* (2014) also recorded a mean FCR of 2.71 ± 0.08 in a pond growth assessment of tilapia fingerlings. The present study nevertheless recorded higher mean FCR in comparison to studies conducted by Bahnasawy *et al.* (2003) and El-Sayed and Kawanna (2004) with Nile tilapia fingerlings and fry who reported 0.82 and 1.78 ± 0.07 , respectively. Since FCR is based on fish weight gain, any factor influencing weight gain ideally affects FCR. In this study, the environmental factors did not differ significantly ($P > 0.05$) between the treatments neither month hence ruling out the effect of water quality. Quality of feed used, pellet quality, size and palatability have been documented to affect FCR. Additionally, the type of pellet whether sinking or floating will too contribute to the FCR of the reared fish. The food conversion rate and specific growth rate help to optimize the feeding of fishes based on the requirement for the fish diets (Chepkirui-Boit *et al.* 2011).

Length-weight relationship (LWR) of fish changes based on the condition fish is subjected to i.e., the water quality and food availability. A length-weight relationship is an important tool that may explain the growth pattern in fish. The current study revealed that the Nile tilapia fingerlings followed the cube law in all group size treatments. Statistical analysis of the pooled length-weight relationship of Nile tilapia fingerlings showed that obtained regression slope "b" from the length-weight relationship was indicative of near isometric growth. The mean value of "b" (2.92) did not significantly differ from the standard value isometric value "b"=3.0, implying that the growth of the fingerlings followed the cube law of growing isometrically (Wootton, 1990). According to Wootton (1990), growth of fish is categorized as positive allometric when the weight of an organism increases more than length ($b > 3$) and negative allometric when length increases more than weight ($b < 3$) lower than the critical isometric value i.e., 3. The mean values of "b" recorded in the present ranged between 2.88 - 2.99, still were within the range of 2 to 4 recommended as appropriate for fresh-water fishes and are sufficient for fish to retain the shape $b=3$ (Froese, 2006). In line with the findings of this study, several authors have reported negative allometric growths. The study by Gómez-Ponce *et al.* (2011) on *O. niloticus* recorded allometric coefficients in the range of 2.5 to 3.5. Elsewhere,

Kullander (2003) working on a Cichlidae fish attained allometric coefficient values between 2.9 and 3.3.

Further, the coefficient of correlation (R) range of $R=0.95$, 0.98 and 0.97 for treatments 1, 2 and 3, respectively and the linear form relationship of length and weight conformed to the general formula expressing the relationship between the length and weight of fishes hence corroborating the isometric growth pattern. Additionally, the mean indices for condition factors (K) were higher than 1 in all treatments indicating that the health and growth of the Nile tilapia fingerlings were good and indicative of the robustness and well-being of the experimented fish. The recorded condition factor is similar to those recorded by Opiyo *et al.* (2020) while working with sex-reversed Nile tilapia. The condition factor (K) in fish reflects on the physiological state of the fish concerning with its welfare and shows the degree of feeding activity of a fish hence a better understanding of whether a fish is making good use of its feeding source (Fagade, 1979).

The present study recorded significantly low survival of $64.4\% \pm 1.93$ in the least weight (0.2 g) fingerling group compared to the other treatments that had more than 95% survival. This study hypothesis that size variability had a significant effect on the survival potential of the fish where the highest weight Nile tilapia fingerlings gained a survival advantage over least weight counterparts due to reduced vulnerability to predators coupled with easy uptake of dry feed pellets. Similar survival rates were reported by Mario *et al.* (2003), Sogard (1997) and Ridha (2006) who recorded survival above 60% on *O. spilurus* fingerlings stocked in grow-out conditions. Although there many studies e.g., effect of stocking density, the effect of photoperiod among others documenting effects on growth and survival of tilapia fingerlings, however, there is hardly any information on the effect of fingerling size at stocking on the output in terms of growth and survival.

Fish growth may be influenced by water quality factors. Dissolve oxygen, water temperature and salinity levels are generally considered to have primary importance in fish culture and considerable fluctuations of these parameters may negatively impact the growth of fish (Islam *et al.* 2006; Houlihan and Boujard, 2001). Throughout the experimental period, measured water quality parameters remained within acceptable limits to the culture of *O. niloticus* under semi-intensive systems (Boyd and Tucker, 1992). Consequently, no significant differences were observed over time in the different parameters. According to Riche and Garling (2003), dissolved oxygen above 5 mg L^{-1} is suitable for optimum growth of Nile tilapia. The lower limit of 3 mg L^{-1} dissolved oxygen concentration should be sufficient to maintain optimum growth in Nile tilapia fish. The present study recorded more than 5 mg L^{-1} in all the treatments. The temperature recorded in this study is comparable to those stated in FAO (2009) which stated 31 and 36°C as the preferred temperatures for warm water tropical species. In a different study, Kausar and

Salim (2006), reported temperatures in the range of 25 to 27 °C were ideal for the optimum growth of Nile tilapia. Even though the recorded salinity in this study was low and suitable for farming Nile tilapia, Tahoun *et al.* (2013) reported that tilapia can tolerate a wide range of 1 ppt to 15 ppt and still maintain optimum development.

The current experiment showed that fingerlings stocked at 5 g produced higher fish yield than those stocked at 1 g and 0.2 g. Although higher SGR was recorded in fingerlings stocked at 0.2 g and 1 g, however, the size difference could not permit the weight gain to surpass that of fingerlings stocked at 5 g. According to Limbu *et al.* (2016) Shoko *et al.* (2019), the yield of reared fish is dependent on growth rates and survival, these observations are consistent with the the present study where fingerlings with the highest growth rate and survival recorded highest NAY and PI. The current NAY results are similar to other previous authors experimenting with Nile tilapia in ponds e.g., Liti *et al.* (2005) who recorded a range of 6.24 to 7.69 tons ha⁻¹ year⁻¹ for fingerlings stocked at 21 g and Bahnasawy *et al.* (2003) recorded 6.9 tons ha⁻¹ year⁻¹ for fingerlings stocked at 6 g. The profit index showed that it was more beneficial in terms of feed input to invest in Nile tilapia fingerlings stocked at 5 g where the highest profit index of 6.6 was achieved. The profit index obtained in this experiment agree with the one recorded by Anani *et al.* (2020) who recorded profit index ranging from 3.14 to 4.75 while experimenting on the growth performance of Nile tilapia fry fed with a commercial feed of four different crude protein levels. Munguti *et al.* (2016) recorded a range of 6.69 to 9.42 while studying the performance of Nile tilapia fingerlings feed diets containing blood meal as a replacement for fish meal. Other studies by Asase *et al.* (2016) and El-Sayed. (1998) recorded profit indices ranging 1.31- 3.96 and 1.65 to 2.3 respectively. In the present study, despite the low feed cost in 0.2 g and 1 g treatments, the harvested weight was not large enough (did not grow fast enough) to achieve a better profit index than 5 g sized fingerlings. The present study indicate that small scale tilapia farmers could benefit more in terms of higher harvested weight and income by avoiding small sized fingerlings during stocking phase.

This study provides the baseline information on the fingerling size effect on the growth, condition, and length-weight relationship of *O. niloticus* fingerlings. These variables are imperative when evaluating the well-being status of the fish under normal pond conditions. Based on the findings of this study, it is possible to conclude that Nile tilapia fingerlings of 5 g provided better growth, yield and profit while staying in good condition while being fed at 3% body weight on commercial tilapia feed. These findings would be beneficial for the Nile tilapia aquaculture industry to improve its growth and also will benefit extension workers in aquaculture.

Ethical Statement

Not applicable

Funding Information

This study was supported by the Association for strengthening Agricultural Research in Eastern and Central Africa (ASARECA) (Grant No. RC10 LFP-02.); through the project "Building public-private sector partnership to enhance productivity and competitiveness of Aquaculture.

Author Contribution

M.W.: Project administration, Methodology, Investigation, Validation, Data curation, Formal analysis, Visualization, Writing - original draft, Writing - review and editing

M.O.: Conceptualization, Funding acquisition, methodology, Validation, Visualization, Writing - original draft, Writing - review & editing,

H.C.-K.: Conceptualization, Funding acquisition, Supervision, Validation, Writing - original draft

P.O.: Resources, Formal analysis, writing-original draft,

B.N.: Resources, Supervision,

Conflict of Interest

No potential conflict of interest was reported by the authors.

Acknowledgements

The authors wish to acknowledge Kenya Marine and Fisheries Research Institute aquaculture technicians for their support during experiment monitoring and data collection. We appreciate the administrative support from the Malindi County Fisheries office during the execution of the research. This study was supported by the Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA) (Grant No. RC10 LFP-02.); through the project "Building public-private sector partnership to enhance productivity and competitiveness of Aquaculture.

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