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Effects of stocking density on growth and feed utilization of grouper (*Epinephelus coioides*) reared in recirculation and flow-through water system

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Stocking density is well known as an important field in aquaculture. Although many studies on stocking density have been conducted, however effects of rearing grouper (*Epinephelus coioides*) with different densities in recirculation and flow-through system are not well known. In this study, grouper juveniles (14.22 ± 0.67 g) were reared into 100-L aquaria at 15, 20 and 25 fish/aquaria and cultivated for 70 days. Statistical analyses showed that the highest growth performances observed in group reared at high densities (25 fish/aquaria) in recirculation system (R25), with an average final body weight and length were 95.82 ± 4.24 g and 18.72 ± 1.40 cm, respectively. Significant increase in weight gain and specific growth rate and decrease in food conversion ratio were observed in R25 after 10 weeks. However, no statistically significant different was found in survival rate and condition factor in all treatments. This study found that the effects of stocking density on growth and feeding ratio were higher in recirculation system compared with flow-through system. Further analysis determined that high stocking density in recirculation system and medium density in flow-through could affect the growth, feeding and fish behavior of this species.

Key words: Feeding ratio, high stocking density, specific growth rate, water system, weight gain.

INTRODUCTION

Recently, research on stocking density is receiving a great attention (Turnbull et al., 2005) since this study is necessary to provide information on a better aquaculture management in relation to the fish welfare. Although some studies on stocking density have been published, it is still difficult to obtain information on better densities for each species, because the best densities are affected by different culture systems, fish species and fish age (Ellis et al., 2002; Jorgensen et al., 1993; Greaves and Tuene, 2001). In aquaculture system, mostly aquaculturists cultivate their fish in high stocking density in order to maximize productivity (Iguchi et al., 2003). Therefore, knowing appropriate stocking density is recognized as an essential aspect because it plays a big role in increasing the fish production to meet the continuous increase in fish demand and maintain the profitable and economic sustainable for aquaculturist (Rafatnezhad et al., 2008).

Generally, stocking density is well known as the weight of fish per unit volume (Ellis, 2001) or the number of fish stocked at the beginning of experiment (Ruane et al., 2002). Stocking density is identified and may affect fish growth performances, physiology and fish behavior (Holm

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et al., 1990; Wedemeyer, 1997; Schreck et al., 1997; Ellis et al., 2002), influence feeding activities, metabolism distortion and digestive utility (Vijayan et al., 1990; Holm et al., 1990; Adams et al., 1998; Dibattista et al., 2005), feed utilization (Jorgensen et al., 1993; Alanara and Brannas, 1996), hormonal alteration (Kebus et al., 1992); and immunological activities (Pottinger and Pickering, 1992; Yin et al., 1995; Tort et al., 1996). In high stocking density, usually fish exhibit aggressive behavior, especially when food availability is limited. This condition often leads to fish stress; particularly it may affect the fish health. Therefore, food availability is very important when it concerns fish density (Holm et al., 1990). Moreover, disproportionate density may cause poor fish welfare, hence affecting the profitableness of the commercial fish industry (Ellis et al., 2002; Conte, 2004; Turnbull et al., 2005; Huntingford et al., 2006; North et al., 2006). Stocking density not only reportedly affects the fish productivity, but also affects water availability, land requirements and production costs.Previous studies have also reported on positive and negative effects of stocking density; for example, in Arctic charr, *Salvelinus alpines* (Jorgensen et al., 1993) and halibut, *Hippoglossus hippoglossus* (Bjornsson, 1994). Those species showed positive impacts when stocked at high densities. In contrast, gilthead sea bream, *Sparus auratus* (Montero et al., 1999) and sea bass, *Dicentrarchus labrax* (Vazzana et al., 2002; Gornati et al., 2004) showed negative impact when reared at high densities, because high densities lead to increased stress level; consequently resulting in poor growth rate and feeding behavior.

Based on the attempts mentioned above, it is worthy to note that studies on fish density have been carried out on some species; however, to this day information on stocking density is still limited (Turnbull et al., 2005), especially in groupers. Thus, this study was designed to investigate the effects of stocking density of grouper, *Epinephelus coioides,* cultivated in different water systems: recirculation and flow-through system.

MATERIALS AND METHODS

Experimental fish preparation

Grouper (*E. coioides*) weighed 14.22±0.67 g body weight were obtained from Aquatic Animal Center and then acclimated in the hatchery of the Department of Aquaculture, National Taiwan Ocean University, for two weeks prior to experimentation. Fish were reared and fed twice a day by feeding commercial diet. Fish of each group were distributed into 100-L total water volume (60 cm length, 50 cm wide, 35 cm height) at 15, 20 and 25 fish/aquarium. Well aerated water was provided from a storage fiberglass. Water quality parameters were maintained at temperature 29.0±1°C; pH 8.0±1 and salinity 34±1 ppt. These ranges are considered within optimal values for grouper juveniles.

Experimental design

The experimental facility was composed of 18 aquaria (100-L each); whereas nine aquaria run- in recirculation water system, others run in flow-through system. Well-aerated water was provided from storage of fiberglass tank, filtered and supplied to the system. Those two water systems were equipped with mechanical filter (spongy), UV light, automatic heater and supplied with compressed air via air-stones from air pumps.

Water flows in experimental aquaria were measured and adjusted before the experiment in order to be proportional to the fish density and to ensure sufficient water circulation. The experimental site was maintained under 24-h photoperiods. The fish were stocked into aquaria based on densities treatments and randomly allocated to triplicate aquaria.

Fish growth measurements

Measurements on parameters such as growth performances, feeding activities, survival rate and water quality was carried on based on required data. Data on growth rate was recorded regularly every two weeks by weighed and measured individual fish from each aquarium. On each sampling day, each individual fish was caught from aquarium using a small net. Then the fish were quickly weighed and measured.

The body wet weight was measured using an analytical balance (Ohaus Navigator, no. 4120, Canada) and the total length using digital caliper (Mitutoyo, Absolute Digimatic, Japan). Immediately after measurements, the fish were carefully returned to its original aquaria. Growth performances were calculated as following:

Specific growth rate (SGR, %/day) = 100 x $(\ln W_2 - \ln W_{1)} / T$

where: W_1 and W_2 are initial weight and final weight, respectively and T is the number of days in the feeding periods.

Weight gain (WG, %) = 100 x [(final weight (g) – initial weight (g)] / initial weight (g)

and

Condition factor (K) = [(10^5 x weight of fish (g) / (length of fish)^3 (cm)]

In experiment on survival rate, all treatments were observed daily and the data was calculated by the following formula:

Survival rate (SR, %) = (Final no. of fish / initial no. of fish) x 100

Feed utilization measurements

Fish were handfed twice daily (at 08:00 and 17:00) at 3% of the biomass by feeding commercial diet. Consequently, the total number and feed size changed as fish grew and as a result of mouth gape. During the experiment, uneaten pellets were collected and measured after each feeding time. For feed utilization, the amount of food consumed was calculated as the difference between dry diet given and dry diet remained.

Feed intake (FI, g/fish/days) = [dry diet given (g) – dry diet remained (g)] / no. of fish

Feed conversion ratio (FCR) = dry feed intake (g) / [final body weight (g) – initial body weight (g)]

and

Feed efficiency ratio (FER) = [final body weight (g) – initial body weight (g)] / dry feed intake (g).

Table 1. Growth responses, condition factor and survival rate of grouper reared at different stocking density.

Variables		Length (cm)		Weight (g)		WG (%)	SGR (%)	K	SR (%)
		Initial	Final	Initial	Final				
				Recirculation system					
Density	R15	9.43 ± 0.58	15.87 ± 1.12^{c}	14.23 ± 0.67	64.17 ± 3.78^{c}	351 ± 20^{c}	2.15 ± 0.06^{c}	1.61 ± 0.10	100
	R20	9.42 ± 0.58	16.13 ± 0.90^{bc}	14.22 ± 0.67	73.53 ± 3.62^{b}	417 ± 25^{b}	2.35 ± 0.07^{b}	1.67 ± 0.15	100
	R25	9.43 ± 0.58	18.72 ± 1.40^{a}	14.24 ± 0.67	95.82 ± 4.24^{a}	574 ± 30^{a}	2.72 ± 0.06^{a}	1.46 ± 0.08	100
				Flow-water system					
Density	F15	9.43 ± 0.58	15.47 ± 1.0^{c}	14.24 ± 0.67	59.82 ± 3.05^{c}	321 ± 14^{c}	2.05 ± 0.05^{c}	1.56 ± 0.11	100
	F20	9.43 ± 0.58	16.72 ± 1.10^{b}	14.23 ± 0.67	75.99 ± 3.70^{b}	435 ± 26^{b}	2.39 ± 0.07^{b}	1.63 ± 0.08	100
	F25	9.42 ± 0.58	15.82 ± 0.90^{c}	14.22 ± 0.67	62.00 ± 3.46^{c}	336 ± 17^{c}	2.10 ± 0.06^{c}	1.57 ± 0.09	100

WG: weight gain; SGR: specific growth rate; K: condition factor; SR: survival rate; R: recirculation system; F: flow-through system. Values are means of triplicate groups' ± S.D. Within a column, means with different letters are significantly different ($P < 0.05$). Means with the same letters or absence of letters indicate not significantly different between treatments.

Water quality measurements

Water parameters including dissolved oxygen (DO), temperature, pH, salinity, ammonia and nitrite were sampled every five days. DO and temperature were measured *in situ* using DO meter (DO600: Waterproof ExStick, Extech Instument Corp. USA), pH with a pH meter (PH100: ExStick, Extech Instument Corp. USA), salinity using refractomoter (ATAGO S/Mill-E, ATAGO CO. LTD, Japan). Data collection was conducted by placing the detector (at the tip of equipments) into the water surface. All displayed number was recorded as water quality parameters. Total ammonia nitrogen (TAN) was examined using phenol-hypochlorite method. It was carried on by prepared 1000 µL of filtered water samples in 1.5 mL tube; and then 40 µL of phenol alcohol solution and 40 µL of sodium nitroprusside were added and mixed into the samples, followed by added 100 µL prepared oxidation solution. After mixing, all samples were stored at 22-27°C for 1 hour, and then samples were analyzed at 640 nm absorbance using spectrophotometer (Ultrospec 8000, Biochrom, Cambridge, UK). Nitrite (NO_2-N) was determined using Wood-Armstrong-Richard method. It was conducted by prepared 1000 µL of filtered water sample in 2 mL tube; 20 µL sulfanilamide was added followed by 20 µL of N-(1-naphthyl)-ethelene diamine solution and mixed. Then samples were stored at 22 to 27°C. After 15 min, all samples were analyzed at 543 nm absorbance using spectrophotometer. During the whole experiments, sea water was changed around 10% daily in recirculation system, whereas in flow-through system, sea water changed approximately 200% daily.

Statistical analysis

Data were analyzed using two-way analysis of variance (ANOVA) with water system and fish densities as factors. When the differences were significant at $P < 0.05$ level, Tukey's test was used to compare the means between individual treatments. Statistical analysis was performed using the SAS software (SAS Inc. Cary, NC, USA).

RESULTS

Fish growth performances

Fish growth was significantly affected by water system and fish densities. A summary of growth responses, condition factor and survival rate in each trial is provided in Table 1. In recirculation system (R), the highest growth performances were observed in group reared with high density (R25). It showed a significantly different ($P < 0.05$) compared to R15 (low density) and R20 (medium density), with an average final body weight 95.82 ± 4.24 g and final length 18.72 ± 1.40 cm. In flow-through system (F), group F20 (juveniles reared in medium density of 20 fish/aquaria) showed the best growth rate. The mean body weight and final length were 75.99 ± 3.70 g and 16.72 ± 1.10 cm, respectively. Among treatment groups, the lowest fish growth was occurred at F15 (juveniles reared in low density of 15 fish/aquaria) in flow-through system. However, it found that there is no significant difference between groups F15 and F25 in their final weight and length. During the whole experimental periods, all groups increase the weight and length steadily every week. Both R-system and F-system showed the greatest weight increments, with fish cultivated in R-system tended to have a better growth performances compare with fish in F-system (Figure 1A, B). However, no statistical differences were observed on the survival rate (SR, %) and condition factor (K) during the 10-weeks rearing (Table 1).

The greatest improvements in weight gain (WG) and specific growth rate (SGR) were observed in group R25 ($P < 0.05$), maintaining a significantly higher WG and SGR than F20 and R20 (Figure 2A, B). The poorest fish growth was obtained in group F15, with the percentage of weight gain and specific growth rate being 321 ± 14 and 2.05 ± 0.05, respectively. There was a decrease of SGR in both cultivation systems. In R-system, at the first measurements (2 weeks) the mean SGR was 2.73%/day (ranging from 2.65 to 2.86%), then decrease steadily to 2.40%/day (ranging from 2.15 to 2.69%) after 10 weeks. Similarly, in F-system, the mean SGR value reduced from 2.54%/day (ranging from 2.38 to 2.67%) to 2.18%/day

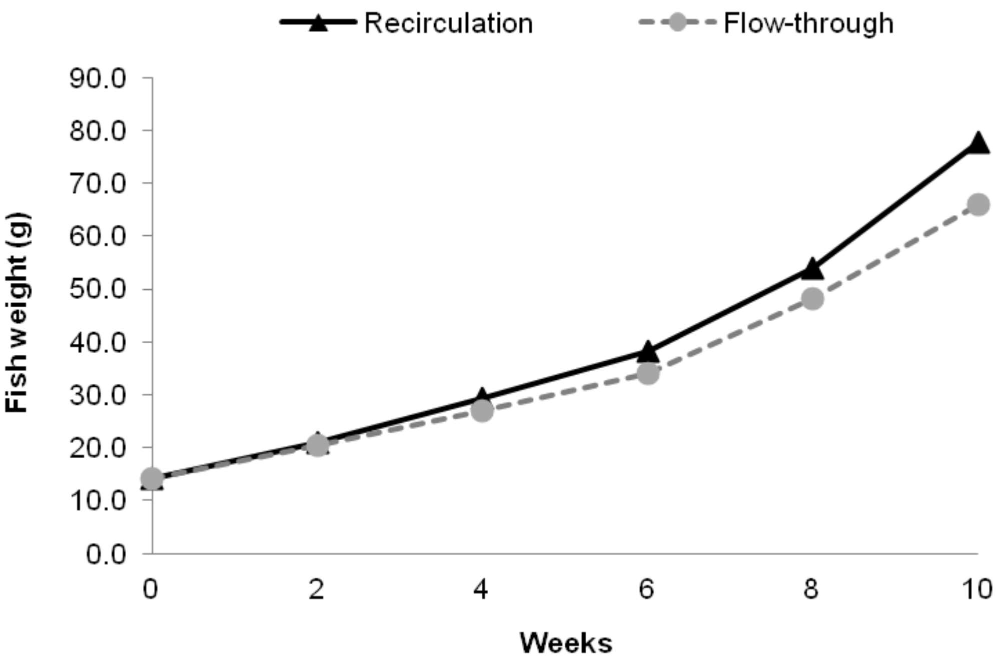

Figure 1A. Comparative of fish weight reared at different stocking density in recirculation and flow-through water system. Values are means of three treatments in each water system.

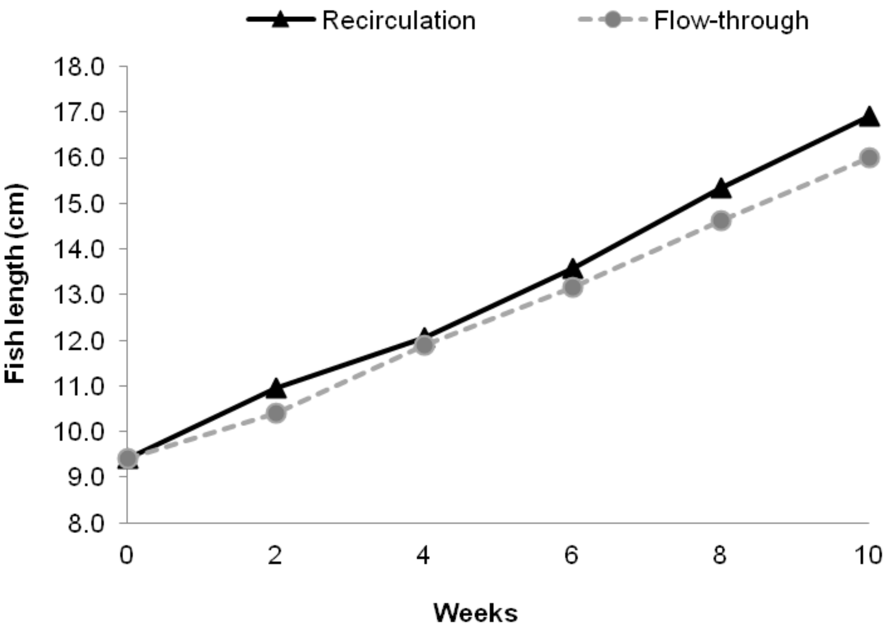

Figure 1B. Comparative of fish length reared at different stocking density in recirculation and flow-through water system. Values are means of three treatments in each water system.

(ranging from 2.05 to 2.39%) at the end of experiment. In contrast, the increased in WG was observed during the experimental time. Mean WG increased from 47% (ranging from 45 to 49%) to 448% (ranging from 351 to 574%) in R-system; and increased from 42% (ranging from 37 to 45%) to 364% (ranging from 321 to 435%) in F- system, respectively. Result on the WG_s and SGR_s indicated that fish cultivated in R-system could enhance overall relative

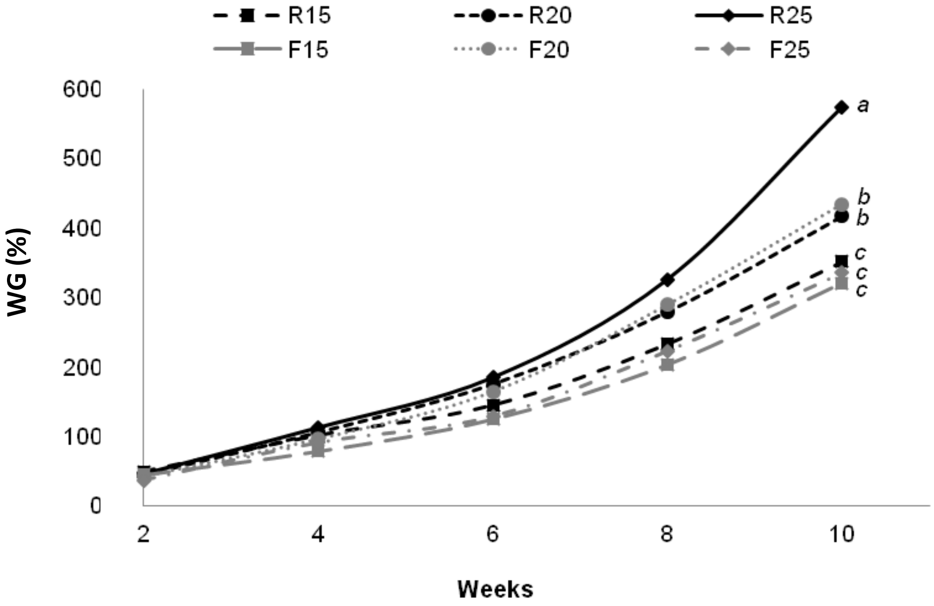

Figure 2A. Mean weight gain (WG) of grouper reared at different stocking density in recirculation and flow-through water system. Values are means of triplicate groups. Different superscripts at the end of each line chart indicate significant different ($P < 0.05$) among treatments.

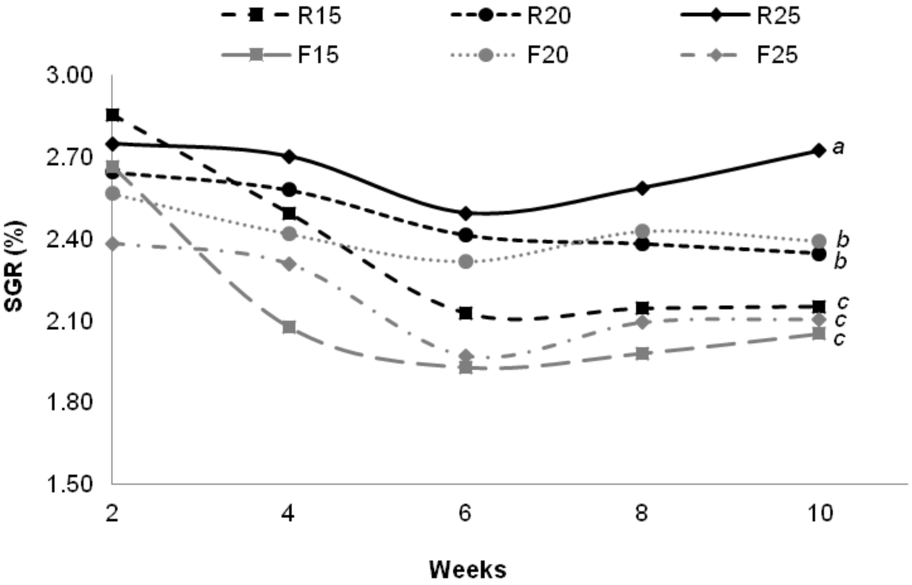

Figure 2B. Mean specific growth rate (SGR) of grouper reared at different stocking density in recirculation and flow-through water system. Values are means of triplicate groups. Different superscripts at the end of each line chart indicate significant different ($P < 0.05$) among treatments.

growth rate compare with fish cultured in F-system. However, statistical analyses showed that there were no significant differences between R20 with F20 and between R15 with F15 and F25.

Feeding performances

Feeding parameters such as feed intake (FI), feed conversion ratio (FCR) and feed efficiency ratio (FER)

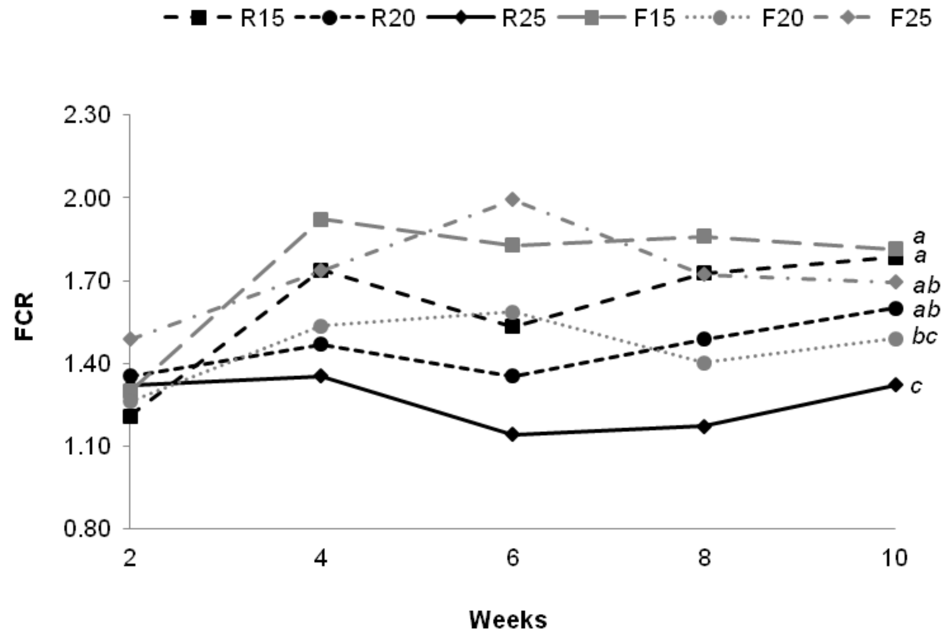

Figure 3. Mean feed conversion ratio (FCR) of grouper reared at different stocking density in recirculation and flow-through water system. Values are means of triplicate groups. Different superscripts at the end of each line chart indicate significant different ($P < 0.05$) among treatments.

Table 2. Feed utilization of grouper reared at different stocking density.

Variables		FI	FCR	FER
		Recirculation system		
	R15	89.05 ± 0.85^{c}	1.79 ± 0.18^{a}	0.56 ± 0.03^{c}
Density	R20	94.68 ± 1.07^{b}	1.60 ± 0.12^{ab}	0.63 ± 0.04^{bc}
	R25	107.8 ± 4.04^{a}	1.32 ± 0.05^{c}	0.76 ± 0.03^{a}
		Flow-water system		
	F15	82.69 ± 0.96^{d}	1.82 ± 0.18^{a}	0.55 ± 0.02^{c}
Density	F20	91.92 ± 0.63^{bc}	1.49 ± 0.12^{bc}	0.67 ± 0.04^{ab}
	F25	80.81 ± 0.77^{d}	1.69 ± 0.19^{ab}	0.59 ± 0.03^{bc}

FI: feed intake (g feed/fish/day); FCR: feed conversion ratio; FER: feed efficiency ratio. Values are means of triplicate groups' ± S.D. Within a column, means with different letters are significantly different ($P < 0.05$). Means with the same letters indicate not significantly different between treatments.

were significantly affected by water system and fish densities ($P < 0.05$). In R-system, the best feed utilization was obtained by R25, with FCR showing significant difference ($P<0.05$) compared to R15 and R20 (Figure 3). Similarly, in F-system, group, F20 showed the best feeding activity with the mean of FI and FCR being 91.92 ± 0.63 and 1.49 ± 0.12, respectively (Table 2). This study found that, fish cultured in R-system tended to consume more feed compared with fish in F-system (Figure 4). The highest FI was found at R25 (107.8 ± 4.04 g), while the lowest one was at F25 (80.81 ± 0.77 g). In R-system, the FCR decreased and FER ratio increased significantly in group R25 compared to other groups. Similar result showed in F-system; the FCR decreased and FER ratio increased significantly in group F20 compared to F15. In contrast, group R15 showed increasing FCR and decreasing FER ratio from 4 weeks until the end of experiment. In both culture systems, the total FCR values were lower in fish cultivated in R-system than fish cultivated in F-system. Furthermore, overall FER values were higher in fish cultured in R-system compare with F-system. Data analyses found that the best FCR and FER were obtained by R25 at 1.32 ± 0.05 and 0.76 ± 0.03, respectively; while the poorest FCR and FER were seen

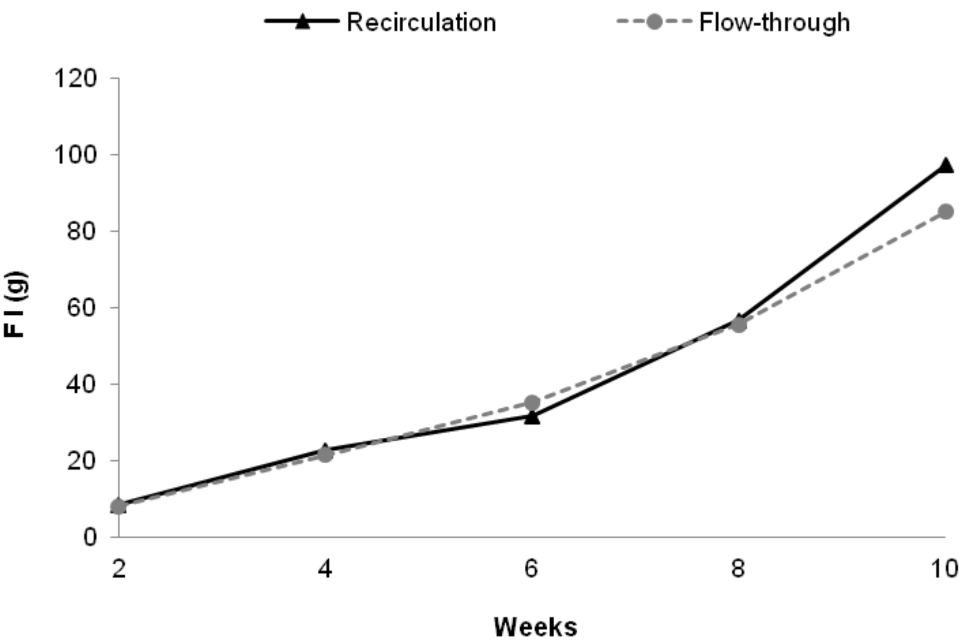

Figure 4. Comparative feed intake (FI) of grouper reared at different stocking density in recirculation and flow-through water system. Values are means of three treatments in each water system.

at F15 at 1.82 ± 0.18 and 0.55 ± 0.02, respectively.

Water quality parameters

A summary of water quality parameters in each trial is provided in Table 3. Water quality parameters such as: salinity, dissolved oxygen (DO), temperature and pH were checked regularly every five days until the end of experiment. During the experiment, temperature in R-system ranged between 30.69 to 30.76°C, with the highest temperature found in higher density compare with lower density. In F-system, the temperature remained stable within the range of 29.09 to 29.10°C. The data showed that temperature in F-system tended to be lower than R-system. This low temperature is estimatedly caused by regularly water changes up to 200% daily. In R-system, DO levels were between 5.81 to 5.96 ppm, while in F-system, it ranged from 6.37 to 6.44 ppm. Significant differences were observed in both water systems, whereas DO concentration seems to be high in F-system than R-system. The highest DO was obtained by F15, whereas the lowest one was occurred in R25. The data also indicated that the higher stocking density tended to be lower in DO concentrations.

pH was significantly different in each treatment, with the lowest pH occurring in R25. This low pH value was predicted due to high fish stocking density. Observation on water quality also found that there was no obvious effect of stocking densities on salinity in the treatment groups. Mean water salinity was dependent on the daily supply of seawater. During 70 days of experiment, the range of salinity in R-system was 34.15 to 34.28 ppt, while in F-system, salinity was around 32.87 to 33.08 ppt. These ranges are considered within optimal values for fish culture (Barnabe, 1990; Shepherd and Bromage, 1992; Boyd, 2000; Ismi et al., 2012).

On the first two sampling times, total ammonia nitrogen (TAN) was low in both groups. In R-system, mean of 3 treatments ranged at 0.11 to 0.12 ppm, whereas in F-system ranged from 0.09 to 0.10 ppm. The differences between groups increased with time (Figure 5). In R-system, TAN increased steadily and reached a peak at day 45, with the mean value of 3 treatments 1.20 ppm, however TAN slightly lower until the final sampling day. In contrast, TAN were continuously increasing in F-system with the highest concentration found at the end of sampling time; with the mean of 3 treatments being 1.04 ppm. During the experimental days, the highest TAN was found at R25 with the mean value being 1.39 ppm. In the present study, nitrite concentration was always lower than TAN concentration in both culture systems. It is found that during 5 times of samples measurements, nitrite was almost similar in each group (<0.14 ppm). In all groups, there was a steadily increasing nitrite with time (Figure 6). In R-system, nitrite reached a peak (mean of 3 treatments: 0.33 ppm) on the tenth sampling date, followed by a gradual decline. On the other hand, nitrite showed an increase in F-system until the end of experiment, with the highest mean value attained by

Table 3. Mean Salinity, dissolved oxygen, temperature and pH.

Parameters		Salinity (ppt)	Dissolved oxygen (ppm)	Temperature (°C)	pH
			Recirculation system		
	R15	34.21 ± 0.67^a	5.96 ± 0.35^b	30.69 ± 1.40^a	7.76 ± 0.29^c
Density	R20	34.28 ± 0.62^a	5.88 ± 0.37^{bc}	30.72 ± 1.42^a	7.75 ± 0.29^c
	R25	34.15 ± 0.59^a	5.81 ± 0.39^c	30.76 ± 1.47^a	7.70 ± 0.30^d
			Flow-water system		
	R15	33.05 ± 0.62^b	6.44 ± 0.48^a	29.09 ± 1.72^b	8.02 ± 0.19^a
Density	R20	33.08 ± 0.64^b	6.43 ± 0.48^a	29.10 ± 1.66^b	8.01 ± 0.18^{ab}
	R25	32.87 ± 0.58^b	6.37 ± 0.47^a	29.10 ± 1.62^b	7.99 ± 0.20^b

Values are means of triplicate groups' ± S.D. Within a column, means with different letters are significantly different ($P < 0.05$). Means with the same letters indicate not significantly different between treatments.

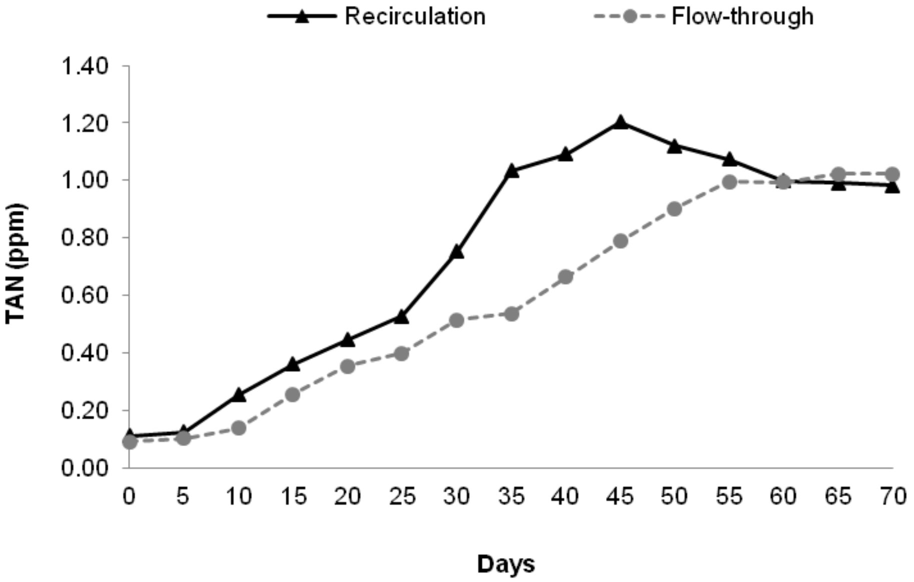

Figure 5. Comparative total ammonia nitrogen (TAN) between recirculation and flow-through water system of grouper reared at different stocking density. Values are means of three treatments in each water system.

F20 at 0.41 ppm.

DISCUSSION

This present study indicated that water system and stocking density had greater effects on fish growth performances, water quality and feed utilization. Growth rate and feed utilization were higher in R-system than in F-system. Presumably, this was caused by a more stable water quality in R-system compare to F-system due to the ability of this system to maintain a constant water quality (Roque d'Orbcastel et al., 2009), improve waste management and nutrient recycling (Piedrahita, 2003), and hygienic (Summerfelt et al., 2009; Tal et al., 2009).

The results showed the positive effect of high stocking density and medium stocking density of *E. coioides* reared in R-system and F-system, respectively. Significant differences in the value of growth were observed among different groups in all the growth parameters studied. The fish cultured at a higher density in R-system reached significantly higher weight and length value than those at lower densities. Further, it found that the greatest improvements in WG and SGR in group R25 were 574 and 2.72%, respectively. It is assumed that higher density caused less swimming due to limited space availability. This condition has been attributed to metabolic savings and low energy expenditure. As a result, consumed nutrients can be utilized for growth maximization. This finding was similar

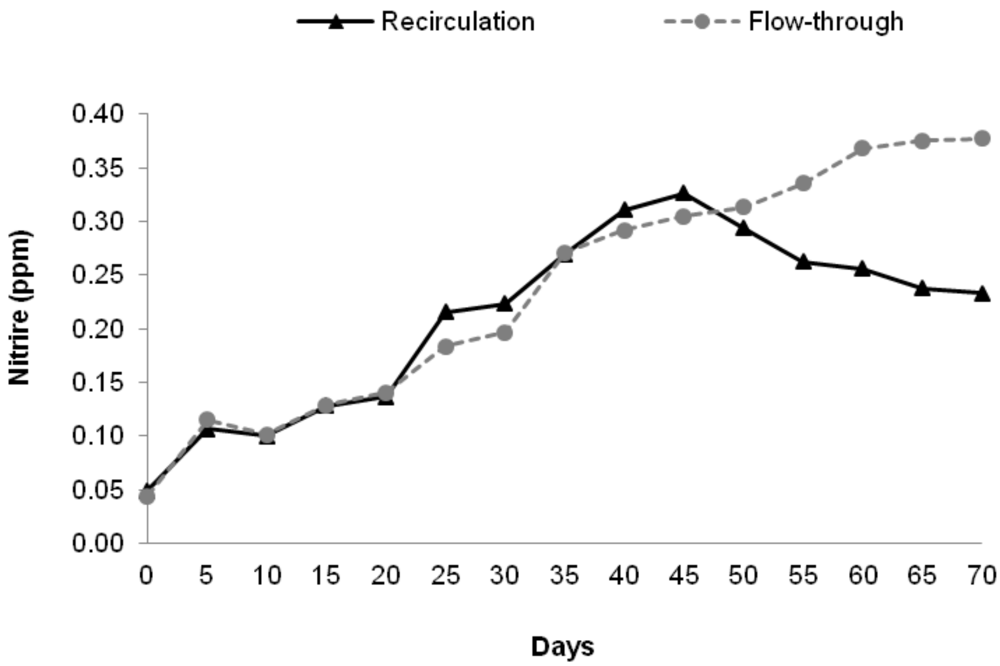

Figure 6. Comparative of nitrite between recirculation and flow-through water system of grouper reared at different stocking density. Values are means of three treatments in each water system.

to previous studies conducted by Jorgensen et al. (1993) on the Arctic charr *S. alpines*; North et al. (2006) on Rainbow trout *Oncorhynchus mykiss*; Papoutsoglou et al. (1998) on sea bass *D. labrax,* and Howell (1998) on turbot (*Scophthalamus maximus*), which described an increment on growth with the increasing of stocking density. However, some studies reported on negative effect when fish is cultivated in higher density, for example in Brook charr *Salvelinus fontinalis* (Vijayan et al., 1990), Rainbow trout and Brown trout (Sirakov and Ivancheva, 2008), and Rainbow trout juvenile (Procarione et al., 1999). On the other hand, fish reared in F-system at medium density (F20) showed the highest growth increment compared with higher (F25) and lower density (F15). It is suggested that medium density is the optimal stocking density of this species when reared in F-system. This may be due to individuals acquiring space, food and water fluctuation during the experiment. It has been demonstrated that appropriate stocking density depends on species, social interaction, water quality and environmental conditions, therefore studies on stocking densities need to be determined in each species for management efficiency in increasing productivity and profitability (Salari et al., 2012; Baldwin, 2010; Ellis et al., 2002).

In these trials where different stocking density had shown effects on juveniles' growth rate, the feed utilization was also observed. The results exposed that FI, FCR and FER were better in R-system compared with F-system. It is assumed that more constant water condition and long-day photoperiods during this experiment affected the fish appetites and feed intake. Similar studies have been reported on the effects of water circumstances and photoperiods in increasing appetite (Taylor et al., 2006; Saunders et al., 1994; Trippel and Neil, 2003), feed intake (Imsland et al., 1995), feeding ratio (Nordgarden et al., 2003; Boeuf and Le Bail, 1999; Boujard et al., 1995), and reducing food costs in commercial farming (Trippel and Neil, 2003). Based on the results, it is alleged that continuously light and schooling behavior seems playing a big role in increasing fish appetence, therefore feed intake can be improved in R25 and F20 in order to gain a maximum growth increment. This finding was in agreement with studies conducted by Webster et al. (2001) and Nordgarden et al. (2003); both suggested that growth may be enhanced by increasing feed consumption to meet the energy demand in maintaining continually growth. In this aspect, we recommend that increase in feeding percentage should be considered in recently studied species; however, well feeding administration is acquired to avoid FCR and FER values deterioration.

Studies on stocking density involve many interrelated parameters including water quality and food availability (Hastein et al., 2005; Ellis et al., 2002). During the experiment, measurements on water quality on both R-system and F-system were examined in the morning. All water samples including TAN and nitrite were taken before the feeding activity, while diurnal water changes were not measured. This practice was similar with the experiment on juvenile cod, *Gadus morhua* (Bjornsson and Olafsdottir, 2006) in agreement with an assumption that all water quality measurements must be checked at minimum values (Burel et al., 1996). The present experiments found that, water quality parameters were significantly different in both R-system and F-system. Deterioration seemed to be affected by different stocking density in R-system, with higher density which showed poorer in DO, pH and ammonia concentration. However, R-system was provided with good controlling water facilities including aeration and filters supported by relatively low water exchange (around 10% daily), which caused more constant and stable water in this system. Boyd (2000) and Agarwal (1999) mentioned that in high stocking density, the problems such as oxygen deficiency, ammonia-nitrogen and carbon dioxide accumulation and other organics pollution are frequently occurring; by then aeration is considered the best way to solve this matter. On the other hand, although some water parameters such as salinity, DO and temperature were not significantly affected by different densities in F-system, it is however observed that pH, TAN and nitrite were influenced by stocking density where each value was slightly decreased with higher density. It is estimably caused by fish excretion in the culture media as a result of metabolic activities.

In the present study, both TAN and nitrite showed a variation in different culture systems. In R-system, TAN increased steadily at the beginning of experiment followed by slightly decreasing until the final sampling day. In contrast, TAN were continuously increasing in F-system with the highest concentration found at the end of sampling time. It is presumably caused by bacteria and other microorganisms' activities. Those microorganisms usually form a complex community interactions commonly known as biofilms. Biofilms seemed to be formed earlier in R-system rather than in F-system; it is estimably caused by lower water exchange compare to F-system which undergoes high water exchange. Although the true function of biofilms is still not yet understood (Flemming, 2008) especially in fish culture system, it has long been recognized that biofilms is essential in water considering its ability in providing protection against environmental stressor (Kokare et al., 2009). In general, biofilms also believed to be involved in biogeochemical cycles of nitrogen, hydrogen, oxygen, carbon, sulphur and phosphorus (Ehrlich, 2002); mineral weathering processes, oxygen production, ammonia and nitrite oxidation (Flemming, 2008); enhancing nutrient availability and removal toxic metabolites (Decho, 1990). These studies imply that bacteria and other microorganisms may affect water quality. In this study, even though no specific experiment was carried out to determine the effect of microorganisms in both R-system and F-system, observation found that microorganisms play a big role for maintaining good water quality in culture system.

Conclusion

The present study indicates that growth performances, feed utilization and water quality were affected by culture systems and stocking densities. Thus, for grouper juveniles, it is suggested to be reared in high stocking density in recirculation system to attain a maximum growth. However, maintaining acceptable water quality and providing enough food is required to sustain fish health and to prevent aggressive behavior.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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