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The influence of different stocking densities on the performance and behavior among the hatchery reared sea bass (*Lates calcarifer*) juveniles in recirculating system

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Abstract. Asian sea bass juveniles (62 days post hatching) with a mean body weight of 0.69 ± 0.05 g and mean total length of 3.45 ± 1.02 cm were distributed randomly among 12 units of 100 L cylindroconical tanks in a recirculating aquaculture system (RAS) with 70 L of filtered seawater for 56 days. Four different stocking densities S2, S4, S6, S8 fish/L^{-1} (140, 280, 420, 560 fish per tank) at three replicates were determined. Light was provided by fluorescent tubes on a 12 h light and 12 h dark photoperiod per day. A commercial dry 2 mm pellets were used to feed the fish at 8 – 10 % biomass, four times a day at 2 h intervals during the experiment. Weekly sampling was done for length and weight measurement and fish mortality was recorded daily. Significant increase in weight gain, specific growth rate, condition factor and the lowest food conversion ratio ($P<0.05$) were observed in sea bass at 4 individuals/L^{-1} (S4) stocking density as compared to the other groups of fish. Statistical analyses showed that there were no significant differences on survival rates after 56 days of culture period among groups S2 and S4, but survival rate was significantly lower in S6 and S8 groups of sea bass ($P<0.05$). During day 35 of culture period, it was observed that sea bass juveniles reared under stocking densities of 6 and 8 fish/L^{-1} showed competition among the individuals (aggressive shooters) for feed and space resulting in size variation that influenced fish behavior that leads to cannibalism. Size difference is considered a major factor which caused cannibalism among juvenile fish even though sufficient food was being provided. In the present investigation, cannibalism was observed to some extent in high stocking density groups due to various attacks from their tails. Water quality parameters were monitored and maintain within the optimum ranges for sea bass at temperature of 29.5 to 30 ^oC; salinity range of 31 to 32 ppt; dissolved oxygen of 8.2 to 8.5 ppm and pH from 7.5 to 8.0. The unionized ammonia (NH_3) and nitrite (NO_2-N) levels in the tanks stocked with 2, 4, 6 and 8 fish/L^{-1} were within the ranges of 0.04 to 0.09 ppm and 0.02 to 0.05 ppm respectively and these values are considered optimal for the growth of sea bass. The water was changed daily around 15 % in the recirculation system. This study found that homogeneity in the growth of juvenile sea bass, improved the feeding performance and survival rate of sea bass juveniles. It is concluded that growth of juvenile sea bass can be significantly increased by stocking the fish at lower density of 4 fish/L^{-1} in a recirculating aquaculture system.

Key Words: Sea bass, food conversion, recirculating system, sinking pellets, cannibalism.

Introduction. Aquaculture remains a growing and important production sector for high protein food and is estimated at US$ 98.4 billion in 2008. China dominates the world aquaculture production in terms of food fish supply providing 31M tonnes from aquaculture and 6M tones from capture fisheries (www.thefishsite.com/articles/257).

In the Philippines, aquaculture involves many species and culture systems, however in contrast to China, its contribution to the global farmed fish production was only 1.0 % today as compared to five percent previously (www.fao.org/fishery/countrysector/naso.philippines/en).

Sea bass is an economically important food fish in the tropical and subtropical regions of Asia and the Pacific. Among various species of finfishes Asian sea bass, *Lates calcarifer* is considered as one most potential candidate species suitable for culture in marine ecosystems, freshwater and brackish water ponds and cages (Barlow et al 1996; Rimmer & Russell 1998; Anil et al 2010; Mojjada et al 2013; Ganzon-Naret 2013). Sea bass exhibits high cannibalistic behavior which may occur at day 30 to 40 after hatching and such phenomenon is believed to be caused by genotypic differences among the larvae (De Angelis et al 1979; Hecht & Pienaar 1983).

Previous publications of Sakakura & Tsukamoto (1998, 1999) on juvenile yellowtail, *Seriola quinqueradiata* and Mojjada et al (2013) on the larviculture of Asian sea bass, *L. calcarifer* had demonstrated that stocking density is one of the essential factors found to influence social dominance that causes size variation, aggressiveness which ultimately lead to hierarchical territoriality and cannibalism. According to Kestemont et al (2003), cannibalism is a common phenomenon among teleost species that has been observed at various ages or sizes depending on fish species and environment. Among the cultured fish species that demonstrate cannibalism are the common carp *Cyprinus carpio* (van Damme et al 1989); dorada *Brycon moorei* (Baras et al 2000); orange-spotted grouper *Epinephelus coioides* (Hseu et al 2003); giant grouper *Epinephelus lanceolatus* (Hseu et al 2004) and barramundi *Lates calcarifer* (Qin et al 2004; Salama 2007; Appelbaum & Arockiaraj 2010).

In sea bass culture, frequent size grading of fish in hatcheries is very important to reduce the rate of cannibalism in order to enhance total fish yield (Hoakanson & Lien 1986; Smith & Reay 1991). The relationship between stocking density and growth performance had been reported to have a positive and negative impacts on several finfish species. For instance, species which showed positive impacts when stocked at high densities were observed in Atlantic halibut (*Hippoglossus hippoglossus)*; European sea bass (*Dicentrarchus labrax)* by Bjornsson (1994) and Papoutsoglou et al (1998) respectively. Previous studies have also reported on the negative effects of high stocking densities like in rainbow trout (*Oncorhynchus mykiss*); estuary grouper (*Epinephelus salmoides*); coho salmon (*Oncorhynchus kisutch*) and juvenile turbot (*Scophthalmus maximus*) according to Refstie (1977), Chua & Teng (1979), Blackburn & Clarke (1990) and Irwin et al (1999) respectively. This was attributed to the increased susceptibility to disease, metabolism distortion and aggressive behavior in fish due to cannibalism particularly when the availability of food is limited, thus affecting fish productivity (Vijayan et al 1990; North et al 2006). In halibut, *H. hippoglossus* L., Bjoernsson (1994) observed that under crowded condition, fish eats less; growth is retarded and suffers stress as a result of aggressive feeding interaction. These results are also similar to that reported by Cruz & Ridha (1991) and Ellis et al (2002).

The behavioral pattern of larvae and juveniles is greatly influenced by several environmental factors such as light intensity, food availability, population density, feeding frequency and feeding rate (Li & Mathias 1982). In contrast to the study conducted by Hatziathanasiou et al (2002) where the feed intake in the post larvae of *D. labrax* was independent of the stocking density and survival rate was found to be higher at 5 and 10 fish/L^{-1} as compared to fish stocked at 15 and 20 fish/L^{-1}. Baldwin (2010) and Salari et al (2012) opined that stocking density is a very important aspect in increasing fish production and this depends largely on species, food availability, water quality, environmental factors and social interaction. Several efforts had been already exerted to determine the appropriate stocking densities to elude certain limitation but until now such recommended stocking densities vary considerably (Russell et al 2008).

The present study aimed to determine the effect of different stocking densities on the growth, survival rate, feed conversion and behavior of hatchery reared Asian sea bass *L. calcarifer* in a recirculating aquaculture system.

Material and Method

Experimental fish and feeding. A number of 4,200 hatcheries reared *L. calcarifer* juveniles (62 days post hatching) with a mean body weight of 0.69 ± 0.05 g and mean total length of 3.45 ± 1.02 cm were stocked in 12 units 100 L cylindroconical fiberglass tanks. These tanks were filled with 70 L of filtered aerated sea water in a recirculating aquaculture system (RAS) located at the UPV Multi-Species Hatchery in Miag-ao, Iloilo, Philippines. The juveniles were counted and stocked at four densities 140, 280, 420 and 560 juveniles per tank (2 fish/L^{-1} (S2), 4 fish/L^{-1} (S4); 6 fish/L^{-1} (S6) and 8 fish/L^{-1} (S8) respectively) at three replicates in a completely randomized design (CRD) and reared for a period of 56 days. Feeding was done four times a day at 2 h intervals with 2 mm size commercial dry pellets (Table 1) containing 50 % protein content at 8 – 10 % of fish biomass during the experiment. During the rearing period a 12 h light and 12 h dark photoperiod was set up using fluorescent bulbs. Mortality was recorded daily and dead fish were removed.

Table 1

Proximate composition (%) of the commercial dry pelleted feed used during the experiment for sea bass juveniles over a period of 56 days

Proximate composition	*%*
Crude protein	50.0
Crude lipid	12.0
Crude fiber	4.4
Crude ash	6.9
Calcium	1.6
Phosphorus	1.3
Vitamin A	6280 IU/kg
Vitamin D3	1500 IU/kg

Water quality parameters. Water quality parameters such as salinity, temperature, dissolved oxygen and pH were measured daily in all tanks. Samples for total ammonia and nitrite were recorded on a weekly basis following the standard method (APHA 1998). These parameters were within the acceptable limits for the indoor production of *L. calcarifer* in the recirculating system (Boyd 1997; Rimmer et al 1994). Fifteen percent of the water volume in each tank was changed daily before feeding throughout the experiment. Tanks were cleaned and uneaten remains were removed daily by siphoning.

Growth parameters and feed utilization. About 100 juvenile fish from each tank were sampled randomly every two weeks for its length and weight measurements. During sampling, each individual fish was caught using a scoop net, quickly weighed, measured and carefully returned to its designated individual tank. The data collected during the study period were used to calculate the variables such as weight gain, specific growth rate (SGR % day^{-1}), condition factor, survival rate (SR %), feed conversion ratio (FCR) and protein efficiency ratio (PER) using the following formulae:

Weight gain = Wf – Wi

where: Wf = final weight (g); Wi = initial weight (g)

Specific growth rate (% day^{-1}) = [(ln final weight – ln initial weight)/time (days) x 100]

Condition factor = (W/L^3) x 100

where: W = final weight (g)
L = final length (cm)

Survival rate (%) = No. of fish survived/total no. of fish at the beginning x 100

FCR = total dry weight of feed (g)/total wet weight gain (g)]

Protein Efficiency ratio = wet weight gain/protein intake

where: protein intake = food supplied x crude protein (%)

Data analysis. The statistical analyses were carried out using the SPSS Software Program for Windows, Version 16.0 at the end of the rearing period. All data were subjected to one-way analysis of variance (ANOVA) and differences among treatments were compared by the Duncan's Multiple Range Test (DMRT) and considered significant at $P<0.05$. Survival was calculated using the arcsin square root.

Results and Discussion. Water quality parameters in the recirculating aquaculture system (RAS) during the course of this experiment are presented in table 2. The water temperature was between 29.5 and 30.0 ^{0}C. The salinity in all tanks ranged from 31 to 32 ppt. Dissolved oxygen did not show much variation ranging from 8.2 to 8.5 ppm; pH of 7.5 to 8.0 whereas the total ammonia-nitrogen (TAN) in ppm ranged from 0.04 -0.09 and nitrite-nitrogen (NO_2-N) 0.02 to 0.05 ppm in different treatments.

Table 2

Water quality parameters for juvenile sea bass, *Lates calcarifer*, reared for 56 days in a recirculating aquaculture system (RAS) at different stocking densities

Water quality parameters	*Stocking densities*			
	S2	*S4*	*S6*	*S8*
Temperature ^{0}C	29.5 ± 0.20	29.5 ± 0.10	30.00 ± 0.20	30.00 ± 0.12
Salinity (ppt)	31.00 ± 0.66	31.00 ± 0.5	31.00 ± 0.48	32.00 ± 0.64
DO (ppm)	8.50 ± 0.12	8.42 ± 0.16	8.35 ± 0.21	8.20 ± 0.17
pH	8.00 ± 0.03	7.85 ± 0.07	7.62 ± 0.08	7.50 ± 0.06
NH_3-N (ppm)	0.04 ± 0.012	0.05 ± 0.008	0.08 ± 0.006	0.09 ± 0.001
NO_2-N (ppm)	0.02 ± 0.001	0.03 ± 0.001	0.04 ± 0.002	0.05 ± 0.002

The data showed that the highest temperature was found in higher density (S8 and S6) groups compared with fish at lower densities (S2 and S4). It was observed that stocking densities had no effect on the salinity. The salinity of the water was totally dependent on the water change up to 15 % daily and regular supply of seawater. The highest DO (ppm) was obtained in S2 groups whereas the lowest one occurred in S8 groups of fish. In the present study, although the concentration (ppm) of NH3-N and NO2-N increased at higher stocking densities, the values were within the limits cited by Rimmer & Russel (1998) for the rearing of juvenile sea bass in the nursery and grow-out culture. Further, it was observed that the fish adapted well to the water environment and no diseases so far occurred when the experiment was conducted for 56 days. Cristea et al (2004) and Vasilean et al (2008) concluded that the dynamics of water quality in a recirculating system is highly dependent on a number of factors; stocking density, feeding rate and feeding frequency, biochemical composition of the feed, morphology and hydraulics of the rearing units.

Initial and final body weights and lengths, weight gain, specific growth rates, food conversion ratios, protein efficiency ratio and survival rates are presented in table 3. The final weights of the fish stocked at a density of S2, S4, S6 and S8 were recorded to be 7.92 ± 0.14, 8.06 ± 0.17, 6.97 ± 0.05 and 6.55 ± 0.05 g, respectively. Statistical analysis showed that the highest mean final weights were observed in groups reared at low densities, S2 and S4 groups and these were significantly different ($P<0.05$) compared to S6 and S8 groups of fish. The same trend was also observed for the mean final length, wherein the highest value was in low stocking density of 4 fish liter^{-1} (8.13 ± 2.2 cm) followed by 2 fish liter^{-1} (8.00 ± 1.8 cm) and were found to be significantly different ($P<0.05$) between 6 fish/L^{-1} (7.63 ± 3.2 cm) and 8 fish/L^{-1} (7.58 ± 2.6 cm) stocking densities. Comparison of the mean weight gain (WG) between treatment groups, it clearly shows that S4 groups of juvenile sea bass (4 fish/L^{-1}) had the highest weight gain and the lowest was observed in the S8 groups of fish. There were no significant differences ($P>0.05$) found between S2 and S4 groups of fish in terms of their final weight, final lengths and weight gain at the end of the study, however they were significantly higher ($P<0.05$) as compared with the two other groups, S6 and S8.

Table 3

Effect of different stocking densities on the growth performance, feed conversion ratio (FCR), protein efficiency ratio (PER), condition factor (CF) and survival rate of juvenile sea bass, *Lates calcarifer* reared for 56 days in a recirculating aquaculture system (RAS)

Parameters	*Stocking densities*			
	S2	*S4*	*S6*	*S8*
Initial weight (g)	0.69 ± 0.05	0.69 ± 0.05	0.69 ± 0.05	0.69 ± 0.05
Final weight (g)	7.92 ± 0.14^a	8.06 ± 0.17^a	6.97 ± 0.27^b	6.55 ± 0.11^b
Weight gain	7.23 ± 2.1^a	7.37 ± 1.8^a	6.28 ± 3.5^b	5.86 ± 2.5^c
Initial length (cm)	3.45 ± 1.02	3.45 ± 1.04	3.45 ± 1.02	3.45 ± 1.01
Final length (cm)	8.00 ± 1.8^a	8.13 ± 2.2^a	7.63 ± 3.2^b	7.58 ± 2.6^c
SGR (% day^{-1})	1.90 ± 0.24^a	1.91 ± 0.38^a	1.79 ± 0.44^b	1.74 ± 0.25^c
FCR	1.29 ± 0.01^c	1.27 ± 0.01^c	1.39 ± 0.03^b	1.42 ± 0.02^a
PER	1.55 ± 0.23^a	1.57 ± 0.18^a	1.44 ± 0.42^b	1.40 ± 0.25^c
CF	1.55 ± 0.05^a	1.50 ± 0.04^a	1.56 ± 0.06^a	1.50 ± 0.03^a
Survival rate (%)	71.42 ± 2.5^a	73.21 ± 3.6^a	50.00 ± 1.9^b	47.32 ± 4.8^c

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

The highest SGR (1.91 % day^{-1}) after 56 days of rearing was recorded for the S4 group which was similar for sea bass juveniles from S2 (1.90 %), and higher (P<0.05) compared with fish from S6 and S8 (Table 3). The FCR in the present work ranged between 1.27 and 1.42 and differences among the treatment groups were found significant (P<0.05) at low stocking densities. The PER values were significantly higher in groups S4 and S2 than S6 and S8 groups. On the other hand, condition factor of fish juveniles calculated at the end of the study was very similar ranging from 1.50 to 1.56. Survival rates were from 47.32 ± 4.8 to 73.21 ± 3.6 for all groups (Table 3). There were no significant difference on survival among groups S4 and S2, but survival rate was significantly lower in S6 and S8 groups. Consequently, the lowest value of survival rate (%) was obtained in S8.

The present study clearly demonstrated that appropriate stocking density affects the growth response, feeding and fish behavior of sea bass after 56 days of rearing in a recirculating aquaculture system. Among the treatment groups, the effect of stocking density on growth (WG and SGR) was highly significant at stocking density S4 S2 groups (P<0.05) as compared with growth at higher stocking densities (S6 and S8). The poor growth of sea bass juveniles in high densities (S6 and S8) maybe attributed to its loss of appetite due to stress as fish were exposed in a crowded condition and deterioration in the water quality (Wendelaar-Bonga 1997). This finding was similar to the previous studies conducted by Sahoo et al (2004) on *Clarias batrachus*; Jha & Barat (2005) on *Cyprinus carpio* larvae; Rahman et al (2005) on *Tor putitora* larvae; Pangni et al (2008) on the African catfish *Chrysichthys nigrodigitatus,* Claroteidae (Lacepede 1803) and Samad et al (2014) on grouper, *E. coioides*. In contrast with our results Howell (1998) on turbot, *S. maximus*; Papoutsoglou et al (1998) on sea bass, *D. labrax* and North et al (2006) on rainbow trout *O. mykiss* reported that high stocking densities for these species had positive effect on their growth performance. Stocking density is considered a very crucial variable to the growth performance in relation to the fish welfare in order to maximize productivity and the minimum incidence of physiological and behavioral diorders (Ellis et al 2002; Turnbull et al 2005; Ashley 2007; Sammouth et al 2009; Ayyat 2011).

In the present investigation, fast growing individuals (shooters) were noticed at higher stocking densities of S6 and S8 groups of fish after the 35 days of rearing period in the recirculating aquaculture systems (RAS). High mortality of sea bass was recorded on the 35th day for these 2 treatments and further observation showed that the size of the fish varied from one another, with the tendency of the large fish to become aggressive. Thus, the presence of the shooters or large-sized fish, which preyed or attacked on the smaller ones resulted to the low survival of the fish in groups S6 and S8 and this was attributed to social dominance that influenced the behavioral pattern of the juveniles that ultimately lead to their cannibalistic behavior. Mackinnon (1985), Paller & Lewis (1987) observed stress in smaller-sized fry of sea bass with the presence of shooters that tend to cannibalize the smaller ones, thus reducing their immune competence towards social dominance and hierarchical territoriality.

The present study found that water quality parameters were not significantly affected (P>0.05) by the different stocking densities namely S2, S4, S6 and S8 of sea bass juveniles in the RAS system after a period of 56 days. On the other hand, it was observed that values for DO (8.20 ± 0.17 to 8.50 ± 0.12) and pH (7.50 ± 0.06 to 8.00 ± 0.03) slightly decreased as stocking densities increased. TAN and nitrite were influenced by different stocking densities in RAS system, of 0.04 ± 0.012 to 0.09 ± 0.001 ppm and 0.02 ± 0.001 to 0.05 ± 0.002 ppm respectively. This study was in agreement with that of Boyd (1997) who reported that at high stocking densities problems such as ammonia nitrogen, carbon dioxide accumulation and organic pollutants are usually encountered.

Conclusions. From the aforementioned results, it could be concluded that the best growth performance, FCR, PER, condition factor of sea bass juveniles were obtained in the RAS system stocked at 4 fish/L^{-1} over a culture period of 56 days. Further, maintaining good water quality in the RAS and providing adequate food is required to sustain fish welfare in order and to prevent from its extreme cannibalistic behavior to optimize sea bass productivity.

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