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Effects of feeding frequency on growth, survival rate and body composition in sea bass (*Lates calcarifer*) juveniles fed a commercial diet under laboratory condition

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Abstract. A 60 day feeding trial was conducted to investigate the effects of different feeding frequencies on the growth, survival rate and body composition of sea bass (*Lates calcarifer)* juveniles fed commercial diet, reared under laboratory condition. The average initial weight of fish was 4.59 g and its initial total length of 7.11 cm was determined for the conditions factors. Ten fish were stocked in each 12 - 100 L conical fiberglass tanks filled with 90 L seawater at three replicates per treatment. The four feeding frequency treatments consist of feeding the fish once a day (F1), twice a day (F2), four times a day (F3), and six times a day (F4) at 5 % body weight with commercial diet (47.85 % crude protein, 7.10 % crude lipid, 0.77 % crude fiber, 1.62 % ash, and 7.70 % moisture) throughout the feeding trial. Final average body weight (ABW) and weight gain of sea bass fed a commercial diet at a feeding frequency six times daily (F4) were superior than those fed at F3 (four times a day), F2 (two times a day) and in F1 groups (once a day). In terms of specific growth (SGR) and relative growth rate (RGR) it can be concluded that the best growth rates of sea bass were obtained from the fish fed six times a day. Feed conversion ratio (FCR), feed efficiency (FE) and protein efficiency ratio (PER) were within the ranges of 1.42 - 3.99, 0.27 - 0.76 and 0.57 - 1.62 respectively. The FCR was significantly affected by different feeding frequencies, with a significantly better FCR value of 1.42 in fish fed six times daily ($P<0.05$) compared with the other three treatment groups fed once, twice and four times daily. The feeding frequency had a significant effect on the crude protein, crude lipid whole body composition, however survival rate of the fish during the experiment was highest in the F4 groups. The results showed that growth performance, increased utilization of feed and survival rates for *Lates calcarifer* juveniles were greatly enhanced by feeding the fish six times daily with a commercial diet containing crude protein level of 47.85 %.
Key Words: Sea bass, *Lates calcarifer* growth, survival, commercial diet.

Introduction. Asian sea bass (*Lates calcarifer*) is considered an important marine food fish with high commercial value (Appelbaum & Arockiaraj 2010; Arockiaraj & Appelbaum 2010; Caipang et al 2011), owing to its rapid growth rate and ability to accept formulated feeds. They are strictly carnivorous and fast-growing marine fish cultured extensively in Southeast Asian countries and the Indo-Pacific region (Thirunavukkarasu et al 2004). This species has contributed approximately 20,066 metric tones annually to the total world aquaculture production of 3.6 million tones (Tucker et al 2002; Thirunavukkarasu et al 2004). According to Wang et al (2006) the global annual production of sea bass in 2006 was currently 400,000 metric tones.

Feed accounts approximately 40 – 50 % in the production cost for intensive aquaculture (Anderson et al 1997). Therefore, more attention shall be given to the optimum feeding management in order to reduce overfeeding, feed wastage, environmental pollution and increase fish production efficiency (Lee et al 2000; Bolliet et al 2001; Dwyer et al 2002). Several authors had already studied the effect of feeding frequency on food intake and growth for various species (Jobling 1995; Bascinar et al 2001), however there is a limited information available for the optimum frequency to feed young sea bass. So, feeding frequency becomes more imperative in the culture of

marine and freshwater fish to optimize the amount of nutrients and metabolic fuel for the rapid fish growth and minimize waste production (Kaushik & Gomes 1988; Kaushik & Meadale 1994). The optimal feeding frequency may vary depending on species, age, size, environmental factors, stomach capacity, fish biorhythm, husbandry and feed quality (Tsevis et al 1992; Goddard 1996). The effects of feeding frequency on fish growth and feed conversion efficiency have been studied for several species. Feeding frequency and timing have been reported to affect feed intake, fish growth and survival rate in rainbow trout, *Oncorhynchus mykiss* (Grayton & Beamish 1977; Reddy et al 1994), juvenile tilapia, *Oreochromis niloticus* (Riche et al 2004), channel catfish *Ictalurus punctatus* (Noeske et al 1985), juvenile turbot *Psetta máxima* (Turker 2006), Korean rockfish Sebastes schlegeli (Lee et al 2000) and camouflage grouper *Epinephelus polyphekadion* fingerlings (Al Zahrani et al 2013). Piper et al (1982) reported that the recommended feeding frequency for rainbow trout fingerlings should be three times daily for 15.1 - 45.0 g and a feeding frequency of once a day for fish having average body weight of 7.6 - 16.0 g. Some of the experimental data on feeding frequency for the optimal growth of different fish species had been already summarized (Chiu 1989).

Asian sea bass was considered a potential prospect for intensive aquaculture from fry to marketable size (Barlow et al 1996), therefore the application of feeding frequency on the fish would insure optimum feed consumption with minimum waste, improves nutritional efficiency and food conversion efficiency, decreases production cost and reduces water pollution.

The present study was carried out to assess the effects of feeding frequency on growth, survival rate and body composition in sea bass (*Lates calcarifer*) juveniles fed on commercial diet under laboratory condition.

Material and Method

Experimental fish and rearing condition. Juvenile sea bass were obtained from SEAFDEC, AQD, Tigbauan, Iloilo, Philippines and then transported to the Multi-species Hatchery at UPVisayas in Miag-ao, Iloilo. The fish were acclimated for two weeks in 1000 L circular fiberglass tanks, fed with a commercial extruded feed (2 mm) prior to the start of the feeding experiment. Sea bass juveniles (mean weight 4.50 g, mean total length 7.11 cm) were randomly distributed at a stocking rate of 10 fish per tank at three replicate tanks per treatment in 12 - 100 L capacity conical fiberglass tanks containing 90 L filtered aerated seawater. The tanks were connected in a semi-closed recirculating system provided with sand and biological filters with continuous aeration. Dead fish were removed and recorded daily during the experiment. The pellet residues and feces in each tank were siphoned to maintain good water quality under controlled laboratory condition.

Growth rates (weight gain) were determined by weighing the fish individually (± 0.01 g) using a weighing scale (Sartorius) and the total lengths (± 1mm) of 10 fish from each tank were measured to estimate the condition factors every 10, 20, 30, 40, 50 and 60 days. Feeding was done manually and the amount of feed given daily at each meal was recorded.

Dissolved oxygen and water temperature was measured using oxygen meter (YSI model 58, Yellow Spring Instrument Co.) daily, which ranged from 6.2 – 7.3 mg L^{-1} and 26 – 32 ^{0}C, respectively. The water pH ranged between 6.4 - 7.8 during the experiment and salinity ranged 28.5 - 30.5 ppt. Total ammonia-nitrogen and nitrite-nitrogen of the water samples were determined every two weeks according to Strickland & Parsons (1972) and the water quality parameters measured were within the acceptable level for the fish growth (Boyd 1984).

Experimental design. Four different treatments of feeding frequencies were randomly assigned to triplicate tanks in this experiment with specific timing as shown in Table 1. Fish were fed once (F1), twice (F2), four (F3) and six (F4) times a day, with commercial diet (2 mm) at 5 % biomass.

Table 1

Different treatments of feeding frequencies with specific timing for sea bass during the experiment

Treatment	*Feeding frequency*	*Timing*
F1	1	09:00
F2	2	09:00-16:00
F3	4	09:00-11:00-14:00-16:00
F4	6	09:00- 10:30-12:00-13:30-15:00-16:30

Chemical analyses and calculations. Commercial diet was analyzed for proximate composition (AOAC 1995). Dry matter was determined by drying the feed sample to a constant weight at 105 ^{0}C. Crude protein was analyzed according to the micro-Kjeldahl method (N x 6.25). Crude lipid was determined by 40 to 60 ^{0}C petroleum ether extraction in a Soxhlet apparatus and ash by incineration at 550 ^{0}C for 12 h, in a muffle furnace. Crude fiber was determined using the Hot Extractor (Fibertec System). A sample of ten individuals was collected at the start of the experiment for the analysis of initial body composition. Subsequently, a batch of ten fish from each treatment group was sacrificed for determination of the final whole body composition.

The data obtained for growth and feed utilization parameters were evaluated by calculating weight gain (WG), specific growth rate (SGR), relative growth rate (RGR), feed conversion ratio (FCR), feed efficiency (FE), protein efficiency ratio (PER) condition factor (CF) and survival rate (%) using the following formulae:

WG = final weight (g) – initial weight (g)

SGR (% day $^{-1}$) = [In final weight (g) – In initial weight (g) / no. of culture days] x 100

$$RGR = \frac{\text{wf - wi}}{\text{wi}} x100$$

Where: wf = final weight (g), wi = initial weight (g)

FCR = feed intake (g) / weight gain

FE = (weight gain / feed intake)

PER = wet weight gain (g)/dietary protein intake (g)

Condition Factor (CF) (%) = fish weight (g) / fish length^3 (cm) x 100 (Ai et al 2006)

Survival rate (%) = No. of fish recovered / No. of fish stocked x 100

Statistical Analysis. All the data were subjected to one-way analysis of variance (ANOVA). Duncan's multiple range test (DMRT) was used as a *post hoc* test to compare between means at P≤0.05. The statistical analyses were performed using the SPSS version 16 for Windows (SPSS Inc. Chicago, IL USA) Software package.

Results and Discussion. The growth performance, feed utilization and survival of sea bass on four different feeding frequencies fed with commercial diets for 60 days are shown in Table 2. During the feeding experiment fish readily accepted and consumed the commercial diet. The average final weights of the fish in different treatments reached 13.49 ± 0.37 g, 14.02 ± 0.15 g, 12.70 ± 0.33 g and 14.00 ± 0.28 g after 60 days of feeding trial in groups F1, F2, F3 and F4, respectively. Fish in the F4 groups (six meals a day) showed the best growth performance. Weight gains in F2 and F4 treatments were significantly higher (P<0.05) than those treatments F1 and F3. The growth data indicated

that the highest RGR (236.53 ± 8.2) and SGR (0.86 ± 0.018) values were obtained from groups of fish fed six meals a day (F4) and was significantly higher than those of other groups F1, F2 and F3. Sea bass that were fed commercial diet once daily (F1) had the lowest RGR and SGR after 60 days of feeding trial. FCRs did not differ significantly between F3 and F2 groups ($P<0.05$), however the best and the poorest FCRs were obtained from groups of fish fed six meals (F4) a day and once a day (F1) respectively.

Table 2

Growth, feed utilization and survival of *Lates calcarifer* at four different feeding frequencies fed with commercial diets for 60 days

Parameter	*Treatment*			
	F1	*F2*	*F3*	*F4*
Initial weight (g)	4.74±0.09	4.76± 0.11	4.70±0.14	4.16±0.13
Final weight (g)	13.49 ± 0.37^{b}	14.02 ± 0.15^{a}	12.70 ± 0.33^{c}	14.00 ± 0.28^{a}
Weight gain	8.74 ± 1.5^{b}	9.18 ± 2.9^{a}	8.00 ± 2.4^{c}	9.84 ± 1.1^{a}
RGR (%)	170.31 ± 6.2^{c}	187.99 ± 12.1^{b}	173.15 ± 5.7^{c}	236.53 ± 8.2^{a}
SGR (%/day)	0.70 ± 0.03^{b}	0.72 ± 0.025^{b}	0.72 ± 0.027^{b}	0.86 ± 0.018^{a}
FCR	3.99 ± 0.06^{a}	2.60 ± 0.03^{b}	2.22 ± 0.04^{b}	1.42 ± 0.039^{c}
FE	0.27 ± 0.013^{c}	0.44 ± 0.03^{b}	0.26 ± 0.025^{c}	0.76 ± 0.018^{a}
PER	0.57 ± 0.054^{c}	0.92 ± 0.02^{b}	0.95 ± 0.017^{b}	1.62 ± 0.036^{a}
CF	2.23 ± 0.088^{b}	2.56 ± 0.103^{b}	1.99 ± 0.031^{c}	2.99 ± 0.025^{a}
Survival (%)	66.67 ± 2.08^{c}	80.00 ± 2.85^{b}	83.33 ± 3.31^{b}	93.33 ± 1.89^{a}

Data represent the mean of three replicates. Values in the same row with different superscript are significantly different ($P<0.05$).

The PER and FE values in treatment F4 were significantly higher than those of other groups in F3, F2 and F1. The condition factors (CF) of fish fed the different feeding frequencies ranged from 1.99 ± 0.031 to 2.99 ± 0.025. The lowest value was recorded for fish on feeding frequency of four times daily while the highest value was obtained in fish fed six times daily. The survival rate in F4 feeding frequency was the highest (93.33 ± 1.89) while the lowest (66.67 ± 2.08) was obtained from groups of fish fed once daily (F1). There were no significant difference at $P<0.05$ in the survival rates between treatments F2 and F3.

The initial and final body compositions of sea bass at four different feeding frequencies during the feeding trial are presented in Table 3.

Table 3

Whole body composition (%) of sea bass (*Lates calcarifer*) juveniles at four different feeding frequencies fed commercial diets for 60 days

Composition	*Initial*	*F1*	*F2*	*F3*	*F4*
Moisture (%)	53.81±0.65	53.72 ± 0.57^{b}	53.74 ± 0.66^{b}	53.78 ± 0.62^{b}	54.9 ± 0.76^{a}
Crude protein (%)	15.28±0.7	17.62 ± 0.5^{b}	17.68 ± 0.42^{b}	17.65 ± 0.44^{b}	18.42 ± 0.33^{a}
Crude lipid (%)	4.05±0.31	5.59 ± 0.22^{b}	5.68 ± 0.19^{b}	5.62 ± 0.38^{b}	6.24 ± 0.08^{a}
Crude ash (%)	1.36±0.08	1.87 ± 0.5^{a}	1.85 ± 0.33^{a}	1.83 ± 0.22^{a}	1.81 ± 0.04^{a}

Values in the same row with different superscript are significantly different ($P<0.05$).

The highest crude protein (18.42 %) and crude lipid (6.24 %) were observed on the feeding frequency of six times daily (F4) for sea bass and these were significantly higher than in the treatments F1, F2 and F3 groups. The lowest value for ash content (1.81 %) was achieved by group of fish in F4 feeding frequency and the highest value was recorded at once daily (F1) feeding frequency, however all the groups in terms of ash content did not display any significant differences ($P>0.05$).

The present study demonstrated that feeding frequency (once, two, four and six times daily) significantly improved the growth performance and feed utilization of Asian sea bas. The effects of feeding frequency may vary with the different species, size, age of

fish, dietary protein, energy levels and feeding time as well as the environmental factors (Wang et al 1998; Lee et al 2000; Mihelakakis et al 2001). For instance, Andrews & Page (1975) and Marian et al (1981) reported that the optimum feeding frequency for stinging catfish (*Heteropneustes fossils)* and channel catfish (*I. punctatus*) respectively was once a day, three times per day for common carps (*Cyprinus carpio*) (Charles & Sebastian 1975) and six times daily for the red spotted grouper (*Epinephelus akaara*) (Kayano et al 1993).

In this study a restricted daily ration of 5 % body weight using commercial diet provided maximum efficiency for the fish. The highest weight gain, specific growth rates and relative growth rate in juvenile sea bass were obtained ($P<0.05$) by feeding the fish frequently six times a day. It is evident that growth increases with the increase of feeding frequency and the highest quantity of feed was consumed in multiple feeding resulting in higher feed efficiency indices. Similar results were observed in the red-spotted grouper (Kayano et al 1993), hybrid sunfish (Wang et al 1998), *Clarias gariepinus x Heterobranchus longifilis* hybrids (Ndome et al 2011), common carps (Kiaalvandi et al 2011) and in rainbow trout (Turker & Yildirim 2011) that feeding frequency between three and six times daily improved body weight gain, survival rate and feed utilization. In contrast to the findings of Tsevis et al (1992) who reported that increasing feeding frequency resulted in an inferior feed efficiency for sea bass reared at 20 ^{0}C. The results of Tiril & Alagil (2009) investigation showed that when feeding was increased from twice to six times daily in rainbow trout, there was no significant difference in growth.

Food conversion ratio was best in six times daily feeding frequency because it had the lowest FCR (1.42) followed by the fish fed four times daily (2.22), twice daily (2.60) and once daily (3.99). There was an observed decrease in the FCR and FE when the group of fish was fed four times daily (F3) among the feeding frequencies, but non specifically between feeding frequency F2 (twice daily feeding) ($P>0.05$). For this reason, it is essential to determine the optimum feeding frequencies for various fish species on practical application (Zakes et al 2006). The ability of the organism to utilize nutrients particularly protein will positively enhance its growth rate (Sogbesan & Ugwumba 2008). This is justified by the best growth and low FCR in the F4 groups. Similarly, the same trend was also observed for the PER, the highest (1.62) was recorded at the highest feeding frequency (F4) and the lowest (0.57) was observed in frequency F1, thus the protein intake which was highest for fish on feeding frequency of six times daily contributed to the best growth. The condition factor was used to assess the physiological and nutritional status of sea bass in this experiment. Condition factor in F4 group displayed a significant difference ($P<0.05$) among the groups and data obtained were much higher than that reported by Ballestrazzi & Lanari (1996). This was probably due to the fact that they used “standard length”, for the computation of CF whereas “total length,” was used in the present investigation. Survival rates were affected by the number of feeding frequencies.

The whole body composition of the fish showed an increase in the values of crude protein, crude lipid and ash content at the end of the experiment over the initial fish samples. The increase in the crude protein level was attributed to the adequate protein level (47.85 %) of the commercial diet used and the protein intake of fish. Garling & Wilson (1976) reported that dietary protein intake resulted in an increase in the fish intake protein level. The feeding frequency had a significant effect on the crude protein and crude fat on the whole body composition of fish in F4, however there was no significant difference in body composition among treatments F1, F2 and F3.

Conclusions. To sum up, it can be concluded that increasing feeding frequency in sea bass resulted in a better growth performance, body composition and survival rates under controlled laboratory condition after 60 days of feeding trial. The success of sea bass depends on effective feeding frequency. A feeding frequency of six times a day (F4) compared to other experimental groups is suggested to be optimum for achieving optimum growth, feed conversion efficiency and high survival rates among *Lates calcarifer*.

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