EFFECT OF STOCKING DENSITY ON PERFORMANCE OF GOLDLINED RABBITFISH Siganus lineatus AND THE ENVIRONMENTAL QUALITY IN A CLOSED CULTURE SYSTEM

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ABSTRACT

The experiment was conducted to determine adaptability of rabbitfish Siganus lineanus under rearing condition that similar a closed earthen pond and to assess the effect of stocking density on fish growth performance and the environmental quality. Rabbitfish (5.7 g) were stocked in 3 treatments with different densities, including low density (LD) (7 fish.m^{-2}), medium density (MD) (14 fish.m^{-2}), and high density (HD) (21 fish.m^{-2}) with four replicates per treatment. After 8 weeks of experiment, survival was 100% in LD and MD treatments, while high mortality occurred in one replicate of HD treatment. There was no significant difference in growth performanceof rabbitfish reared at different densities. The fish biomass was significantly lower in the LD treatment than those in other treatments whereas there was no significant difference between MD and HD treatments. Some water and sediment parameters such as turbidity, Chl a, TAN and SRP were significantly higher in HD than those in LD treatment. The environmental variation increased following the increase of stocking density that led to phytoplankton bloom in the HD treatment at the end of the experiment.

Our results suggested that increasing stocking density from 7 to 14 fish.m² does not decrease fish growth and the environmental quality, while increases fish final biomass. High survival and good growth rate of rabbitfish S. lineatus illustrate that rabbitfish is a suitable candidate for reareing in closed earthern ponds.

Keywords: Siganidae, closed system, growth, environment, biomass

I. INTRODUCTION

Siganidae (Rabbitfishes) is a family consisting of 28 marine herbivorous species. They are widely found in the Indo-Pacific region (Duray, 1998; Borsa et al., 2007). Rabbitfishes traditionally contribute a major part to commercial fisheries production in several Pacific countries and are considered high potential candidates for mariculture. Many studies have been conducted on biological and ecological aspects of rabbitfish species for mariculture (Gundermann et al., 1983; Wassef and Addul Hady, 1997; Duray, 1998; Bariche, 2005; Jaikumar, 2012). Rabbitfishes possess most of the desirable characteristics for aquaculture, such as high tolerance to different environmental factors, rough handling and crowding, palatability and high demand and market prices for both local consumption and export. In addition, rabbitfishes are primarily herbivores but may turn to other diets readily. Thus, in captivity they have shown to feed on a wide variety of foods offered, and grow rapidly on a variety of natural foods or artificial food pellets (Lam, 1974). Some species are gregarious and thus may be able to tolerate crowded conditions (Duray, 1998). Many species of Siganidae have already been farmed in coastal ponds in the Philippines either in monoculture or co-culture with milkfish (*Chanos chanos*) (Duray, 1998). Nowadays, rabbitfish mariculture has been widely expanded in many countries such as Guam (Brown et al., 1994), Taiwan (Nelson et al., 1992), the Red sea and Mediterranean region (Stephanou and Georgiou, 2000; El-Dakar et al., 2010), UAE (Yousif et al., 2005), East coast Africa (Bwathondi, 1982), India (Jaikumar, 2012) and New Caledonia

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(SPC, 2008), under diversity of suitable designed structures of earthen ponds and sea cages. Yet rabbitfish aquaculture has not advanced on a commercial scale, possibly due to the slow growth rate but mature early at the small size and are difficult to handle (Von Westernhagen and Rosenthal, 1976; Duray, 1998). Furthermore, many aspects of rabbitfish performance in different grow-out facilities remained unsolved (Yousif et al., 2005). Nearly all studies on rabbitfish grow-out were conducted in the cages or ponds/ tanks with water flow through and mainly focused on production performance. The environmental variations in culture systems as well as the mutual effects between the environment and rabbitfish production have not been well reported. So, we conducted the study "The effect of stocking density on performance of goldlined rabbitfish *Siganus lineatus* and on environmental quality in a closed culture system". The objectives include to estimate the adaptive capacity of S. lineatus under culture conditions such as an earthen pond and to estimate the effects of different stocking densities on *S. lineatus* performance e.g. survival and growth rate and on environmental quality in a closed system. The results of this study would be useful for determining whether *S. lineatus* is a good candidate for commercial culture in earthen ponds.

II. MATERIALS AND METHODS

1. Experimental design and setup

The experimental closed system included 12 – 700 L outdoor circular fiberglass tanks (1.0 m^2 in area, 70 cm in height). Sediment taken from salt-marsh was mixed and spread evenly in all tanks up to 10 cm (per tank). The tanks were filled with fresh seawater one week before stocking up to 50 cm (500 L in volume). Aeration was continuously supplied into the tanks via 4 cm diameter spherical air-stones hanging 5 cm above bottom centers, one air-stone per tank. No water exchange was applied during the experiment.

S. lineatus juveniles (5.7 ± 1.2 g, 6.8 ± 0.5 cm TL), hatchery-reproduced, were randomly stocked at three different densities to form three treatments, including low density (LD) (7 fish.m^{-1}, also 7 fish.$tank^{-1}$); medium density (MD) (14 fish.m-2) and high density (HD) (21 fish.m-2). All treatments were randomly distributed among tanks with four replicates per treatment. Fish were fed with commercial pellet feed (35 – 40% protein, SICA Manufacturer), twice a day at 8:00 and 16:00, with a feeding rate of approximately 3–5% of fish biomass per day. Feed quantity was adjusted using feeding trays (30 cm diameter) placed 10 cm above tank bottoms at each time of feeding. Feed consumption on the tray was closely observed to determine and adjust the feed ration. The experiment lasted 8 weeks from stocking to harvesting.

2. Sampling and analyzing

At stocking, 30 fish were randomly sampled, individually weighed and measured. At harvesting, all fish in each tank were counted, individually weighed and measured. The weight was scaled to the nearest 0.1 g using an electronic balance, and the total length (TL) was measured to the nearest 0.1 cm using a technical ruler.

Fish performance was evaluated in terms of survival rate (SR), daily weight gain (DWG), specific growth rate (SGR), and yield.

SR (%) = harvesting number/stocking number*100

DWG (g.day^{-1}) = Weight gain (g)/time (days)

SGR (%.day^{-1}) = (Ln Wf – Ln Wi)/time (days)*100

Yield (g.m^{-2}) = harvesting biomass (g)/area of culture tank (m^2)

where Wi: initial mean weight (g), Wf: final mean weight (g)

Fulton's condition index: K = 100 * W/ TL^3, where W is the weight (g), TL is the total length (cm).

The coefficient of variation CV = SD/ mean*100 (%)

Food conversion ratio (FCR) was calculated as followed:

FCR = total feed fed (dry weight, g)/total weight gain (fresh weight, g)

Water temperature and dissolved oxygen (DO) concentrations were recorded twice a day (07:30 am and 15:00 pm) at mid depth of each tank using an OxyGuard meter (Handy Polaris, Birkerød, Denmark). Salinity was measured daily (08:00 am) using refractometer (Cond 3210, Welheim, Germany). Turbidity, fluorescence and pH were measured twice a week using turbidimeter (TN-100, Eutech Instruments, Singapore), Aquafluor (Turner Designs, Sunnyvale, CA. USA), and pH meter (pH 197i, Welheim, Germany), respectively. On the day before fish stocking and one a week thereafter, water samples (1 L from each tank) were collected in all tanks (08:00-08:15 am) and filtered through pre-combusted (450 ºC, 4 hrs) GF/C Whatman fiberglass filters (ϕ: 47 mm, pore size: 1.2 µm). Water parameters were analysed, including total ammonia nitrogen (NH_4^+-NH_3)-N, (TAN) (Koroleff, 1976) and soluble reactive phosphorus (SRP) (Murphy and Riley, 1962). To estimate chlorophyll *a* (Chl *a*) and phaeopigments (Phaeo), water samples of 25 mL were filtered through GF/F Whatman fiberglass filters (ϕ: 25 mm; pore size: 0.7 µm) and then analyzed using a fluorometer (TD 700) following Holm-Hansen et al. (1965).

Sediment samples were sampled on the day before fish stocking and one every three weeks thereafter from 1 cm deep core using 50 ml cut-off syringes (ϕ: 2.3 cm). The samples were collected at three different points within each tank and pooled for the analysis of organic matter content, pH and nutrient concentrations in pore water. pH was directly measured by pushing the glass electrode (pH 197i, Welheim, Germany) into freshly collected sediment in the sample vials. The samples were centrifuged at 814 *g* for 20 minutes. The supernatant parts (pore water) were used to analyze TAN and SRP following the methods as described above for water. The sediment samples were dried at 60 °C for one week and then analyzed for loss on ignition in a muffle furnace at 350 °C for 8 h (Nelson and Sommers, 1996). Sediment Chl *a* concentration was analyzed from three different samples (1cm core layer) per tank. Frozen sediment samples were freeze-dried (lyophilized) for 24 h and analysed using a TD-700 fluorometer (Holm-Hansen et al. 1965). The concentration of sediment Chl a was expressed in mg/m².

3. Statistical analysis

All data were checked for normality (Kolmogorov-Smirnov test) and homogeneity of variances (HOV, Brown Forsythe test), and statistically analyzed using one-way ANOVA with IBM SPSS software version 16.0; with possible differences among data being tested by Duncan's multiple range tests. Percent data were arcsine-transformed before statistical analyses, but non-transformed data are presented in tables. Statistical comparisons of experimental data among treatments were performed for overall mean values and for each time of analyses. Non-parametric test (Kruskal-Wallis test, H test) and Tamhane's T2 (Post-hoc, one-way ANOVA) were used when data were not normally distributed or the variances were heterogeneous.

III. RESULTS AND DISCUSSION

1. Environmental variation

Mean values of temperature, DO, salinity and pH were similar in all treatments throughout the experiment (Table 1). Temperature varied in ranges that seemed to be lower recommended suitable temperatures for rabbitfish growth while DO, salinity and pH remained in suitable ranges for rabbitfish growth during the experiment.

Mean turbidity was not significantly different between the MD with the other treatments, while it was significantly higher in the HD treatment than that in the LD treatment. Chl *a* concentration and mean value of TAN was significantly higher in the HD treatment than those in the other treatments. Mean value of SRP was significantly higher in the HD treatment than that in the LD treatment, whilst there was no significant difference between the

Table 1: Water parameters in the experimental treatments of rabbitfish culture at different stocking densities. Values are means ± SD.

	Treatment		
	Low density (7 fish.m^{-2})	Medium density (14 fish.m^{-2})	High density (21 fish.m^{-2})
T (07:30) ($^{\circ}$C)	19.7 ± 0.1	19.7 ± 0.1	19.7 ± 0.1
T (15:00) ($^{\circ}$C)	22.8 ± 0.2	23.0 ± 0.2	22.9 ± 0.2
DO (07:30) (mg.L^{-1})	6.5 ± 0.1	6.4 ± 0.1	6.3 ± 0.1
DO (15:00) (mg.L^{-1})	8.0 ± 0.2	8.1 ± 0.2	8.2 ± 0.2
Salinity	33.2 ± 0.4	33.1 ± 0.1	33.1 ± 0.2
pH	8.1 ± 0.0	8.0 ± 0.1	8.0 ± 0.1
Turbidity (NTU)	6.4 ± 3.5^{a}	7.1 ± 1.6^{ab}	12.6 ± 3.0^{b}
Fluorescence (µg.L^{-1})	23.5 ± 9.7^{a}	18.6 ± 6.0^{a}	44.1 ± 24.0^{a}
Chl *a* (µg.L^{-1})	9.8 ± 5.1^{a}	7.4 ± 0.6^{a}	27.5 ± 11.8^{b}
Phaeopigment (%)	38.3 ± 1.1^{a}	43.7 ± 2.6^{b}	36.0 ± 3.0^{a}
TAN (µmol.L^{-1})	4.6 ± 1.0^{a}	18.0 ± 5.3^{a}	52.4 ± 10.6^{b}
SRP (µmol.L^{-1})	1.8 ± 0.6^{a}	4.8 ± 1.6^{ab}	7.8 ± 2.6^{b}

Mean values in a same row with different superscript letters are significantly different (P<0.05).

HD and the MD treatments, as well as between the MD and the LD treatments (Table 1).

Sediment pH was similar among treatments, and relatively stable throughout the experiment. Sediment Chl *a* concentration was significantly higher in the HD treatment than those in the MD and the LD treatments. Mean value of pore water TAN was significantly higher in the HD treatment than that in the LD treatment. There was no significant difference in mean pore water SRP among treatments (Table 2).

The significant differences in some major

Table 2: Sediment parameters in the experimental treatments of rabbitfish culture at different stocking densities. Values are means ± SD.

	Treatment		
	Low density (7 fish.m^{-2})	Medium density (14 fish.m^{-2})	High density (21 fish.m^{-2})
pH	7.4 ± 0.1	7.3 ± 0.1	7.2 ± 0.2
Chlorophyll a (mg.m^{-2})	107.5 ± 21.9^{a}	137.6 ± 32.9^{a}	221.6 ± 59.2^{b}
Phaeopigment (%)	27.4 ± 2.1^{a}	29.2 ± 3.2^{a}	30.3 ± 0.5^{a}
Loss on ignition (%)	1.8 ± 0.1^{a}	1.9 ± 0.2^{a}	1.8 ± 0.2^{a}
Pore water TAN (µmol.L^{-1})	188.4 ± 51.0^{a}	313.8 ± 116.2^{ab}	386.0 ± 52.7^{b}
Pore water SRP (µmol.L^{-1})	6.7 ± 2.6^{a}	6.9 ± 1.3^{a}	7.2 ± 1.7^{a}

Mean values in a same row with different superscript letters are significantly different (P<0.05).

environmental parameters between the HD and the LD treatments, (Table 1&2) indicated the effects of rabbitfish stocking density on environmental variation in the culture tanks. These effects were possibly derived from the amount of food feeding daily and rabbitfish activities. Boyd and Tucker (1998) stated that most of the feed were eaten directly by fish, but usually only 10 – 30% of phosphorus (P) and 20 – 40% of nitrogen (N) applied in feed were retained by cultured animals. The remainder of the N and P entered pond ecosystems in faeces or other metabolic products. Depending on the species and culture techniques, up to 85% of P and 52 – 95% of N input into a marine fish culture system as feed might be lost into the environment through feed wastage, fish excretion, faeces production and respiration, and some of 21% of N and 53% of P of feed input accumulated in the bottom sediments (Wu, 1995). N in sediment organic matter may be mineralized to ammonia and recycled to the pond water. P released by decomposition of organic matter in pond bottoms is rapidly adsorbed by sediment and little of it enters the water (Boyd et al., 2002). As the experiment was carried out in the closed tanks, all released waste and nutrients were retained and accumulated in the water columns and sediments over the course of the experiment. The accumulation of waste and nutrients led to increasing and variation of some of the environmental parameters in the culture tanks, especially in the HD treatment. The high

increases of TAN and SRP in the HD treatment were probably derived from larger quantity of waste, fish excretion, nutrients loading from larger amount of feed used in comparison with the lower quantities in the MD and the LD treatments. High concentrations of TAN and SRP might bring about well development of phytoplankton and microphytobenthos in water column and sediment (Table 1&2). In aquaculture ponds, N and P are the two most important nutrients because they are often present in short supply and limit phytoplankton growth (Boyd, 1998). The nutrient concentrations likely increased following the stocking density, and thus got the highest values and wide ranges of variations in the HD treatment (Table 1&2). However, these values still lied in acceptable ranges for ammonia, NH^+_4 0.2 - 2 mg.L^{-1} (14.3 – 143.0 µM), NH_3< 0.1 mg.L^{-1} (7.1 µM), and phosphorus, 0.005 – 0.2 mg.L-1 (0.2 – 6.5 µM) in pond aquaculture water (Boyd, 1998). Notably, the present experiment was conducted in a closed system without water exchange, so nutrients released by feed loading and metabolic products would be accumulated within the tanks that probably led to degradation of water quality and then effects on rabbitfish growth and survival.

2. Rabbitfish growth performance

There was no significant difference in rabbitfish growth performance among treatments. Fish SR was 100% in the LD and MD treatments, while fish mortality strongly occurred in one of replicate of the HD treatment. Rabbitfish yield was significantly greater in the MD and the HD treatments than that in the LD treatment, but it was not significantly different between the MD and the HD treatments. Food conversion ration (FCR) was not significantly different between the MD and the LD treatments (Table 3).

Table 3: Growth performance of rabbitfish cultured at different stocking densities.

	Treatment		
	Low density (7 fish.m^{-2})	Medium density (14 fish.m^{-2})	High density (21 fish.m^{-2})
Stocking			
Biomass (g.m^{-2})	39.9	79.9	119.8
Initial mean weight (g.$fish^{-1}$)	5.7 ± 1.2	5.7 ± 1.2	5.7 ± 1.2
Initial mean length (cm.$fish^{-1}$)	6.8 ± 0.5	6.8 ± 0.5	6.8 ± 0.5
CV (%)	7.4	7.4	7.4
K	1.80	1.80	1.80
Harvesting			
Final mean weight (g.$fish^{-1}$)	10.9 ± 2.0^a	11.9 ± 2.7^a	10.3 ± 3.4^a
DWG (g.d^{-1})	0.09 ± 0.01^a	0.11 ± 0.01^a	0.08 ± 0.06^a
SGRw (%.d^{-1})	1.16 ± 0.11^a	1.31 ± 0.09^a	0.98 ± 0.67^a
SR (%)	100 ± 0.0^a	100 ± 0.0^a	92.1 ± 13.7^a
Yield (g.m^{-2})	76.5 ± 4.8^a	166.8 ± 8.3^b	205.7 ± 89.5^b
FCR*	2.72 ± 0.43^a	2.44 ± 0.09^a	
Final mean length (cm.$fish^{-1}$)	8.5 ± 0.6^a	8.8 ± 0.7^a	8.4 ± 0.7^a
DLG (cm.d^{-1})	0.03 ± 0.0^a	0.03 ± 0.0^a	0.03 ± 0.0^a
SGR_L (%.d^{-1})	0.39 ± 0.05^a	0.45 ± 0.01^a	0.37 ± 0.03^a
CV (%)	17.9 ± 7.3^a	22.3 ± 4.5^a	21.7 ± 1.5^a
K	1.81 ± 0.06^a	1.77 ± 0.05^a	1.70 ± 0.17^a

Mean values in a same row with different superscript letters are significantly different (P<0.05).

(*): FCR could not be calculated for the high density treatment because of negative weight gain in a replicate where high mortality occurred.

There was no significant difference in rabbitfish survival and growth performance among all treatments, indicating that stocking densities at tested levels had no negative effect on rabbitfish survival and growth. Similar results were recorded by other authors (Yousif et al. 2005; Saoud et al. 2008). Stocking density may or may not cause adverse effects on fish survival and growth, depending on the species of fish being reared and their development stages (Jorgensen et al. 1993, El-Sayed 2002). Since rabbitfish are schooling fish (Lam, 1974) and have tolerance of overcrowding (Ben-Tuvia et al., 1973), little competitive behaviour is expected among individuals reared at high densities.

Rabbitfish mortality occurred in one of four

replicates of the HD treatment without known apparent reason. This phenomenon happened near the end of the experimental period when phytoplankton was blooming in the tank as Chl *a* concentration reached 179.2 μL^{-1}. The toxic gas, such as NH_3, was lower than lethal level for fish (TAN 0.5 – 1.33 $mg.L^{-1}$, and NH_3 0.02 – 0.09 $mg.L^{-1}$, which was probably not a reason of rabbitfish mortality. But this concentration of ammonia could damage gills and reduce growth of fish (Lazur, 2007).

An increase in stocking density is desirable since generally reduce production costs per culture area (Huguenin, 1997). However, as biomass increases, so does the quantity of feed offered, resulting in potential eutrophication and oxygen concentration depletion. The results of this study showed that stocking density had no directly negative effect on growth and survival of *Siganus lineatus* by competing among individuals. High stocking density (in this experiment, 21 $fish.m^{-2}$), however, might cause high environmental variability, as a consequence that adversely affects on fish performance. At low density (7 $fish.m^{-2}$), the environment was well maintained, but low yield was produced. Stocking density at 14 $fish.m^{-2}$ seemed to be more suitable for rabbitfish rearing in a closed system, produced a relative high yield without widely environmental variations. However, further researches need to be carried out for longer period of culture with different stocking densities at *various* size groups of rabbitfish to determine optimal stocking density and size to optimize high production versus low environmental changes in a closed system.

IV. CONCLUSION

The results showed that goldlined rabbitfish *S. lineatus* can well adapt and grow in a closed culture system. The fish has little competitive behavior among individuals when stocked at size and density of 5.7 g, 7 – 21 $fish.m^{-2}$. The density has no effect on growth performance of *S. lineatus*, but when increase stocking density from 7 to 14 $fish.m^{-2}$ can elevate harvested yield. The environmental quality can be adversely affected as increasing stocking density (7 – 21 $fish.m^{-2}$), leading to environmental deterioration by potential eutrophication, high water and sediment nutrient concentrations and phytoplankton bloom. The factors associated with hyper - eutrophication could cause fish mortality and reduce growth.

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