



Thesis for the Degree of Master of Fisheries Science

Effects of Feeding Frequency on Growth,

Body Composition and Size Variation of

Black Seabream, Acanthopagrus schlegelii

by

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KOICA-PKNU International Graduate Program of Fisheries

Science Graduate School of Global Fisheries

Pukyong National University

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사료공급 횟수가 감성돔의 성장, 체성분

및 크기에 미치는 영향

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by

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February 2020

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A dissertation

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Abstract

To evaluate the effects of different feeding frequencies on growth performance, size variability and body composition of juvenile black seabream, *Acanthopagrus schlegelii*, a 35-day experiment was carried out in a semi-closed recirculating system. The experiment set up consists of three treatments in triplicates of nine rectangular tanks (50 cm L x 37 cm W x 24 cm H), a sump, oxygen circulators, a water pump for each treatment. Water temperature was controlled using an electric heater with temperature controller installed for the whole system. Feeding frequencies were set at one, two and three times/day (T1, T2, and T3) respectively. Experimental fish individual weight of 6.3 ± 0.40 g and total length of 5.95 ± 0.25 cm were stocked in each tank.

Water temperature, pH, dissolved oxygen (DO) and salinity were maintained at 25.5±1.5° C, 7.5±0.5, 9.0±1.0 mg/L and 34±1.0 ppt respectively. At the end of the experiment, T3 displayed a significantly better growth (P < 0.1) in weight, total length and specific growth rate (SGR) than T1 but not with T2. Food conversion (FC), survival rates (SR) and condition factor (K) were not significantly different (P > 0.1) among the treatments although they were improved with increasing feeding frequency. For size variability and dispersion, uniformity indexes, coefficient of variation (CV), skewness and kurtoses for body weight, length and K were determined. CV for body weight and length decreased with increasing feeding frequency with significant differences (P < 0.1), while CV for K was fairly uniform but there were no significant differences (P > 0.1) among all the treatments. Skewness for body weight and K for T3 were significantly different from T1, and T2 (P > 0.1). Kurtoses for body weight and K significantly decreased with increasing feeding frequency (P > 0.1) observed among treatments. In this study, the results indicate that, feeding juvenile black seabream three times a day improves their performance in terms of growth, condition factor, survival rates and uniformity than feeding once or twice a day.

Key words: Feeding frequency, Black sea bream, Growth performance and Size variations

1. Introduction

Black sea bream, *Acanthopagrus schlegelii* (Bleeker 1854), also known as black porgy of family sparidae is one of the most commercially important and prevalent fish farmed in China, Japan, Korea and in most parts of South -East Asia regions (Nip et al., 2003). It is a marine fish species with a relatively faster growth rate with high resistance to diseases (Hong and Zhang, 2003). However, during the winter when water temperatures lower, they stop growing and experience a high rate of mortality due to lack of feeding and increased susceptibility to diseases (Park et al., 2009).

Feeding is a key factor determining efficiency and cost in aquaculture, so ,it is important to develop appropriate feeding regimes for cost effective aquaculture (Zhou et al., 2018). Also fish farmers have to feed fish at a level that ensures not only good growth but also minimal waste (Al Zahrani et al., 2013). It is prudent to gather sufficient information about feeding rate and environmental requirements of the culture species before undertaking production. Optimum feeding frequency for maximum fish growth and minimum waste production may vary with species and size of fish, culture conditions, food quality, and quantity provided (Bascinar et al., 2007). When fish are fed insufficiently or excessively, their growth or feed efficiency may decrease, raising up

production cos. It is also especially true that overfeeding becomes a source of water quality deterioration (Lee et al, 2000; Cho *et al.*, 2007).

Water quality in the culture unit is key for growth, health and survival of fish at all stages of production. The type and quantity of feed given to fish have direct impacts on levels of ammonia nitrogen, dissolved oxygen (DO) and pH in the water environment. A less conducive environment for fish leads to stress and disease and in the extreme may cause mortalities. It is necessary to select a diet and quantity that will not lead to water quality deterioration. The major nitrogen metabolic by-product released by fish is the total ammonia nitrogen (TAN), generated from fish excretion and through decomposition of food remains and feces (Park et al., 2015). With high stocking densities and limited water exchange in recirculating aquaculture system (RAS), fish stress and mortalities may increase due to water quality deterioration. Good feeding regimes can be determined based on species feeding behavior, physiology, and culture system.

The main challenges experienced in aquaculture related to a poor feeding regime are a reduced growth rate and production of uneven fish sizes in the same cohort. Narrowing inter-individual variation in food consumption and growth of cultured fish results in production efficiency (Bascinar et al., 2007) Every farmer targets to produce healthy, fatter and fast growing fish that are more uniform in sizes without inflating production costs. This can be achieved by striking a right balance between adequate feeding and avoiding feed wastage. In African catfish, *Heterobranchus longifilis* weight variations due to food competition may be abridged through adequate feeding (Ewa-Oboho & Enyenihi,

1999). Right feeding frequency can significantly reduce size heterogeneity and cannibalism in barramundi (*Lates calcarifer*) (Ribeiro et al., 2015). On the other hand, Biswas et al., (2010) encourage constant sorting and size grading to minimize competition for space and food for production of homogeneous barramundi

Most available feeding charts for fish under farming conditions are not accurate as they tend to over-estimate feed requirements resulting in overfeeding, poor feed efficiency, feed waste, environmental pollution and thus economic disadvantages for fish farmers (Cho et al., 2007) Determining the best feeding frequency for juvenile black seabream generally in aquaculture and particularly in RAS is critical in promoting fish growth performance, and maintaining good water quality. The objectives of this study were to (i) evaluate the relationship between feeding frequency and growth performance of juvenile black sea bream in a semi-closed recirculating system and (ii) asses size distribution of juvenile black seabream at different feeding frequencies

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2. Materials and Methods

2.1. System setup and management

A 35- day feeding experiment was carried out in the Laboratory of Aquaculture Systems in the department of Marine-Bio Materials and Aquaculture, Pukyong National University, Busan, South Korea. The system consisted of three treatments in triplicates, with nine identical (50 cm L x 37 cm W x 24 cm H) rectangular transparent plastic tanks filled with clean natural marine water to a 30 L mark. Three sumps were fitted with same filters consisting seawater media kaldness K1. Hyub Shin water pumps were fitted to pump water to experiment tanks from the sumps. Water temperature for all the treatments was regulated at 25.0°C using electric water heater rods and oxygen was supplied by underwater circulators. Salinity was maintained at 30-35ppt by gradual addition of freshwater. Water flow rate was maintained at 1.5 L/min and daily loss through evaporation, splashing, and siphoning of uneaten feed, feces and other solids replaced by 10% of total system water.

Three treatments were labeled T1, T2 and T3 with daily feeding frequencies of one, two and three times respectively. Juvenile black sea bream require 5% of feed for full satiation. This was determined by weighing the feed given to 150 g fish biomass to full satiation two times a day for a week. After fish were fed to full satiation, the balance of the feed weight was subtracted from the initial weight and calculated as feed percentage to body weight. The daily feed amount supplied was the same 5% body weight for all the treatments distributed varyingly according to the treatment. T1 was fed once a day a total of 5% body weight, T2 was fed 5% body weight two times in 24 hours and T3 was fed 5% body weight three times a day. Siphoning of uneaten food and excreta was done once a day at the end of a feeding cycle before new food was introduced.

2. 2. Water sampling and quality analysis

Water samples were taken from the three sumps for total ammonia nitrogen (TAN) and nitrite nitrogen (NO₂-N) analysis on a daily basis in the first week, then twice a week afterward. TAN and NO₂-N were analyzed with a HACH DR 900 multiparameter colorimeter (HACH, Co., Loveland, CO, USA) using the salicylate method for TAN and azo-dye method was used for NO₂-N. Total organic carbons, (TOC), was measured on a weekly basis by total direct TNT method using HACH 427 organic carbon LR. Total suspended solids (TSS) concentrations were quantified by the filtering and weight method (APHA 1995). DO, pH, and water temperature were monitored daily using portable multi meter (HQ 40D, HACH CO, Loveland, Colorado, USA). Salinity was measured with a salinity refractometer (JA-100) biweekly

2.3. Experiment fish and measurements

A commercial feed, CJ Company, Busan, South Korea for juvenile fish with a composition of 52% crude protein, 10% crude fat, 5% crude fiber, 18% crude ash, 1% Calcium, 2.7% Phosphorus and 14.0% moisture was used to feed the fish. Juvenile black sea bream average weight of 6.3±0.40g and total length of 5.95±0.25cm were obtained from Wonheung Susan fish farm in Goeje, South Korea and transferred to the Laboratory of Aquaculture Systems. Twenty five fish were randomly stocked in each tank and fed the commercial diet according to the feeding frequency for each treatment. Before measurements were carried out, fish were fasted for 24 hours. Individual body weight and total length were measured for all the surviving fish. Food conversion (FC), specific growth rate (SGR), survival rate (SR) and condition factor (K) were calculated. Pooled data from each treatment were used to determine uniformity index (UI₁₀), coefficient of variation (CV), skewness, and kurtoses.

SGR (%/d) = $[(\ln W_f - \ln W_i)/d] \times 100$

where W_f is the final body weight in grams and

W_{*i*} is the initial body weight in grams

Survival rates were determined by subtracting the final number of fish from the initial

SR (%) =
$$(N_i - N_f \times 100)/N_i$$

where N_i is initial fish number and N_f is final fish number

K =
$$(100 \times W_{i \, or f})/L^3$$

L denoting body length in cm

UI₁₀ (%) $= n_{10}/N \times 100$

where, n_{10} is fish number (frequency) between "mean x 0.9" and "mean x 1.1", while N is total fish number measured

Skewness = $[N/(N-1)(N-2)\Sigma(x_i-x/s)^3]$

Kurtosis =[$N(N + 1/(N-1)(N-2)(N-3)\Sigma(x_i-x)^4/s$]-3(N-1)²/(N-2)(N-3)

2.4. Total body composition analysis

Proximate body composition was determined at the end of the experiment. A total of 27 fish; three from each tank were sampled and stored in a freezer at -40° C until time for an alysis. Proximate analysis was conducted based on AOAC (1995) methods. Ash content was determined using a muffle furnace (550°C for 4 h), crude protein content was analyzed using an automatic Kjeldahl system (Foss Tecator, Hoganas, Sweden) acco rding to the Kjeldahl method. Moisture content was measured after drying for 24 hrs at 105° C in a dry oven. Crude lipid was measured using the ether □extraction method.

2.5. Data analysis

Results are presented as means \pm SD. All data were first examined for homogeneity of variance using Levene's test and normality by Shapiro-Wilk test. One-way ANOVA and Duncan's multiple range test were used to analyze the significance of the difference among the means of treatments and probability values of P < 0.1 were deemed to be statistically different. SPSS Statistic 20.0 software (IBM, New York, USA) was used for data analysis.

3. Results

3.1. Water quality

Results for water temperature, pH, dissolved oxygen (DO) concentration, total ammonia nitrogen (TAN), nitrite nitrogen (NO₂⁻N), total organic carbons (TOC) and total suspended solids (TSS) were shown on Table 1. There were no differences (P > 0.1) observed in all the treatments for water temperature, pH, DO, NO₂⁻N, TOC and TSS. TAN concentrations were significantly higher in T2 and T3 than those in T1 (P < 0.1)

aquacui	ture system for 55			
Treatment	T1	Τ2	Т3	P value
TAN	2.10±1.54 ^b	3.95±2.45 ^a	3.60±2.60 ^a	0.016
NO_2^-N	3.93±3.02	4.82±2.69	5.57±3.45	0.472
TOC	12.8±2	13.2±1.4	11.5±4.6	0.933
TSS	0.70±0.17	0.67±0.05	0.42±0.28	0.533
Water	26.15±0.31	26.09±0.22	26.36±0.46	0.322
temperature DO	10.3±0.35	10.38±0.2	10.41±0.16	0.739
pH	7.53±0.17	7.58±0.19	7.57±0.19	0.423
	10.		- VAN	

 Table 1. Water quality parameters at different feeding frequencies for juvenile black sea

 bream Acanthopagrus schlegelii raised in a semi- closed recirculating

 aquaculture system for 35 days

Values with the same superscript within the same row are not significantly different at P > 0.1

TAN, total ammonia nitrogen; NO₂⁻ N, nitrite nitrogen; TOC, total organic carbon; TSS, total suspended solids, DO is dissolved oxygen

3.2. Fish performance

At the end of the experiment, T3 showed a better FC than T2 and T1 but all the treatments were not significantly different (P > 0.1, Table 2). Specific growth rate (SGR) was improved with increasing feeding frequency with T3 having a significantly better SGR than T1 (P < 0.1) but not with T2 (P > 0.1). Survival rates (SR) ranged from 92% to 96% with T3 having the best survival rate but there were no significant differences among

the treatments (P > 0.1).

Table 2. Juvenile black sea bream Acanthopagrus schlegelii growth performance at
different feeding frequencies in a semi-closed recirculating aquaculture system
(mean \pm SD) after 35 days

Treatment	T1	Τ2	Т3	P value
Initial number of fish	75±00	75±00	75±00	1.000
Total initial weight (g)	490±00	490±00	490±00	1.000
Final number of fish	69±1.0	70±0.6	72±1.7	0.609
Final total weight (g)	919±27 ^a	1037±30 ^{ab}	1150±40 ^{bc}	0.075
Feed conversion	1.37±0.33ª	1.07±0.16 ^a	0.95±0.063ª	0.124
Survival rate (%)	92±4 ^b	93.3±2.3 ^b	96±6.9 ^b	0.609
Specific growth rate (%)/day	1.57±0.23ª	1.88±0.21 ^{ab}	2.13±0.25 ^{bc}	0.071

Values with the same superscript in the same row are not significantly different at P > 0.1.

3.3. Fish sizes and variations

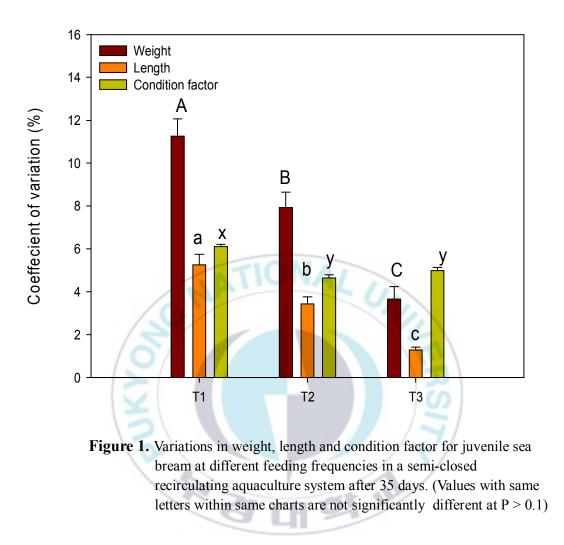
Final body weight and total length increased with increasing feeding frequency, T3 having a significantly higher body weight and total length than T1 (P < 0.1,Table 3) however, there were no significant differences (P > 0.1) between T3 and T2, and between T2 and T1

Treatment	T1	T2	Т3	P value
Final individual length (cm)	9.29±0.49ª	9.71±0.33 ^{ab}	10.14±0.13 ^{bc}	0.065
Final individual weight (g)	13.3±1.50 ^b	14.82±1.18 ^{ab}	15.9±0.6 ^{bc}	0.084
UI_{10} total body length (%)	60±14 ^a	77.2±5.9 ^b	76.4±12 ^b	0.003
UI ₁₀ total body weight (%)	31.8±5.7 ^{ac}	45.6±13.2 ^{bc}	36.9±20 ^c	0.006
UI_{10} condition factor (%)	49.6±11 ^a	55.7±6.9 ^b	61.6±14.4 ^b	0.004

Table 3. Final fish sizes and variations for juvenile black seabream Acanthopagrusschlegeliiat different feeding frequencies (mean \pm SD) in a semi-closedrecirculating aquaculture system after 35 days

Values with the same superscript in the same row are not significantly different at P > 0.1.

Uniformity index for body length and condition factor for T2 and T3 were significantly different (P < 0.1) from T1. Uniformity index for body weight for T2 was significantly different from T1 (P < 0.1) but both were not different from T3 (P > 0.1). Coefficient of variation (CV) for individual body weight and length decreased with increased feeding significantly (P < 0.1) while CVs for condition factor for T1 was significantly higher than for T2 and T3 at P > 0.1 (Figure 1).



Skewness for body weight decreased significantly with increasing feeding frequency (P < 0.1) with T3 having the lowest values (Figure 2). T3 had the highest skewness for body length and lowest condition factor while T2 had the lowest values for skewness for body length but the highest for condition factor, with treatments having significant

differences at P < 0.1.

Kurtoses for body weight decreased significantly with increasing feeding frequency (P > 0.1) with T3 displaying the lowest values. Kurtosis for body length was lowest in T2 with all treatments significantly different from each other (P < 0.1). There were no differences between kurtoses for condition factor for T1 and T2 (P > 0.1) but T3 was significantly different from T1 and T2 (P < 0.1).



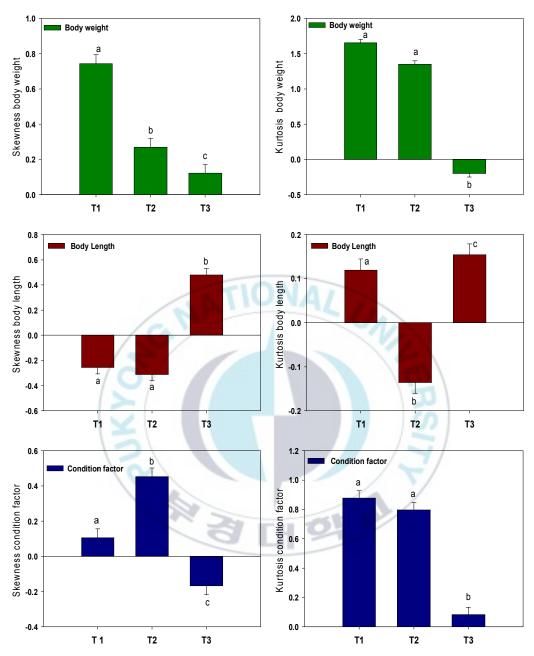


Figure 2. Body weight, lengths and condition factor distribution for juvenile black seabream *Acanthopagrus schlegelii* at different feeding frequencies in a semi-closed recirculating system. Values with same letter in each chart are not significantly different at P > 0.1

3.4. Body composition

Results for body composition are summarized in Table 4. Values for body moisture content at the end of the experiment ranged from 76% to 78%, with T1 having the highest followed by T3 but there were no significant differences (P > 0.1) among the treatments. Crude ash differed significantly among all the treatments (P < 0.1) with T2 having the highest values followed by T1. Crude fat increased with increasing feeding frequency with all treatments showing significant differences (P < 0.1). Crude protein content in T1 was significantly higher (P > 0.1) than T2 and T3.

 Table 4. Body composition of juvenile black seabream Acanthopagrus schlegelii at different feeding frequencies in semi-closed recirculating system

Treatment	Y T1	T2	T3	P value	
Moisture (%)	78.19±0.45	76.84±0.38	77.49±1.66	0.357	
Crude ash (%)	5.38±0.22ª	6.12±0.2 ^b	4.92±0.21°	0.004	
Crude fat (%)	1.14±0.15 ^a	1.71±0.01 ^b	2.61±0.19°	0.001	
Crude protein (%)	15.52±0.72 ^a	13.66±0.56 ^b	14.07±0.92 ^b	0.085	

Values within same row with same superscript are not significantly different at P < 0.1.

4. Discussion

The amount of feed supplied at each interval was lower in T3 which were fed on the 5% daily feeding rate divided three times a day and therefore fish in T3 consumed feed efficiently without leftover. There was no feed wastage meaning all the nutrients in the supplied ratio at each feeding were absorbed by the fish. Fish growth is highly dependent on utilization of the nutrients especially protein in the feed supplied (Sogbesan and Ugwumba, 2008). There is a possibility that fish in T3 spent less energy foraging for food since they were conditioned to frequent meals at relatively shorter intervals, thus, that energy was conserved for growth. Fish in T1 were supplied all the 5% daily feeding rate at once which they could feed up to satiation but fail to finish leading to wastage. The meal intervals were far apart in between, it is likely that fish in T1 spent more energy moving in the tanks looking for food. According to Dwyer et al., (2002) fish fed at lowest frequencies were most active and foraged equally at all feedings. Our results are not different from those reported by Kasir et al., (2011) whose study on angelfish, Pterophyllum scalare revealed that increased feeding frequency resulted in higher growth two years old black sea trout, Salmo trutta labrax weighing rates. Similarly, 155.9±31.56g showed the best growth results when they were fed three times a day as compared to one or two times (Bascinar et al., 2007). Lee et al., (2007) reported that increasing feeding frequency improved weight and length gain in ayu post-larvae *Plecoglossus altivelis,* with the larvae receiving one meal a day showing lowest growth rate due to inadequate feed supply. The effect of feeding frequency on fish growth is entirely dependent on fish species, size and culture conditions and results cannot be replicated in other species and/or conditions. In contrast to our findings, Salama; (2008) reported that growth and utilization of feed for juvenile barramundi were significantly better at the lowest feeding frequency (two) followed by three and four.

FC and SGR, which are growth performance indicators, are comparable with those reported by Partridge and Jenkins; (2002) for another species of black seabream, *Acanthopagrus butcheri* which ranged from 0.85 to 1.08 and 1.87 to 2.09% /day respectively. SGR in T3 was significantly higher compared to T1 (P < 0.1) but not different from T2 (P > 0.1). FC and SGR improved with increasing feeding frequency, this is in agreement with the results of Lee et al., (2007) on ayu post-larvae. Xie et al., (2011) reported that feeding large yellow croaker, *Pseudosciaena crocea*, eight times a day results in a better SGR than feeding two or four times. Liu; (1999) on the other hand, reported that feeding hybrid striped bass *Morone saxatilis* x *M. chrysops* once a day resulted in a better FC compared to feeding two, three and four times a day, although SGR displayed better results in intermediate feedings of two and three compared to one and four feedings a day in their study. They affirm that a better FC could be attributed to less physiological activity and lower endocrine function of fish

Feeding frequency had no effect on survival rates of black sea bream in this study.

Nekoubin and Sudagar, (2012) reported that feeding frequencies did not have a significant effect on survival rates (P > 0.05) of zebra fish, similarly,(Aydın, 2010; Goldan et al., 1997) reported that feeding frequencies did not significantly affect the survival rates of gilthead sea bream, *Sparus aurata* and juvenile black sea turbot, *Psetta maxima* respectively.

Condition factor (K) compares growth conditions, fatness or wellbeing of fish and is directly influenced by the environmental conditions. Biswas et al., (2011) stated that heavier barramundi of a given total length reared in brackish water were in a better condition and condition factor data was useful for better management of culture practices as it is a source of vital information to a farmer about the conditions under which the culture organisms are growing. Feeding frequency did not have an effect on condition factor with treatments showing no significant differences in this study (P > 0.1). Previous studies by Basciner et al., (2007) on black sea trout Salmo trutta labrax, Kasir et al., (2011) on angel fish *Pterophyllum scalare* did show significant differences (P > 0.05) There were reduced inter-individual differences in body weight, length and condition factor as feeding frequency increased. This is linked to increased chances for food accessibility for each individual in T2 and T3, unlike in T1 where food was accessed once a day (Liu, 1999; Phillips et al., 1998). In T1, it is more likely the bigger individuals fed on more food due to their stomach capacity and aggressiveness leading to better growth, further, compounding on the problem of size discrepancy. Uniformity index is expressed as the percentage of individual body lengths, weights and condition factor which occur

within 10% of the mean values Park et al., (2015). The same authors highlighted the benefits of determining the uniformity index in aquaculture especially during the early stages of fish development, as it could improve management regimes in terms of handling, grading, stocking, and also will help to show how fish sizes are distributed in a population.

As feeding frequency increase, feeding opportunities increase for all the fish thus reducing aggressiveness and social hierarchies among the dominant individuals resulting in less size coefficient of variations Wang et al., (1998). Liu, (1999) on hybrid striped bass, Phillips et al., (1998) on walleye fingerlings and Kasir et al., (2011) on angel fish all reported that fish fed at higher frequencies had small size variations compared to those fed at lower frequencies.

Skewness is a measure of asymmetry in fish body weight, length and condition factor. It helps in determining which side of the mean value most fish is found. Kurtosis on the other hand, measures the steepness of a distribution. In this study, body weights were moderately skewed to the right with T3 having the least skewness, this is because there were a few individual fish that were heavier than the mean weight but their difference from the mean was very small. Body lengths for T1 and T2 were moderately skewed to the negative side because the group of fish that formed the mode length were shorter than the mean length while T3 was moderately skewed to the right. Skewness for condition factor was the reverse for body lengths, T1 and T2 were positively skewed while T3 was negatively skewed. (Ribeiro et al., 2015) reported that increased feeding frequency

improved chances for food access for all the fish, this is needed in fish farming to produce less skewed, more homogeneous fish. (Liu, 1999) found that increasing feeding frequencies for hybrid striped bass resulted in more numbers of larger fish, leading to a slightly positive skew while fewer feeding frequencies produced more smaller fish with a negative skew. Condition factor is a ratio of body weight to length; its skewness depends on the skewness of body weight and body length.

An increase in feeding frequencies improves chances for each individual to access food (Liu, 1999) resulting in a more symmetrical size distribution (Goldan et al., 1997), implying that tails of the distribution are close to each other, this resulted in a significantly reduced kurtosis for body weight

Body composition analysis revealed moisture content was not significantly affected (P > 0.1) by feeding frequencies despite being slightly higher in T1 followed by T3. Crude ash was significantly different (P < 0.1) among all the treatments with T2 being highest followed by T1. Crude fat on the other hand, increased linearly with feeding frequency significantly (P < 0.1) while crude protein was significantly higher in T1 (P < 0.1) compared to T2 and T3. Some studies have reported that low feeding frequencies lead to increased moisture content, (Kim et al., 2007) on juvenile flounder, *Paralichtys olivaceus*, (Sang Min Lee et al., 2000) on juvenile rockfish. Fish in T3 had significantly more crude fat and reduced crude protein because during the process of energy partitioning, energy is retained as crude fat and crude protein from the combined source, where these fish utilized the frequently available protein energy source while

conserving crude fat energy; this trend was the inverse for the fish in T1 which conserved crude protein while utilizing crude fat energy sources (de Almeida Ozório et al., 2009; Lupatsch., et al 1998). Furthermore, in higher feeding frequencies, there is minimum activity and reduced mobility thus leading to minimum utilization of energy that is stored as crude fat (Cui et al., 2004; Liu, 1999; Rahman Md Mizanur et al., 2014)

The conclusion in this study is that feeding juvenile black seabream three times a day results in a better growth and reduces size variations thus producing more homogeneous fish than feeding once or twice.



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