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Research Article

Performance of Nile tilapia *Oreochromis niloticus* fingerlings in a hyper-intensive recirculating aquaculture system with low water exchange

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ABSTRACT. The aim of this paper was evaluate the performance of Nile tilapia fingerlings (*Oreochromis niloticus*) raised at hyper intensive stocking density in a recirculating aquaculture system (RAS) with minimum water replacing. The experimental system was performed in a single-batch nursery system to obtain 50 g fish size in 60 days. Fish (2.07 ± 0.04 g) were stocked in triplicate at 400 (T1), 500 (T2) and 600 (T3) fish m^{-3} (0.84, 1.05, 1.22 kg m^{-3}). RAS functioned with 12,000 L of recirculating water and 252 L $day^{-1}$of water replacing (2.1% daily). The efficiency of the biofilter for removing the total ammonia nitrogen (TAN) was 48 ± 12.5 mg L^{-1}. Stocking density did not affect significantly the survival (89.5 to 93.6%). The growth rate of T1 (0.96 g day^{-1}; 5.01% day^{-1}) and T2 (0.92 g day^{-1}; 4.95 % day^{-1}) was significantly higher than T3 (0.83 g day^{-1}; 4.80% day^{-1}). The specific growth rate (SGR) of T1 was 41% influenced by temperature. For T2 and T3 the SGR were influenced by the variation of dissolved oxygen (DO) that explained 47 and 44% of the fish weight variation, respectively. The SGR from T3 was also affected by the concentration of ammonia nitrogen (31%). The high stocking density affected the overall size of fish and the size homogeneity, but had no negative effect on the length–weight relationship (L-W). Data support the conclusion that fingerling stocked at 400 and 500 fish m^{-3} shows high performance during 9-weeks when the biomass not exceed 37 kg m^{-3}. At this time fish have reached the desired final nursery weight (50 g) for transfer to grow-out facilities.

Keywords: Nile tilapia, recirculating aquaculture system, low water exchange, intensification.

Rendimiento de juveniles de tilapia del Nilo *Oreochromis niloticus* en un sistema híperintensivo de recirculación acuícola con mínimo recambio de agua

RESUMEN. El objetivo de este trabajo fue evaluar el rendimiento de juveniles de tilapia del Nilo *Oreochromis niloticus* sembrados en densidades hiperintensivas en un sistema de recirculación acuícola (SRA) con mínimo remplazo de agua. El sistema experimental consistió en un sistema de precría de cosecha única para obtener peces de 50 g en 60 días. Los peces (2,07 ± 0,14 g) se sembraron por triplicado a densidades de 400 (T1), 500 (T2) y 600 (T3) peces m^{-3} (0,84; 1,05; 1,22 kg m^{-3}). El SRA funcionó con 12.000 L de agua recirculante y un remplazo diario de 252 L (2,1% por día). La densidad de siembra no afectó significativamente la supervivencia (89,5-93,6%). El biofiltro removió el amonio nitrogenado total con una eficiencia del 46,9 ± 7,0%. La tasa de crecimiento de T1 (0,96 g $día^{-1}$; 5,01% $día^{-1}$) y T2 (0,92 g $día^{-1}$; 4,95% $día^{-1}$) fue significativamente mayor que T3 (0,83 g $día^{-1}$; 4,80% $día^{-1}$). La temperatura influyó 41% en la tasa de crecimiento específico (TCE) de T1. La concentración de oxígeno disuelto (DO) influyó en la variación de peso en T2 (47%) y T3 (44%). La TCE de T3 también se vio afectada por la concentración de nitrógeno amoniacal (31%). El aumento de la densidad de siembra afectó el tamaño y la homogeneidad de tallas de los peces, pero no afectó la relación longitud-peso (L-P). Los datos respaldan la conclusión que los juveniles sembrados a densidades de 400 y 500 peces m^{-3} tienen alto rendimiento durante nueve semanas consecutivas, siempre y cuando la biomasa no se exceda de 37 kg m^{-3}. En este tiempo los peces alcanzan el peso final esperado de precría (50 g) y pueden ser transferidos hacia las instalaciones de engorde.

Palabras clave: tilapia del Nilo, sistema de recirculación acuícola, bajo recambio de agua, intensificación.

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INTRODUCTION

Tilapia culture has traditionally relied on extensive and semi-intensive systems in earthen ponds or cages. The expansion of tilapia culture across the world, together with the shortage of freshwater and competition for it with agriculture and with urban activities has gradually shifted tilapia culture from traditional semi-intensive systems to more intensive production systems (El-Sayed, 2006).

Tilapia culture in Mexico is widely diversified in terms of geography and methods of production. Semi-intensive culture is practiced in small water bodies and ponds, with stocking density range of 4 to 6 fish m^{-3} and yields of 1 to 4 ton ha^{-1} *per* production cycle (Hernández-Mogica *et al.*, 2002; Ponce-Marbán, 2006). Intensive culture is practiced in cages, raceways, ponds, and secondary or tertiary irrigation channels (Arredondo & Lozano, 2003). The stocking densities vary from 80 to 100 fish m^{-3} and the annual production ranges from 1.5 ton ha^{-1} in rustic ponds to 25 ton ha^{-1} in raceways (Camacho-Berthely *et al*., 2000). In 2006, the production of tilapia reached a maximum of 81,250 ton, of which the 98% came from nine states of Mexico. Although the favourable mean annual temperature (28.5°C), the Yucatan state located at the southeast Gulf of Mexico occupied the place number 28 in the national production ranking (CONAPESCA, 2010). Its particular geological and hydrographical characteristics, such as the lack of rivers and surface water, and the karst and impermeable soils, have prevented producers to adopt tilapia farming technologies from other regions of Mexico. In this sense, the development of the commercial-scale aquaculture in the Yucatan state requires the construction of tanks and pumping of groundwater, which would bring an increase in investments and production costs, making this activity not always profitable. At present, 82% of farmers are growing tilapia at intensive levels (80 fish m^{-3}) and 100% use circular tanks (Vivanco-Aranda *et al.*, 2011). However, the intensification should be increased over 150 fish m^{-3} to meet financial outcomes (Arámburu-Adame, 2011).

Recirculating aquaculture systems (RAS) are characterized by the ability to support extremely high stocking densities and high net production compared with open aquaculture systems (Timmons *et al.*, 2002). For example, using a RAS, it is possible to produce over 45,000 kg of fish in a 464 m^2 building, whereas an 8 ha of outdoor ponds would be necessary to produce an equal amount of fish, with the traditional open pond culture (Helfrich & Libey, 1990). Similarly, since water is reused, the water volume requirements in RAS are only about 20% of what conventional open pond culture demands. RAS offers a promising solution to water use conflicts, water quality, and waste disposal. Raceways, for example require, on the average, 2.9 to 3.6 L min^{-1} of flow available for each kg of biomass (Parker, 2011). Nevertheless, RAS consume approximately 250-1000 L kg^{-1} of fish, and discharge less effluent compared with open systems, reducing the volume and cost of wastewater treatment (Shnel *et al*., 2002). In RAS the water is reconditioned by clarification, biological filtration and re-aeration, so that most of the water is reused, and only 10% of the total daily flow is replaced by new water, to compensate water evaporation and for diluting the nitrate concentration (Timmons *et al*., 2002). The productive capacity of RAS depends on the ability of the biological, chemical and physical treatment units to remove waste, as well as on the volume of replacement water used to improve water quality.

Performance data on tilapia in RAS are scarce, particularly at intensive stocking densities. Two production modalities have been reported for recirculating tilapia systems with different performance; nursery (1 to 50 g), and grow-out phase (50 g to market-size). Most authors report performance data from the grow-out stage. Suresh & Lin (1992), reported a relatively low growth (0.77, 0.65 and 0.64 g day^{-1}) of red tilapia *Oreochromis aureus* (75 g) stocked in RAS, at 50, 100 and 200 fish m^{-3} (3.6, 7.5, 15.3 kg m^{-3}) for 70 days. Ridha & Cruz (2001), reported high growth rate (1.17 g day^{-1}) and 97.6% of survival in Nile tilapia *Oreochromis niloticus* (62 g) reared in RAS at 166 fish m^{-3} (10.3 kg m^{-3}). Initially, 20-30% of the system water was changed with new water, to dilute the high levels of ammonia and nitrites; however, as the experiment progressed, the daily water exchange rate was reduced to 10-15%. Performance data about the intensification of Nile tilapia at nursery phase is even scarcer. Bailey *et al.* (2000) performed an experiment to study the intensive production of Nile tilapia fingerlings (4.3 g) in RAS. Fish stocked at 200 fish m^{-3} grew slightly faster (0.78 g day^{-1}) than those stocked at 450 fish m^3 (0.60 g day^{-1}). The total ammonia nitrogen (TAN) ranged from 0.82 to 1.33 mg L^{-1} and nitrate from 1.3 to 83.8 mg L^{-1}. The heterogeneity of size was pronounced in both treatments, where over 50% of the population in each treatment was greater than the desired 50 g-size.

Good aquaculture practices should minimize the increase of size heterogeneity over time, in order to minimize food wastage that may result from the establishment of dominance hierarchies as size heterogeneity soars (Jobling & Baardvik, 1994).

Partial harvesting is useful in some types of systems, where only large individuals are caught and the smallest are left in the system to grow larger. However, besides the increase in labour and facilities, partial harvest in RAS increases the water losses associated for harvesting procedures and also makes difficult the correct supply of food with appropriate pellet sizes. The aim of the present study is to evaluate the performance of Nile tilapia fingerlings, raised at hyper intensive stocking density, in a RAS with minimum water replacing. The experimental system is performed for a nursery with a single-batch, for obtaining a desired 50 g-fish size in 60 days harvest. The study recorded grow-out and survival data of fish and monitored water quality parameters as indicators of the system performance.

MATERIALS AND METHODS

Experimental fish

Sex-reversed Nile tilapia *Oreochromis niloticus* fingerlings (2.07 ± 0.04 g), were acquired from a commercial farm (Tabasco, México) and transported in oxygenated plastic bags to the experimental unit of the Universidad Marista de Mérida (México).

Experimental facilities and design

The experiment was performed in a RAS, which consisted of nine circular indoor tanks (1.2 m^3 each), containing 12,000 L of total recirculating water. The system included a 2.7 m^2 concrete settling basin, two 5 µm-capacity filter cartridges (10 inch Big Blue^{TM}), and a wet/dry trickle filter with 405 m^2 m^{-3} of substrate area (Ultra Fibra, México). Water was continuously recirculated from the rearing tanks through the sedimentation area and pumped (Venus, 1 HP) through the filter cartridges to the biological filter. A protein skimmer (200 x 800 mm) was installed between the biofilter and the aeration system. The water flow rate was adjusted to 80 L min^{-1}. The dissolved oxygen (DO) concentration was increased using a 1 HP blower (Sweetwater) with air diffusers. Ion profile of groundwater using in the RAS was: 86.1 mg $L^{-1}Ca^{2+}$, 27.6 mg L^{-1} Mg^{2+}, 126 mg L^{-1} Cl, 376.2 mg L^{-1} $CaCO_3$ of hardness, 325.5 mg L^{-1} $CaCO_3$ of alkalinity and pH = 7.0. The biofilter was inoculated with 45 mg L^{-1} of ammonium chloride (NH_4Cl) and 70 mg L^{-1} of sodium nitrite ($NaNO_2$) twice during the first week prior to stocking with fish. The water level indicator placed into each tank was used to estimate daily water loss in the RAS. Accordingly, an equal volume of water was replaced three times a week.

Nile tilapia fingerlings (2.07 ± 0.14 g) were stocked at T1 = 400, T2 = 500 and T3 = 600 fish m^{-3} (0.84, 1.05, 1.22 kg m^{-3} respectively) with three replicates of each density, and fed three times a day for 70 days. Commercial floating pellet containing 40% crude protein and 3400 kcal kg^{-1} metabolizable energy (Nutripec, Purina) was used until the fish reached 35 g of body weight (BW). After that, fish were fed with 32% protein and 3,152 kcal kg^{-1}ME until harvest. The weekly feeding rate was adjusted according to a 1:5 feed conversion ratio (FCR), declining from 4.5 to 3.8% BW at the 5 week, and continuing at 3.1% BW until harvest.

Fish growth

Each week, 45 fish from each tank were individually weighed and their total length measured. Fish were removed from each tank using a minnow seine, and returned to the tank following measurement. The sample size (n) required for estimating growth was calculated with a 15% relative error and 0.05% confidence level (Zar, 1999). A digital scale (Ohaus, 0.01 g) was used to record fish weight and a vernier caliper (Westward, 0.01 mm) was used to estimate length. At the end of the 11th week all fish were harvested, weighed and counted. The absolute growth rate (AGR, g day^{-1}) was estimated for each treatment as a function of W_t–W_1 over time (t) where: W_t and W_1 is the final and initial weight, respectively, and t is the number of days in the experimental period. The specific growth rate (SGR, % day^{-1}) was estimated as $(\ln W_t - \ln W_1)100t^{-1}$. Mortality was recorded every day, and calculated according to the difference between the number of fish stocked and harvested. Gross yield (kg m^{-3}) was calculated as the sum of individual weights of harvested fish. The ratio of feed fed (kg) to wet fish weight gain during the feeding period was expressed as FCR.

Water quality parameters

Water temperature and dissolved oxygen (DO), in each tank, were measured three times a day using an oxymeter (YSI 200). Weekly measurements were taken of ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), pH and total organic carbon (TOC). All water samples were collected in triplicate and analyzed in the laboratory. Total ammonia nitrogen (TAN) was determined using the salicylate hypochlorite method (Solorzano & Sharp, 1980). The concentration of un-ionized ammonia nitrogen (NH_3-N) was calculated according to Thurston *et al*. (1979). Nitrite nitrogen was determined using sulphanilamide in an acid solution. NO_3^-N was measured as NO_2^-N after its reduction in a Cd-Cu column (Strickland & Parsons,

1972). TOC was determined using the heated-persulfate oxidation method (Clescerl *et al.*, 2000). The pH was measured with a pH meter (Oaklon 510). At harvest, three effluent samples were collected in quadruplicate at the beginning, middle, and end of the discharge. Samples were pooled to analyze total phosphorus and total nitrogen concentrations, using Kjeldahl and ammonium molybdate methods (APHA, 1999).

Removal efficiency of solid and soluble waste

Weekly water samples were also collected in triplicate from the pre- and post-settling basin and from the pre- and post-biofilter, to estimate the TOC, TAN and the C/N ratio (TOC/TAN). The loading rate and the removal efficiency of solids and TAN was determined using the formula proposed by Suresh & Lin (1992); loading rate = C_i x Q; and removal efficiency (%) = $[(C_i - C_e)$ x Q / $(C_i$x Q)] x 100, where C_i: affluent concentration, C_e: effluent concentration, Q: flow rate.

The abundance of total heterotrophic bacteria (HB) was determined in the laboratory by the standard plate count method (Norrell & Messley, 1997). Samples were blended at 16,000 rpm for 1 min to achieve the greatest recovery of HB attached to suspended solids. Serial 10-fold dilutions were made transferring 1 mL of sample into 0.9% NaCl sterile solution. For each sample, five dilutions were plated over Nutrient Agar (Difco, 213000), in triplicate. Incubation was performed at 28 ± 1°C for 48 h (Isotemp 625D, Fisher Scientific). The number of surviving HB was evaluated as the mean number of colony-forming unit (CFU) per dilution, multiplied by the dilution factor. Results were expressed as CFU mL^{-1} of water and correlated with TOC concentration.

Data analysis

Survival percentage data were converted to arcsine x square root, before statistical analysis. Analysis of variance (one-way ANOVA) was used to test the effect of stocking density on fish growth rate and survival. Final weight, was tested via analysis of covariance (ANCOVA) using the initial body weight as the covariate. Significance of water quality parameters, between treatments and over weeks, was evaluated at the 0.05-probability level using ANOVA and Tukey test. Before performing the ANOVA and ANCOVA, the data were checked for normality and homogeneity of variance using a Shapiro-Wilks test and F distribution, respectively.

The statistical significance of the effect of each water quality variable (predictors) on SGR (response variable), was evaluated by forward stepwise regression. The quality of the regression model was judged by the SGR variability described by the systematic predictor in order to present only the significant water quality variables. The Monte Carlo permutation, with 5000 permutations, was used to assess the quality of each potential predictor to extend the subset of the response variable used in the regression model (Braak & Smilauer, 2002). Significant water parameters were added to the Gompertz model

$$w(t) = ae^{be^{ct}}$$

to obtain the predicted trend line of growth, where *a* represents the initial weight, *b* is the weekly growth rate, and *c* is the fitting parameter number (Ricker, 1975). Curve fitting parameters were estimated using the Rosenbrock and quasi-Newton method ($P < 0.05$). The predicted curve reliability was examined for likelihood and accuracy, using Theil's inequality coefficient (Pindyck & Rubinfeld, 1991). Thiel´s inequality coefficient (U) can be interpreted as follows: U = 0, means that the estimated data has the same trend line as the observed data; U = 1, means the worst fit. Therefore, a Thiel´s U close to zero corresponds to the best data-fitting curve.

The relationship between total length and weight (L-W), in each treatment, was analyzed using the statistical significance of the isometric exponent *b* and adjusted using the potential model: $Wi = a\ TL_i^b$, where TL is the total length, *a* is the proportionality constant and *b* the isometric exponent (Pauly, 1984). In order to define the isometric or allometric L-W relationship, the *b* value was tested by the expression $t_B = |b-3|/S_B$ and checked on a one-tailed test. Statistical analysis was conducted using Statistics 5 and CANOCO software.

RESULTS

Effect of density on fish growth and survival

Stocking density had a significant effect on the absolute growth rate (AGR) ($F_{(2,6)} = 13.3$, $P < 0.05$), specific growth rate (SGR) ($F_{(2,6)} = 10.6$, $P < 0.05$) and the final weight ($F_{(2,6)} = 12.7$, $P < 0.05$). The growth and final weight were higher at the lower densities (T1 = 400 fish m^{-3}, T2 = 500 fish m^{-3}) than at the highest density (T3 = 600 fish m^{-3}). Total gross yield increased with increasing stocking density but fish from T3 were significantly smaller than those in T1 and T2 (Table 1). Fish survival decreased with increasing stocking density but no significant differences were found between treatments (Table 1). Cumulative mortality was less than 5% during the first month, after which the rate increased, particularly with medium and high densities (Fig. 1). According to the slope

Table 1. Performance of Nile tilapia fingerlings (*Oreochromis niloticus)* cultured in a recirculating system for 70 days.

Stocking density (Fish m^{-3})	Stock data		Growth data				Harvest data							
	Initial weight (g)		AGR (g day^{-1})[a]		SGR (% day^{-1})[b]		Final weight (g)		Survival (%)		FCR[c]		Gross yield (kg m^{-3})	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
400 (T1)	2.10 [a]	0.05	0.96 [a]	0.01	5.01 [a]	0.02	68.8 [a]	1.04	93.6 [a]	1.81	1.27	0.03	37.1 [a]	1.27
500 (T2)	2.09 [a]	0.10	0.92 [a]	0.02	4.95 [a]	0.09	66.3 [a]	1.20	91.8 [a]	1.43	1.39	0.04	43.8 [b]	1.48
600 (T3)	2.03 [a]	0.04	0.83 [b]	0.02	4.80 [b]	0.03	59.8 [b]	1.19	89.5 [a]	0.67	1.78	0.02	46.4 [b]	1.14

Different letters indicate significant difference ($P < 0.05$) between data in the same column, [a] Absolute growth rate, [b] Specific growth rate, [c] Food conversion ratio.

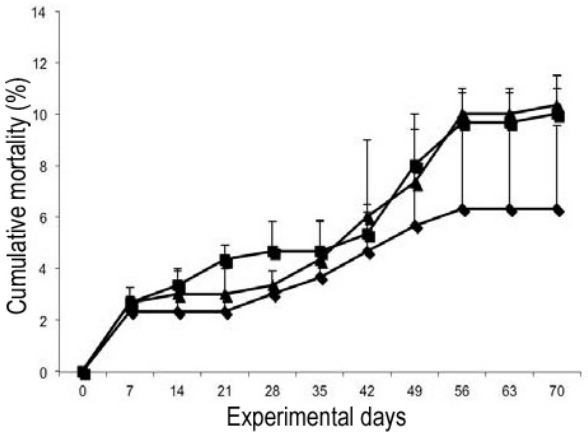

Figure 1. Cumulative mortality of Nile tilapia *Oreochromis niloticus* at three stocking densities in a recirculating system. ♦ 400 fish m^{-3}, ■ 500 fish m^{-3}, ▲ 600 fish m^{-3}.

value (b), the fish length-weight (L-W) relationship of all treatments showed allometric growth ([Ho = b = 3, Ha = b > 3]; t-value = 43.8 (T2) and 34.3 (T1, T3), $P < 0.05$), indicating a higher rate of growth in weight than in length (Tesch, 1968) (Fig. 2).

Effects of water quality on fish weight and heterogeneity of population

Weekly variations in water quality parameters are presented in Table 2. The mean values were 25.6 ± 1.9°C; 5.1 ± 0.6 mg L^{-1} DO; 0.20 ± 0.16 mg L^{-1} NH_3-N; 0.61 ± 0.55 mg L^{-1} NO^-_2-N, 22.3 ± 9.9 mg L^{-1} NO^-_3-N and 7.79 ± 0.33 pH. Water parameters explained 71, 77 and 83% of the SGR variability for T1, T2 and T3, respectively (Table 3). The SGR of T1 was mainly influenced by temperature ($P < 0.05$), which explained 41% of the variance in the fish growth. Fish growth from T2 was influenced by DO variation that explained 47% of the variance. The SGR from T3 was significantly affected by both DO (44%) and ammonia nitrogen (31%). Each significant water variable was added in the Gompertz model to plot the weekly increase of fish BW (Fig. 3). Thiel´s inequality coefficient (U) was 0.174, 0.195 and 0.181 for T1, T2 and T3, respectively. Data clearly show a high standard deviation of fish weight that increased during the experiment (Fig. 4). The estimated growth rate, taking into account the heterogeneity in size, was 6.52, 4.68 and 4.44 g $week^{-1}$ for T1, T2 and T3 respectively

Performance of waste treatment system

RAS functioned during 70 days with 12,000 L of recirculating water and replacing an average of 252 L day^{-1}. Daily water loss was 2.1% on the average. The

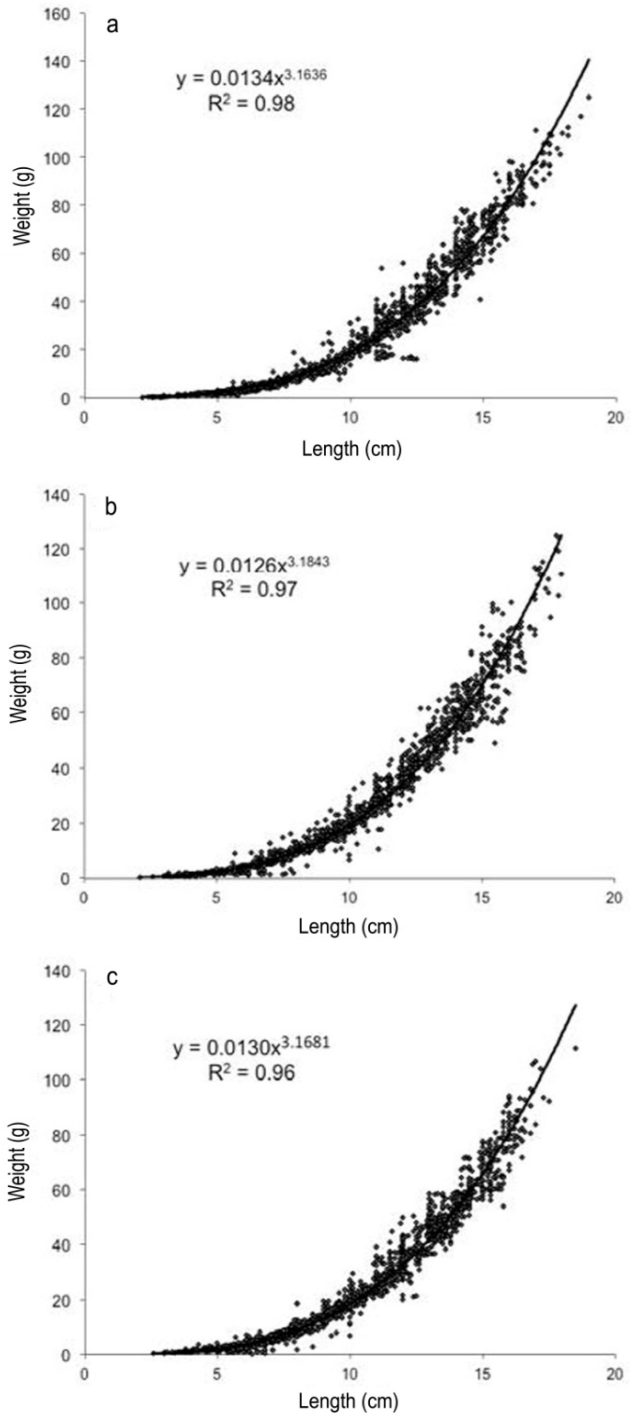

Figure 2. Length-weight (L-W) relationship of Nile tilapia *Oreochromis niloticus* at three stocking densities in a recirculating system. a) 400 fish m^{-3}; b) 500 fish m^{-3}; c) 600 fish m^{-3}. Point: mean data; Line: trend line.

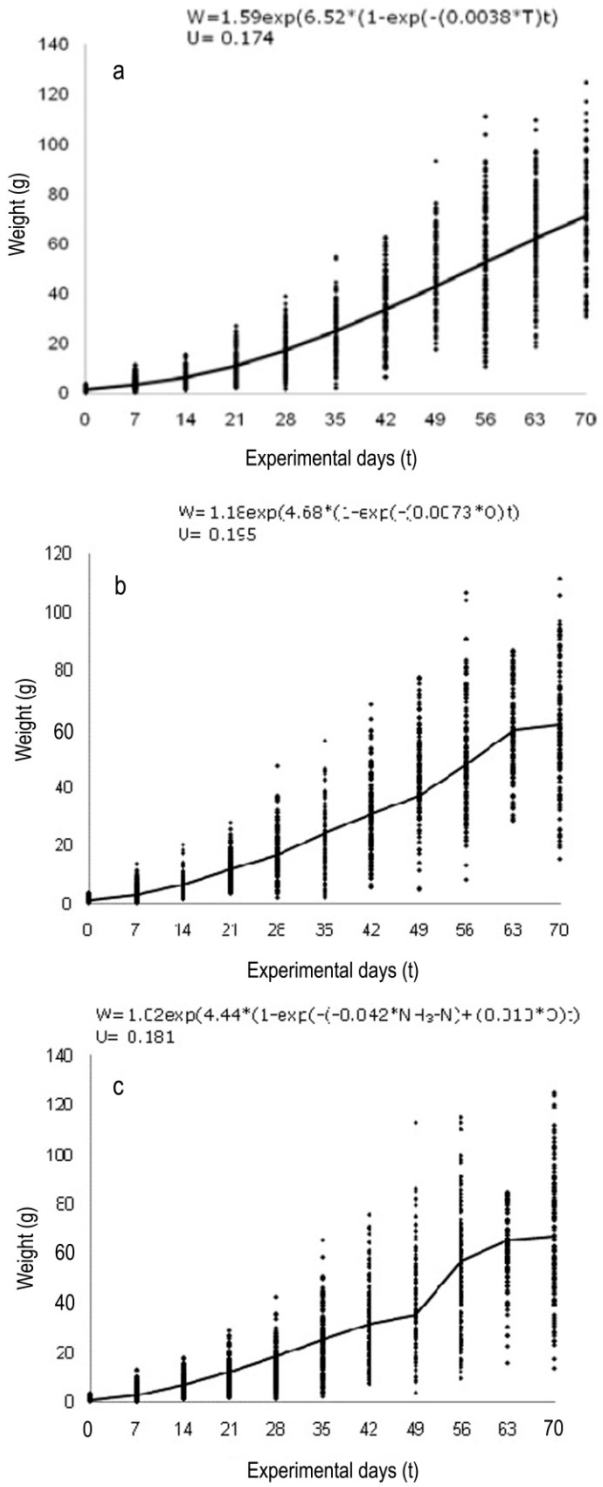

Figure 3. Growth of Nile tilapia *Oreochromis niloticus* at three stocking densities in a recirculating system. a) 400 fish m^{-3}, b) 500 fish m^{-3}, c) 600 fish m^{-3}. Point: observed data, Line: predicted data.

specific water consumption was 108.7 L kg^{-1}. The efficiency of the settling basin and the bio-filter for suspended solids removal and TAN varied during the experimental period. Increasing TOC indicated a decrease in the ability of the settling basin to remove

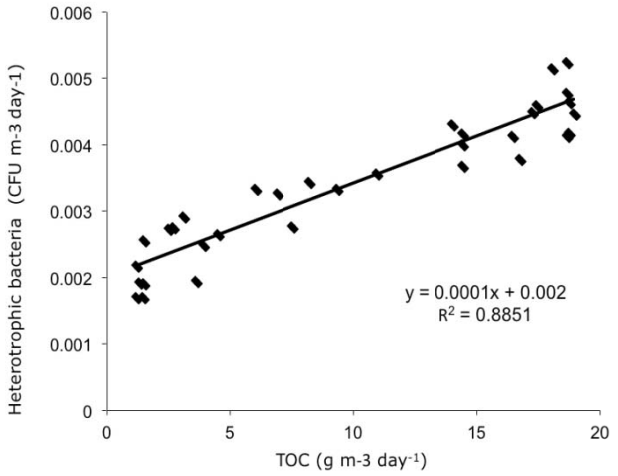

Figure 4. Relationship between total number of heterotrophic bacteria and total organic carbon (TOC) from the biofilter outlet of the recirculating tilapia system.

suspended solids. After the 6th week, rates of TOC removal were low (Table 4). The concentration of heterotrophic bacteria increased by 40% from the beginning (0.0019 CFU m^3 day^{-1}) up to the end of the trial (0.0047 CFU m^3 day^{-1}), and it was directly related to the increase of TOC (Fig. 4). The removal efficiency of TAN was relatively constant throughout the experiment (Table 4). The weekly loading of TAN was 48 ± 12.5 mg L^{-1} and the removal efficiency of the biofilter was 46.9 ± 7.0%. The level of TAN in the water of different treatments was 0.36 ± 0.33, 0.52 ± 0.56 and 0.62 ± 0.41 mg L^{-1} for T1, T2 and T3, respectively. At harvest, the concentration of total nitrogen and total phosphorus from the effluent was 25.6 ± 5.1 mg L^{-1} and 1.75 ± 0.13 mg L^{-1}, respectively.

DISCUSSION

Results showed the biological feasibility of culturing tilapia fingerlings at hyper intensive densities in RAS. The simple design-RAS used was successful in maintaining water quality within acceptable limits, requiring only 2.1% of daily water replacement. Mean temperature (25.6 ± 1.9°C), ammonia nitrogen (0.20 ± 0.16 mg L^{-1} NH_3-N), and nitrite nitrogen (0.61 ± 0.55 mg L^{-1} NO_2^--N), remained within the optimal range for the growth of Nile tilapia (Popma & Masser, 1999; Ross 2000). Stocking density did not affect significantly the survival (89.5 to 93.6%). Consequently T3 had the highest feed conversion ratio (FCR), given its high biomass. In general, the RAS supported 37.1-46.4 kg m^{-3}, with a specific water consumption of 108.7 L kg^{-1}.

Data of this study demonstrated that growth rate differences between stocking densities is due to the effect of weekly variation of the water parameters, in particular DO, NH_3-N and temperature. The SGR of T2 and T3 was influenced by the DO variation, 47 and 44% respectively (Table 3). The influence of DO over the growth rate has been well established for tilapia species. Historical report of Tsadik & Kutty (1987), indicates a positive correlation between DO demand and AGR (r = 0.99), and assimilation efficiency (r = 0.98) of Nile tilapia. These authors reported a higher AGR (0.58 g day^{-1}) at 7.3 ± 2.6 mg L^{-1} of DO than at 3.4 ± 1.4 mg L^{-1} (0.15 g day^{-1}). Sofronios & Tziha (1996), reared Nile tilapia in RAS for 7 months obtaining an AGR of 1.6, 1.8 and 1.9 g day^{-1} for DO levels of 2.62, 3.75 and 6.51 mg L^{-1}, respectively. The growth rate was higher than our study; however, the stocking biomass is significantly less (0.0079 kg m^{-3}). The decline in individual growth rate with increasing stocking densities is a common occurrence that has been previously documented. This effect has been related to a social stress or chronic stress response, which may impair fish growth due to the mobilization of dietary energy by the physiological alterations provoked by the stress response (Kebus *et al.*, 1992). Thus, in the present study stocking density had a significant effect on the AGR and SGR. The growth rate of T1 (0.96 g day^{-1}; 5.01% day^{-1}) and T2 (0.92 g day^{-1}; 4.95% day^{-1}) was higher than T3 (0.83 g day^{-1}; 4.80% day^{-1}); therefore the final weight of T1 and T2 (68.9 and 66.3 g) was higher than T3 (59.8 g).

Results of the present experiment showed that the SGR of T3 was also affected by the NH_3-N concentration (0.25 mg L^{-1}) that was 58.6% higher than T1 (0.15 mg L^{-1}) and 1.19% higher than T2 (0.21 mg L^{-1}) (Tables 1, 3). Changes in water pH from present RAS indicated that the pH of T3 was higher than others treatments (7.97 ± 0.31), with a range of 7.12 to 8.40. Although that range is within the optimum values of pH recorded for Nile tilapia, there is more concentration of NH_3-N toxic form at pH > 7.0 (Popma & Masser, 1999). The mean value of NH_3-N (0.25 mg L^{-1}) would have no adverse effect on survival of T3, but possibly affected the growth rate. This data are in disagree with report done by Hargreaves & Kucuk (2001), which mentioned that continuous exposure to 0.27 mg L^{-1} NH_3–N (pH 8.0) did not affect SGR of blue tilapia reared at 0.023 kg m^{-3}. However, the SGR reported by those authors (1.84% day^{-1}), was significantly lower than our study (4.95% day^{-1}), even at low stocking density. Bailey *et al*. (2000) reared Nile tilapia (4.3 g) in RAS at the hyper intensive stocking densities of 0.86 and 1.72 kg m^{-3}. The survival rate (91.9%) and the FCR (1.25),

Table 2. Mean water quality parameters in the recirculating tilapia system (*Oreochromis niloticus*) at three stocking densities.

Week	Oxygen (mg L^{-1})		Temperature (°C)		NH_3-N (mg L^{-1})		NO_2^--N (mg L^{-1})		NO_3^- - N (mg L^{-1})		pH	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Stocking density 400 fish m^{-3}												
1	5.73	0.54	28.1	0.0	0.34	0.07	0.88	0.23	6.0	2.10	7.70	0.30
2	5.48	0.06	27.8	0.2	0.44	0.10	1.66	0.68	27.2	9.16	7.40	0.27
3	5.80	0.04	27.2	0.3	0.08	0.05	1.80	0.40	25.5	5.19	7.40	0.24
4	5.80	0.23	27.2	0.4	0.07	0.03	0.10	0.05	14.6	4.88	7.17	0.23
5	5.18	0.14	26.1	0.1	0.07	0.02	0.07	0.06	10.6	9.04	7.52	0.44
6	5.11	0.12	26.5	0.0	0.07	0.06	0.09	0.01	23.3	5.41	7.55	0.44
7	4.91	0.05	25.5	0.0	0.11	0.05	0.16	0.09	33.3	6.98	7.39	0.31
8	5.20	0.23	25.1	0.1	0.14	0.06	0.37	0.21	28.8	2.73	7.63	0.31
9	5.51	0.08	22.2	0.0	0.14	0.07	0.45	0.17	36.0	2.69	7.68	0.31
10	4.63	0.15	23.0	0.4	0.07	0.01	0.34	0.11	29.0	3.73	7.22	0.33
11	3.82	0.16	24.9	0.1	0.07	0.05	0.32	0.22	27.0	5.83	7.76	0.33
Stocking density 500 fish m^{-3}												
1	5.66	0.03	27.8	0.0	0.74	0.05	0.02	0.01	2.1	1.40	7.80	0.42
2	5.60	0.13	27.7	0.0	0.41	0.40	0.09	0.01	2.2	1.43	7.84	0.36
3	5.76	1.56	27.6	0.1	0.18	0.12	0.95	0.61	17.0	3.54	7.88	0.21
4	5.34	1.34	26.5	0.4	0.18	0.03	1.11	0.65	22.2	7.63	7.93	0.22
5	5.08	1.34	26.2	0.1	0.14	0.02	1.16	0.24	28.4	2.00	7.97	0.32
6	4.91	0.16	26.5	0.0	0.14	0.05	0.09	0.03	12.8	9.02	8.01	0.34
7	4.67	0.19	25.5	0.2	0.12	0.10	0.03	0.03	19.7	5.27	8.05	0.17
8	4.29	0.23	25.1	0.0	0.09	0.02	0.05	0.02	23.5	1.18	8.09	0.06
9	5.37	0.07	22.1	0.2	0.08	0.04	0.15	0.08	34.6	8.21	8.13	0.09
10	5.42	0.34	22.2	0.4	0.12	0.08	0.44	0.08	27.1	8.00	7.76	0.09
11	3.74	0.56	24.5	0.1	0.09	0.04	0.96	0.50	36.2	6.81	7.80	0.03
Stocking density 600 fish m^{-3}												
1	5.60	0.23	28.0	0.3	0.47	0.24	0.83	0.11	5.5	1.20	7.84	0.88
2	5.39	1.76	27.4	0.4	0.42	0.23	1.06	0.23	21.7	8.54	7.89	0.76
3	5.70	0.04	27.1	0.0	0.25	0.15	1.72	1.11	16.1	7.65	7.70	0.67
4	5.50	1.34	26.4	0.8	0.24	0.14	1.17	0.34	13.1	8.45	7.70	0.16
5	5.02	0.07	26.0	0.5	0.32	0.12	0.10	0.01	16.8	3.54	8.40	0.18
6	4.79	0.10	26.5	0.1	0.33	0.12	0.10	0.01	20.3	2.65	7.12	0.18
7	4.55	1.50	25.5	0.6	0.23	0.02	0.20	0.02	23.8	6.12	8.18	0.15
8	4.19	1.20	25.0	0.4	0.12	0.01	0.45	0.34	27.3	5.11	8.22	0.12
9	5.42	1.23	22.2	0.0	0.14	0.03	1.07	0.25	30.8	3.21	8.26	0.12
10	5.58	0.98	22.4	0.1	0.14	0.04	1.09	0.64	34.3	7.45	8.00	0.11
11	3.80	0.20	24.0	0.2	0.07	0.02	1.10	0.74	37.8	12.40	8.34	0.08

Table 3. Significant effect of water quality parameters on the specific growth rate (SGR) of Nile tilapia (*Oreochromis niloticus*) according to forward stepwise regression.

Stocking density (fish m^{-3})	400 (T1)		500 (T2)		600 (T3)	
	Variance (%)	*P*-value	Variance (%)	*P*-value	Variance (%)	*P*-value
Temperature (^oC)	0.41	0.024*	0.02	0.055	0.01	0.706
Oxygen (mg L^{-1})	0.12	0.164	0.47	0.022*	0.44	0.016*
NH_3-N (mg L^{-1})	0.13	0.162	0.07	0.288	0.31	0.030*
NO_3^--N (mg L^{-1})	0.01	0.708	0.13	0.116	0.00	0.930
NO_2^--N (mg L^{-1})	0.00	0.786	0.06	0.312	0.07	0.610
pH	0.04	0.454	0.02	0.614	0.00	0.916
Cumulative % variance	0.71		0.77		0.83	

* Water parameters with *P*-value less than 0.05 are considered significant.

Table 4. Rate of reduction of total organic carbon (TOC) and total ammonium nitrogen (TAN) from the settling basin and biofilter of the recirculating tilapia system (*Oreochromis niloticus*).

	Settling basin				Biofilter				
	TOC (g m^{-3}) Loading rate		TOC (%) Removal efficiency		TAN (mg L^{-1}) Loading rate		TAN (%) Removal efficiency		C/N ratio
Week	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
1	95.2	3.4	32.2	6.7	56.7	3.3	42.2	6.7	1.7
2	119.5	0.9	52.1	1.5	47.5	2.9	51.1	1.5	2.5
3	162.7	68.2	62.7	26.6	42.0	18.2	32.6	16.6	3.9
4	218.7	34.7	39.8	6.7	61.3	14.7	39.6	6.7	3.6
5	608.3	45.2	42.9	11.2	69.3	21.2	40.9	11.1	8.8
6	866.6	87.6	29.2	11.1	56.7	12.3	52.8	11.1	15.3
7	1472.0	27.7	15.1	3.8	56.1	27.7	61.0	3.7	26.2
8	1146.6	43.3	21.7	12.3	39.3	12.4	49.6	12.3	29.2
9	1456.0	69.2	6.0	4.8	48.1	4.3	51.3	4.8	30.3
10	1333.3	21.0	7.4	1.4	30.4	12.3	49.7	1.4	43.9
11	1272.0	23.9	3.4	1.2	29.3	8.2	45.5	0.8	43.4

were similar to that obtained in the T1 in the present experiment, but the AGR was lower than here (0.14 g day^{-1}). The DO from RAS of the cited studied was higher than here, even in the high density (6.79 mg L^{-1}), however the NH_3-N of our RAS (0.20 mg L^{-1}) was significantly lower (0.98 mg L^{-1}). In addition, at high stocking densities the effects of a limited amount of space per organism also increased metabolic rates, and therefore, the oxygen consumption (Kisia & Hughes, 1993). The oxygen consumed and the ammonia excretion rate depends on the fish biomass and the oxygen consumption rate which, in turn, is a function of the average weight and water temperature (Timmons *et al*., 2002). Even when the mean of DO was not different between treatments, the data suggests that the DO concentration supplied in our RAS was not enough for the high stocking densities (T2 and T3), in contrast with T1 that was only influenced by the weekly temperature variation (Fig. 3).

The high stocking density affected the overall size of fish and the size homogeneity, but had no negative effect on the length–weight relationship (L-W). Several authors have reported that crowding intensifies the heterogeneous fish growth, due to the competition for a sparse supply of adequate food or

social dominance, which often coincides with increasing fish densities (Sahoo *et al*., 2004; Jha & Barat, 2005; Barbosa *et al.*, 2006). When growth is density dependent, partial harvest of the standing fish have been use to decrease competition, and thereby increasing individual growth rate. However this procedure increases the water consumption. Martins *et al*. (2009), report that juveniles (up to 150 g) can be raised in RAS at 3.8 kg m^{-3} with water exchange rates of 30 L kg^{-1} feed day^{-1}, without a decrease in feeding motivation. On the contrary, large fish (288.7 ± 34.2 g) stocking at 7.2 kg m^{-3} exhibit growth retardation. Based on those studies we believe that partial harvest does not improve the performance of the juvenile's tilapia in the present RAS.

Tilapia exhibited an expected positive allometric growth ($b > 3$), at the three stocking densities. The classical ontogenetic interpretation of the allometric L-W is that fish increases in weight at a greater rate than required, maintaining constant body proportions (Tesch, 1968). Fish grows isometrically ($b = 3$), during their final growth stanza, while values of $b < 3$ are usually associated with a shortage of suitable food or overcrowding (Ricker, 1979; Murphy *et al*., 1991). The L-W model is sensitive to and reflects small changes in condition, and can alert producers to the onset of disease, stress due to overcrowding, bad water quality, or other physiological effects, before high mortality rates are suffered (Jones *et al*., 1999). Although body weight is commonly recorded for culture management purposes (*e.g.*, estimations of growth rate, feed conversion ratio, harvest weight, and productivity), the application of L-W relationship could be use as a simple alternative to estimate body weight from length measurements that are less variable and more easily measured in the field. Rutten *et al*. (2005), clearly demonstrated the very strong relationship (0.90) between length and fillet weight from raised Nile tilapia. In this context, the L-W relationship was assessed for several commercial aquaculture species (Sofronios & Tziha, 1996; Peixoto *et al*., 2004; Araneda *et al.*, 2008; Gullian *et al*., 2010). Results of the present study represent the first report of L-W relationship for *O. niloticus* at hyper intensive nursery conditions.

Gradual build-up of nitrate (NO_3^--N) was seen along the experimental weeks, indicating nitrification (Table 2). In the final week, NO_3^--N concentration reached 33.7 ± 5.8 mg L^{-1} and the total ammonia nitrogen (TAN) decreased up to 0.06 ± 0.01 mg L^{-1}. Nitrate is relatively non-toxic to tilapia (Segalas *et al*., 2003). In the classic autotrophic RAS, ammonia peaks in the first week, followed by a nitrite peak and the accumulation of nitrate (Timmons *et al*., 2002). Thus, many recirculating tilapia systems reach NO_3^-N concentrations over 50 mg L^{-1} (Bailey *et al*., 2000; Shnel *et al*., 2002). Although data shows that TAN removal efficiency was less than 50%, the NH_3-N level denotes an acceptable function of the biofilter. Twarowska *et al*. (1997) achieved 65% of TAN removal using a high-rate linear-path tricking biofilter in a recirculating tilapia system at 246 min L^{-1}. The biofilter keep the TAN concentration at 0.62 ± 0.37 mg L^{-1} and nitrite nitrogen concentration at 1.62 ± 1.10 mg L^{-1}. Those values are according with the results from the present study at least for T1 and T2, where the biofilter at lower flow rate (80 L min^{-1}) held the TAN at 0.36 ± 0.33 and 0.52 ± 0.56 mg L^{-1}, respectively. The TAN level was slightly higher in T3 (0.62 mg L^{-1} ± 0.41 mg L^{-1}) than the cited study, and even the nitrite nitrogen concentration was relatively higher (1.71 mg L^{-1} ± 2.34 mg L^{1}).

The data suggest that the accumulation of organic matter increased the oxygen-consumer heterotrophic bacteria population (HB) (Fig. 4). The reduction of nitrification rate, with increasing organic matter, has been previously reported. Zhu & Chen (2002), reported 70% reduction in the nitrification rate when the carbon-nitrogen ratio (C/N) increased up to 2.7. Ling & Chen (2005), also reported that the reduction of nitrification rate in the biofilter is about 60-70% when the concentration of TAN inside de biofilter rises to 10 mg L^{-1}, and also the C/N ratio increases up to 8.4. In this study, the C/N ratio overcame 2.7 from the 3th week to the 11th week. However, the TAN removal was 57 ± 7% (Table 4). It is noteworthy that increased C/N ratio, through the consecutive experimental weeks, was not inversely proportional to the reduction nitrification process, possibly due to the available DO in the RAS. Jechalke *et al.* (2011), reported that nitrification is inhibited when the DO is less than 1.2 mg L^{-1}. Data of the present study showed that the minimum value of DO registered in the last week was 3.78 ± 0.03 mg L^{-1}, higher than the inhibition nitrification value. If the experiment would be extended more than 11 weeks, it is likely that the nitrification efficiency will decrease due to increasing fast-growing heterotrophic bacteria competing with nitrifying bacteria for the limited oxygen available. This would have required increasing the oxygen supply or exchanging the water.

In summary, stocking densities and harvest yields are finite and are determined by the carrying capacity of the system. Results showed that Nile tilapia fingerlings grow in low water exchange RAS, at high stocking density, reaching between 60 to 69 g-fish size in 70 days. Data from growth curves suggest the 9th and 8th weeks as optimum harvest time for T2 and T3,

respectively. At this time, the biomass reached by these treatments was 34.4 kg m^{-3} for T2 and 36.1 kg m^{-3} for T3. Considering that T1 reached 37.1 kg m^{-3} and it had the highest SGR (5.01 g day^{-1}), we believe that biomass of the present RAS should not exceed 37 kg m^{-3} with a stocking density exceeding 400 fish m^{-3}. The average total nitrogen (25.6 ± 5.1 mg L^{-1}) and total phosphorus (1.75 ± 0.13 mg L^{-1}) concentration from the final effluent were lower than the long-term trigger values for irrigation concentrations of the nutrients recommended by the Official Mexican Standards (NOM-001-SEMARNAT-1996) (40 mg L^{-1} of total nitrogen, 20 mg L^{-1} of total phosphorus). Based on the data, the nitrogen and phosphorus levels from the wastewater of the RAS can be used for irrigation and it is unlikely to cause a significant impact on the environment.

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