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Full Length Research Paper

Effects of different stocking densities on tiger grouper juvenile (*Epinephelus fuscoguttatus*) growth and a comparative study of the flow-through and recirculating aquaculture systems

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Two experiments at different water-flow conditions and stock densities in the critical 6-week nursery phase determined growth, survival and feed conversion of brown-marble grouper juveniles at 35 Day After Hatching (DAH) with initial mean body weight of 0.045±0.02 g and total mean length of 15.08±0.4 mm. In the first experiment, juveniles of 35 DAH were kept in an 80 L fiberglass tank with the flow-through system for 42 days of the nursery phase at three densities: 1, 3 and 5 ind/L. The highest specific growth rate (P<0.05) and the lowest food conversion ratio (P<0.05) were observed for fish at the 3 ind/L stocking density compared to the other groups. No statistically significant differences (P>0.05) were found in the survival rate of fish groups held in any stocking density. In the second experiment, juveniles of 35 DAH were reared with a re-circulating aquaculture system (RAS) at three densities, 1, 3 and 5 ind/L, to evaluate the effects density has on grouper growth and survival rate in the RAS system, and results were compared with the open flow-through system. No statistically significant differences (P>0.05) were found in total length and body weight between treatments (1, 3, 5 fish/L) in the RAS system, while the weight and total length of the fish reared in open water flow-through conditions were significantly larger (P<0.05) than fish reared in the RAS. This study suggests that optimum stocking density in a flow-through system is 3 fish per liter and flow-through water is preferred to the RAS system.

Key words: Tiger grouper, *Epinephelus fuscoguttatus,* stocking density, re-circulating aquaculture system, flow-through system.

INTRODUCTION

Groupers are a marine species with good growth potential as leading fishery commodities because of their characteristic economic value. However, problems may occur at 30 to 40 days after hatching (DAH) when groupers become extremely cannibalistic. Tiger grouper (*Epinephelus fuscoguttatus*) exhibits higher cannibalistic behaviour compared to *Latescalcarifer* and *Lutjanus sp*. This behavior arises from lack of feed, rearing tank sizes, water system conditions, various fish sizes and stress at high densities. Mortality rate due to cannibalism at this stage can reach 90% in a traditional nursery. In intensive fish culture systems, increasing rearing density is one way of optimizing productivity. However, poor growth and increased incidence of disease have often been observed in fish reared at higher densities. On the other hand, stocking density is one of the most important biotic factors in aquaculture because it directly influences survival, growth, behavior, health, feeding and production. Higher densities may interfere with intra- population interactions and can consequently affect biomass gain.

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The relationship between stocking density and fish growth has been shown to be positive (Papst et al., 1992), negative (Hengsawat et al., 1997; Irwin et al., 1999), density independent (Fairchild and Howell, 2001; Rowland et al., 2004, 2006) or dependent on different experimental density ranges. Stocking density might vary as a function of behavioral adjustments, food availability and water quality. Fish confined at high densities may present chronic stress, potentially resulting in lower growth rates, increased susceptibility to disease and increased mortality rates (Blackburn and Clarke, 1990). On the other hand, there are also species in which growth rate is maximized at higher stocking densities, as observed for *Dicentrarchus labrax* and *Rhamdia quelen* fingerlings by Papoutsoglou et al. (1998) and Piaia and Baldisseroto (2000), respectively. Stocking density is seen as a crucial variable contributing to cultured fish growth performance.

The effects of density on growth are diverse, usually showing a negative correlation in several finfish species like the rainbow trout (*Oncorhynchus mykiss*) (Refstie, 1977) or Atlantic cod (*Gadusmorhua*) (Lambert and Dutil, 2001). The number of fry per square meter could lead to lower productivity in trout farming, maybe due to the existence of a particular disease when there is an excessive number of fry and high mortality rate in rainbow trout fry during the initial trout farming stages. Therefore, excessive numbers of fry in trout ponds might lead to a major decrease in trout production and reduced productivity growth as well. A similar finding was noted by Zarranezhad and Rezaei (2004) and Khayyati and Mashoufi (2007) in the trout farming sector in Iran, or in flatfish species like turbot (*Scophthalmus maximus*) (Irwin et al., 1999). However, it has been noted that excessively low densities can also negatively impact growth of species that present schooling behavior such as the Arctic charr *(Salvelinus alpinus) (*Jørgensen et al., 1993) or sea bass *(D. labrax*) (Papoutsoglou et al., 1998). Most studies on the impact of density on fish performance have been carried out on freshwater species, mainly salmonids, while little information is available for marine species (Turnbull et al., 2005). Studies were carried out on Atlantic halibut (*Hippoglossus hippoglossus*) (Kristiansen et al., 2004), Dover sole (*Soleasolea*) (Schramet al., 2005), Atlantic salmon (*Salmosalar*) (Irwin et al., 1999) and gilthead sea-bream (*Sparusaurata*) (Canario et al., 1998).

In aquaculture systems, classification according to water flow provides key insight with respect to the water quality processes that control fish production (Krom and Van Rijn, 1989). Intensive fish culture systems can be classified as flow-through or re-circulating. In the former, clean water is typically directed in a single pass through the production unit and discharged or dispersed thereafter. These systems are used to produce fresh water, brackish water and marine species. Freshwater flow-through or open systems are found in regions with an ample supply of clean water (for example, trout farming in Idaho, USA). However, comparative studies on the effects of stocking density on tiger grouper social behavior are not available. The objectives of this study were to investigate those effects, as well as the correlation between different stocking densities and growth rates in different water flow-through conditions (open flow-trough and RAS).

MATERIALS AND METHODS

Location

Research on nursery tiger grouper was carried out on juveniles 35-DAH obtained from a back yard hatchery in the Marine Finfish Production and Research Center (MAFPREC), Tanjung Demong Besut, Terengganu. The fish (initial body wet weight and length of 0.044±0.0085 g and 15.33±1.20 mm respectively), were randomly distributed among the experimental units as Experiments 1 and 2.

Experimental design

The first experiment was carried out in 9 round 80 L fiberglass tanks with bottom area of 0.22 m^2 each. The treatments were run in triplicate with densities of 1, 3 and 5 ind/L (80,240,400) per tank, respectively. Fish were kept in a flow-through circuit of sea water flowing into the tanks. A flow rate of 2 to 4 L/min was constant in all tanks, while water parameters (temperature, salinity, pH, DO) were monitored daily. In the second experiment, 35-DAH juveniles were reared in a tank-based, re-circulating aquaculture system for 42 days (the same as in Experiment 1) at three densities (1, 3 and 5 ind/L) to compare local recirculating aquaculture system (R.A.S) with flow-through system on growth, management and production. The closed system was provided with spongy (mechanical) and gravel (biological) filters, the water was treated with UV illumination, water parameters were constant (similar to Experiment 1) and 10% sea water was exchanged each day throughout the experiment.

Fish management and feeding

During the 42 days of culture, fish were fed ad-libitum with pellet (love larvae No. 2 to 7 and chuen shin No.2) of the following composition: love larvae - crude protein 48 to 54%, crude fat 9 to 12%; chuen shin - crude protein 56%, crude fat 10%. The daily amount of food, which was weighed twice a day to calculate food consumed for each treatment was separately recorded. Fish were fed twice per hour in the first week, hourly in the second week, and every 2 h in the 3rd, 4th, 5th and 6th weeks of rearing from 7 am till 9 pm daily.

Measured parameters

Water quality parameters

Temperature, dissolved oxygen, electric conductivity (EC), total dissolved solid (TDS), PH, and salinity were measured daily in all tanks, with an YSI 556 MPS instrument. Debris, feces and dead fish was siphoned from the tank regularly and daily larval mortality was recorded.

Fish performance parameters

Daily fish mortality was recorded and weekly sampling was done for length and weight measurements, before biometric sampled fish were anesthetized with 0.1% clove oil. Fish were taken randomly and weighed (g) as well as measured at total length (mm). Performance was calculated using the following indicators:

1) Feed conversion ratio (FCR) = F/(Bf - Bi).
2) Specific growth rate (SGR% d^{-1}) = (lnWf - lnWi) × 100/n.

Where: F = feed consumption per period (g), n = number of days in a period, Bf = final biomass (g), Bi = initial biomass (g), Wi, f = initial and final body average weight (g) over a period.

Data analysis

The statistical differences in survival, weight gain and total lengths among groups achieved at the end of the rearing period. All data were subjected to normality test using Kolmogorove –Smirnove. One way analysis of variance (ANOVA) and Duncan multiple range test (DMRT) were applied to determine differences among all treatment. Statistical significance was assumed at $P < 0.05$. To compare the flow-through with RAS systems for weight, total length, and SGR, two independent sample t test was used at a significance level of α = 0.05.

RESULTS

Water quality

Temperature was maintained at 26 to 28°C and the water salinity range was 25 to 28%. There was no obvious effect of stocking density on water quality in the treatment groups. DO (≥5.0) and pH (7.8 to 8.2) were stable and within acceptable ranges.

Fish performance

Biological performance

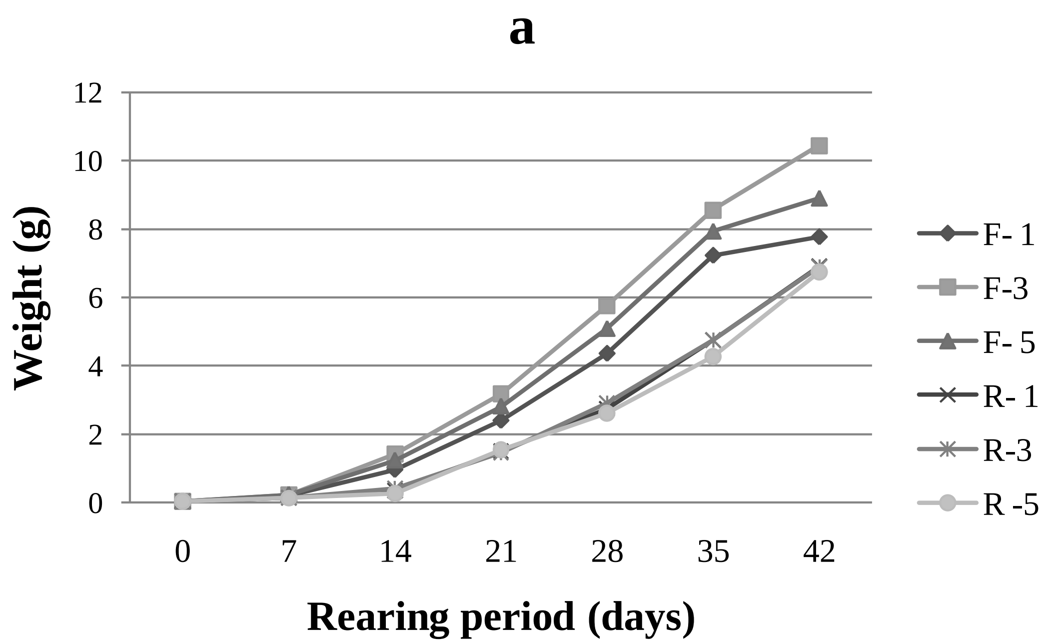
The evolution of weight and length during the whole experimental period (nursery phase) for flow-through and RAS systems are shown in Figure 1a and b, for average weight curves and average length curves respectively. The average total length and weight of treatment 2 (3 ind/L) was significantly larger (P<0.05) than the other groups. Statistically significant differences (P<0.05) for final body weights, total lengths, FCR and SGR were observed between groups at three different stocking densities in flow-through system, and growth characteristics clearly indicate that fish reared at a density of 3 ind/L had the highest final mean body weight, total length, FCR, SGR, of 10.45±0.17 g, 84.92±3.04 mm, 1.22±0.4 and 5.57±0.041, respectively. At the lowest density (1 ind/L) the lowest mean final body weight, total length, FCR, SGR, 7.77±0.27 g, 76.15±0.73 mm, 1.68±0.6, 4.86±0.08, respectively, were found. No meaningful statistical differences (P<0.05) in survival rearing were observed during the 6-week nursery stage in open flow-through water conditions. In the second experiment with RAS, no significant difference was detected for length, weigh, FCR and SGR of fish reared with 3 different treatments at 1, 3 and 5 ind/L (Figure 2a and b). The final mean and standard deviation for growth characteristics, as well as food conversion ratio, survival rate, final weight, total length and specific growth rate for both systems are presented in Table 1.

While comparative growth performance of final mean body weight and final mean total length are shown in Figure 3a and b clearly indicate significant differences between the flow-through water condition and the RAS system.

DISCUSSION

Flow-through systems comprise fish being held in ponds, tanks, raceways or other specially designed systems with a constant water supply passing through. Flow-through systems can be classified as open flow-through water or recirculaiting aquaculture system (RAS). Intensive and semi-intensive flow-through culture systems are those in which fish are stocked at high densities and fed artificial diets. Appropriate stocking densities depend on the species stocked and prevailing environmental conditions. These systems generally require large quantities of water to allow high stocking densities without deterioration of water quality. Therefore, the stocking density and production phase need to be determined for each species to enable efficient management and to maximize production and profitability. Mechanisms linking stocking density and growth are not fully understood, but it is generally accepted that when water quality is not affected by the increased number of fish per cubic meter and sufficient food is provided, differences in growth performance could be attributed to the onset of hierarchies and dominant relationship (Papoutsoglou et al., 1998; Bolasina et al., 2006). Moreover, intrinsic internal factors such as genotype or the interaction among genotypes, and growing environment, could also relate to growth perfor-mance (Bagley et al., 1994). It has been demonstrated that rearing fish at inappropriate stocking densities may impair growth and reduce immune competence due to factors such as social interaction and water quality deterioration which can affect both feed intake and conversion efficiency of the fish (Ellis et al., 2002).

According to this study's results, it is suggested that schooling behavior of the tiger grouper *(Epinephelus fuscoguttatus*) may be a factor in observed differences in fish performance. Feeding rates and frequencies are in part a function of fish size. Small larval fish and fry need to be fed a high protein diet frequently and usually excessively. Small fish have high energy demands and must eat nearly continuously and be fed almost hourly. Excessively feeding small fish is not as much of a problem as overfeeding larger fish because small fish require only small food amounts relative to the water volume in the culture system. Because feed is expensive, feed conversion ratio (FCR) or feed efficiency (FE) are important calculations for the grower. They can be used



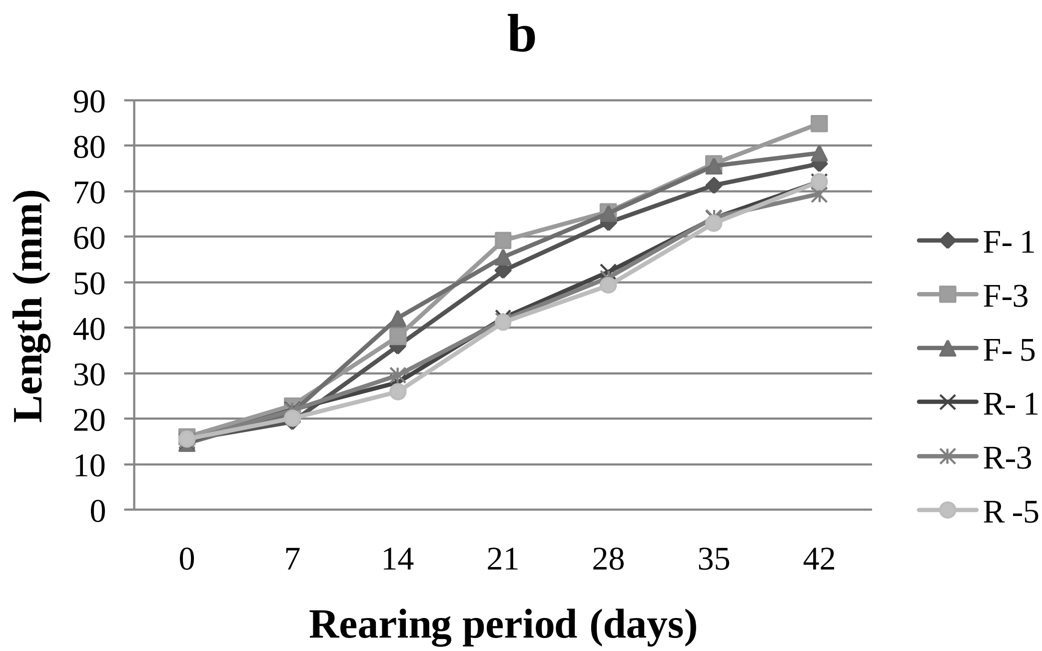


Figure 1. Comparative growth performance of juvenile *Epinephelusfus coguttatus* in open flow-through and RAS systems within 6 weeks of culture (nursery phase) at different stocking densities; a) weight, b) length 1, 3, and 5 represent the densities of fishes per liter. F represents flow-through system, R represents RAS system.

to determine if feed is being used as efficiently as possible. From the present study, it is concluded that within the range of stocking densities tested and at least at nursery stage in an open water flow-through condition, the tiger grouper is influenced by stocking density. This study clearly indicates that optimum stocking density in a flow-through system is 3 fish per liter, and the flow-through water condition is preferred to the RAS system. However, because this system relies on an ample supply of clean water, the water source and amount are important. More detailed studies should be conducted concerning this aspect so the actual cause of growth

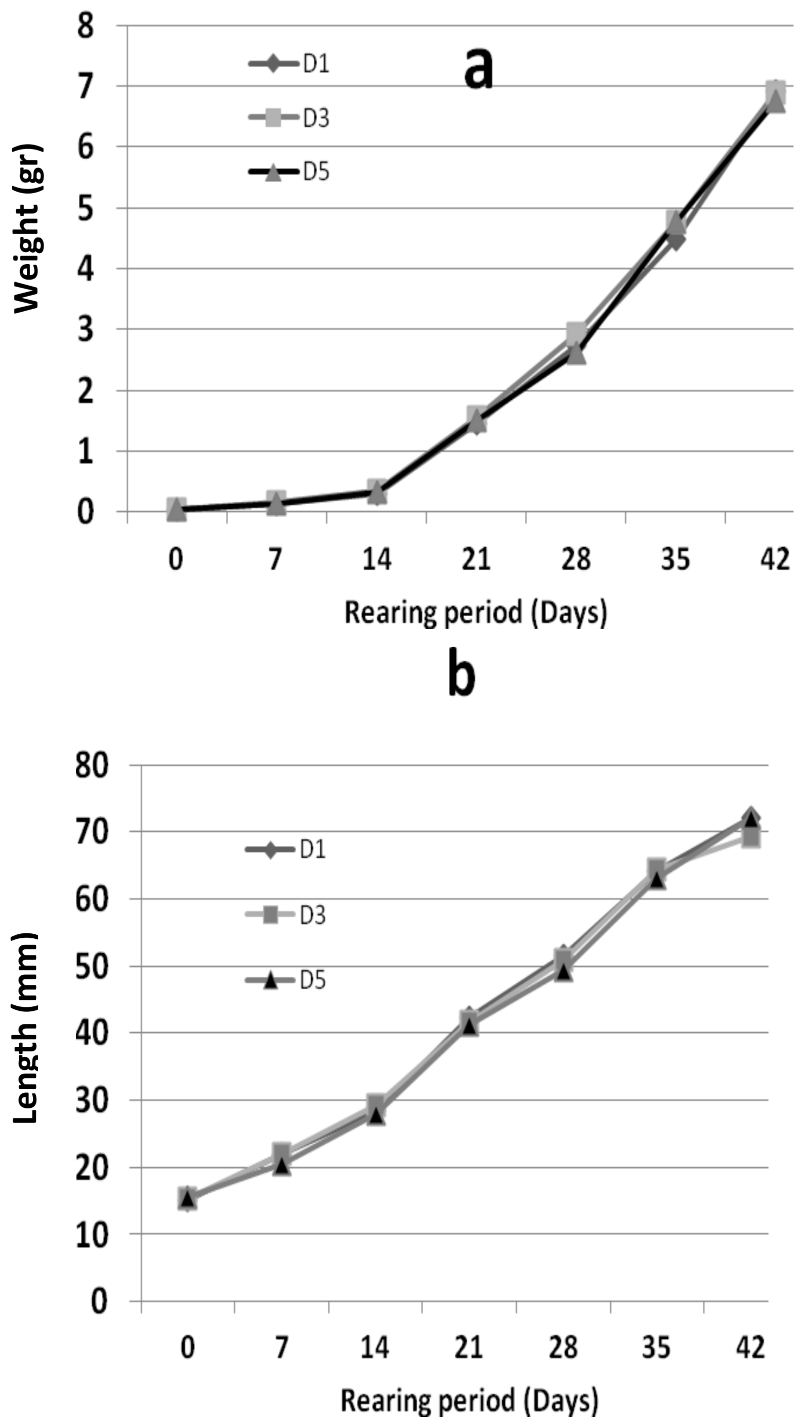


Figure 2: Growth performance of juvenile *Epinephelus fuscoguttatus* in RAS system within 6 weeks of culture (nursery phase) with different stocking density a) weight, b) Length.

suppression at high stocking densities may be evaluated. Techniques for intensive juvenile production have already been developed; however, it is advisable to carry out further studies on larval rearing enhancement to obtain improved survival and greater result predictability.

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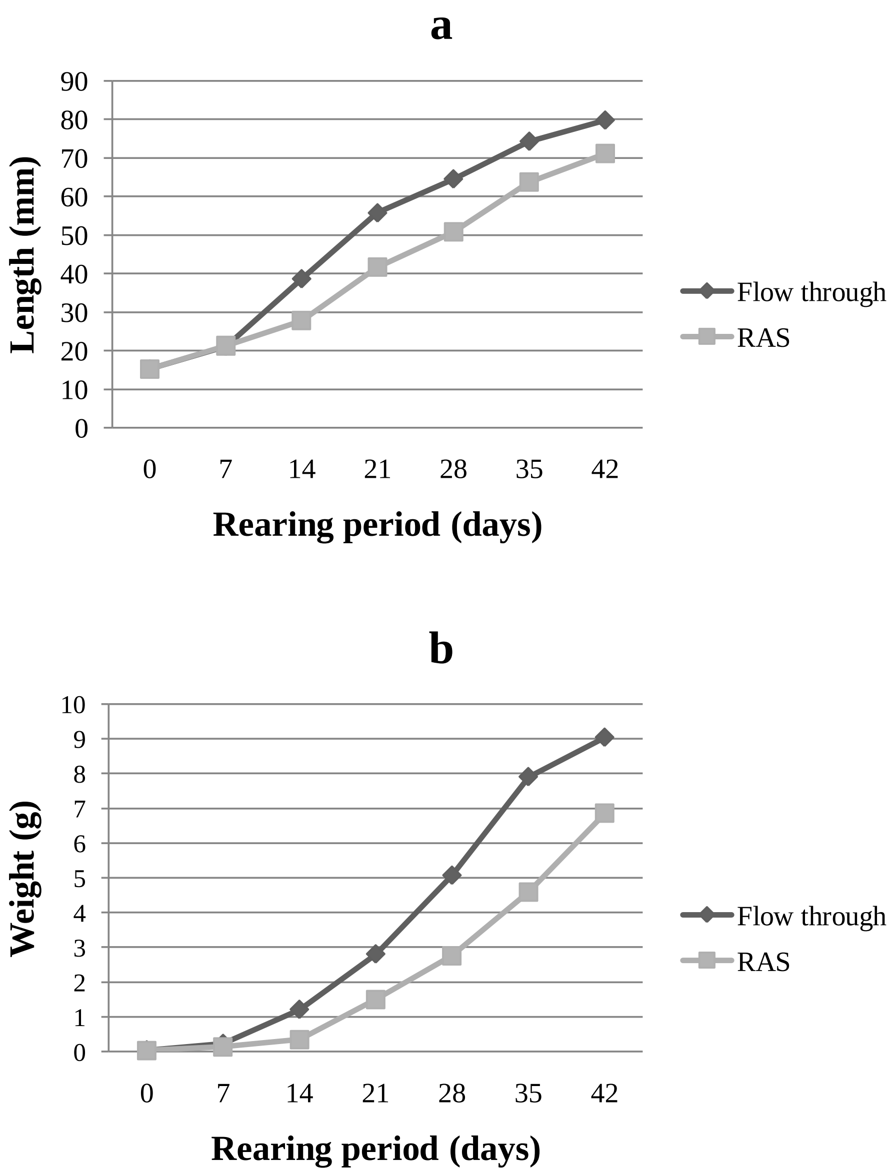

Figure 3. Comparative growth performance of Grouper fish larvae between flow-through and RAS systems within 6 weeks; a) weight, b) length.

Table 1. Effect of different stocking densities on the growth performance of juvenile *Epinephelus fuscoguttatus* in open flow-through and RAS systems during 42 days of culture (nursery phase).

Treatment	T1	T2	T3	T4	T5	T6
Initial mean weight (g)	$0.045±0.001^{a}$	$0.041±0.005^{a}$	$0.041±0.015^{a}$	$0.041±0.006^{a}$	$0.042±0.008^{a}$	$0.042±0.008^{a}$
Final mean weight(g)	$7.77±0.27^{c}$	$10.45±0.17^{a}$	$8.90±0.16^{b}$	$6.92±0.33^{d}$	$6.90±0.19^{d}$	$6.74±0.72^{d}$
Initial mean length (mm)	$15.4±1.88^{a}$	$15.99±1.39^{a}$	$14.60±1.53^{a}$	$15.08±0.72^{a}$	$15.08±1.28^{a}$	$15.62±0.77^{a}$
Final mean length (mm)	$76.15±0.73^{b}$	$84.92±1..36^{a}$	$78.52±0.48^{b}$	$72.11±2.31^{c}$	$69.42±1.37^{d}$	$72.12±1.75^{c}$
FCR	$1.68±0.67^{a}$	$1.22±0.45^{a}$	$1.46±0.78^{a}$	$1.52±0.25^{a}$	$1.38±0.30^{a}$	$1.12±0.32^{a}$
SGR (%)	$12.49±0.34^{bc}$	$13.33±0.18^{a}$	$13.05±0.12^{ab}$	$11.82±0.04^{d}$	$12.17±0.44^{cd}$	$12.07±0.62^{cd}$
Survival (%)	$80.83±3.18^{a}$	$82.77±4.21^{a}$	$80.91±3.31^{a}$	$78.33±3.14^{a}$	$84.86±3.07^{a}$	$79.58±5.21^{a}$

T1: 1 fish per liter, T2: 3 fish per liter, T3: 5 fish per liter, T4: 1 fish per liter, T5: 3 fish per liter, T6: 5 fish per liter, T1, T2, T3 represent flow-through system andT4, T5, T6 represent RAS system, ± SD. Superscript letters in the same row present significant differences. Means in the same row with different superscripts are significantly different (P < 0.05).

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