



ISSN: 0975-833X

Available online at <http://www.journalcra.com>

International Journal of Current Research
Vol. 8, Issue, 02, pp.26540-26547, February, 2016

INTERNATIONAL JOURNAL
OF CURRENT RESEARCH

RESEARCH ARTICLE

THE EFFECTS OF LARGER FISH AND SIZE GRADING ON GROWTH PERFORMANCE OF WHITE SEA BREAM JUVENILES (*DIPLODUS SARGUS*)

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ARTICLE INFO

Article History:

Received 25th November, 2015
Received in revised form
19th December, 2015
Accepted 20th January, 2016
Published online 27th February, 2016

Key words:

Size grading,
Diplodus sargus,
White sea bream.

ABSTRACT

This study was conducted to determine the effects of size grading on growth performance of white sea bream (*Diplodus sargus*) juveniles. Wild-caught fish were size sorted into two different groups: small (5.6 g) and large (7.5 g) and then four experimental groups were assigned as 100% small group (S100), 25% large + 75% small group (S75), 50% small + 50% large group (S50), and finally 75% small + 25% large group (S25). Twenty fish were stocked into each tank (450 L) connected with a flow through system in three replicates per treatment. The juveniles were fed to satiation with a commercial feed for 60 days. According to final results, specific growth rate for all periods was similar in all experimental groups ($P>0.05$). No significant differences were found in survival rate ($P>0.05$). On the other hand small fish final weight of S50 was significantly higher than the others ($P<0.05$). There were significant difference between FCR of graded group (S100) and mixed groups (S25, S50 and S75) ($P<0.05$). The results have demonstrated that the larger individuals in the present study did not suppress growth of the smallest individuals. The fish in S100 were adversely affected by the absence of large fish and this was postulated to be due to poor competition for food acquisition in this group. For this reason size grading seems to be unnecessary to improve growth and survival of white sea bream of around 5.6-7.5 g.

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Citation: Suat Dikel, Orhan Tufan Eroldoğan, Ilgın Özşahinoğlu, Pınar Mumoğullarında and Hatice Asuman Yılmaz, 2016. "The effects of larger fish and size grading on growth performance of white sea bream juveniles (*Diplodus sargus*)", *International Journal of Current Research*, 8, (01), 26540-26547.

INTRODUCTION

The Mediterranean aquaculture mainly marine fish farming of sea bass, sea bream, Atlantic salmon and turbot has been notable for its development and growth in the last decade. However, this dramatic increase especially in the production of sea bass and sea bream has occurred despite technical constraints and limited markets (Basurco and Abellan, 1995; Ozorio et al., 2006). It is now clear that one explicit strategy for step-up of European aquaculture lies in its diversification (Quemener et al., 2002). Currently, about 25 'new' marine fish species are under investigation in the Mediterranean area, including Dover sole (*Solea solea*), blackspot seabream (*Pagellus bogaraveo*) (Ozorio et al., 2004) and white seabream (*Diplodus sargus*) (Ozorio et al., 2006). A special emphasis is

given to the white sea bream farming sector in Southern Europe, because it is considered to be a promising new species, having a high market price and demand (Pousao-Ferreira et al., 2001; Ozorio et al., 2006; Santos et al., 2006; Saaverda et al., 2009). However, a decrease in growth rate was faced when approaching the juvenile stage of this species because of its hierarchic behavior (Cejas et al., 2003). In general, size variability in a batch of same-age fish is common among farmed fish (Huntingford et al., 1990; Stefánsson et al., 2000; Saoud et al., 2005). This hierarchy effect may be due to direct competition for food or the result of social interactions depressing the growth of the smaller members of the population (Jobling 1982). A wide size distribution in aquaculture is disadvantageous due to social growth suppression of small individuals (Ruzzante, 1994). There are many instruments to overcome this problem. One of the commonest tools is size grading, that is used in the culture of many commercial fish species in an attempt to improve growth

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and survival (Gunnes, 1976; Huet, 1986; Baardvik and Jobling, 1990; Popper et al., 1992; Kamstra, 1993; Alev and Dikel 2010, Dikel et al., 2010). The main idea behind size grading in general is to separate small and large individuals from each other to avoid potentially negative effects of social interactions (Jobling, 1982, 1995; Knights, 1987). In some fish species, dominant or large individuals exhibit higher growth rates than subordinates (Brown, 1946; Magnuson, 1962; Jobling, 1985, 1995; Koebele, 1985), and several mechanisms have been proposed to explain how larger dominant individuals suppress the growth of small subordinates (Wirtz, 1974; Koebele, 1985; Wallace et al., 1988). Competition for food seems to be particularly important in governing growth (Magnuson, 1962; Wallace and Kolbeinshavn, 1988; Jobling and Koskela, 1996), and in the absence of competition from larger individuals, smaller individuals avoid the negative effects of a dominance hierarchy and may achieve higher growth rates (Purdom, 1974). However, size grading, in some cases, has been shown to reduce growth, possibly owing to higher levels of interaction between fish of the same size (Doyle and Talbot, 1986; Baardvik and Jobling, 1990; Sunde et al., 1998).

Actually size grading is believed to reduce the harmful effects on small fish, with a consequent reduction in size variation and increased biomass gain. Nevertheless, there is evidence that size grading may not eliminate this effect in all species [e.g., in Arctic charr (*Salvelinus alpinus*), Baardvik and Jobling, 1990; in European eel (*Anguilla anguilla*), Kamstra, 1993; in channel catfish (*Ictalurus punctatus*), Carmichael, 1994; in turbot (*Scophthalmus maximus*), (Sunde et al., 1998) and in silver perch (*Bidyanus bidyanus*) Barki et al., 2000; marbled spinefoot rabbit fish (*Siganus rivulatus*) (Ghanawi et al., 2010). At present there are no clear guidelines about the need for grading in white seabream (*D. sargus*) hatchery operations. Therefore, the following experiment was designed to investigate the effect of size grading and larger fish on growth and survival of juvenile white seabream.

MATERIALS AND METHODS

White sea bream juveniles (*Diplodus sargus*) were caught by beach seine in Yumurtalik Bight and transported to Yumurtalik Marine Research Station of Faculty of Fisheries in Cukurova University. They were then randomly stocked in 2 circular fiberglass tanks (3-m diameter, 1000 L) fitted with a continuous water flow-through system. Fluorescent lighting was provided in addition to natural lighting to maintain a 12-h light: 12-h dark photoperiod with daybreak set at 07:00 h. Water temperature was checked daily. Throughout the 60 days experimental period, the rearing water in each tank was permanently saturated with oxygen by supplying air continuously through air stones from an air blower. Average water temperature ranged from 24.5 to 26.5 °C. The dissolved oxygen and pH were measured every five days, and they averaged 7.2 ± 0.4 mg/L and 7.8 ± 0.5 , respectively.

Fish were acclimated to the system for 1 month before the experiment started. After the acclimation period, the fish were individually weighed and manually graded into two size groups: small and large. Four treatments with three replicate tanks (450 L) per treatment were established.

S25: Mixed as 25% small +75% large (small 5.45 ± 0.26 g and large 7.56 ± 0.43 g),
 S50: Mixed as 50% small + 50% large (small 5.78 ± 0.19 g and large 7.48 ± 0.22 g),
 S75: Mixed as 75% small + 25% large (small 5.68 ± 0.06 g and large 7.21 ± 0.48 g).
 S100: Graded Group 100% small (5.68 ± 0.06 g) (mean \pm SD).

Stocking rate was twenty fish for each tank and maintained in a flow-through system. Water exchanged was $1 \text{ L} \cdot \text{min}^{-1}$. Fish were offered a commercial diet (Çamli Yem Ltd., Izmir, Turkey) containing 50% protein, 15% lipid three times daily (09:00, 13:00 and 18:00 h) until apparent satiation. All the fish in each tank were individually weighed (to the nearest 0.01 mg) every 10 days, after anesthetizing in 0.3-mL/L 2-phenoxyethanol (Sigma, St. Louis, MO, USA). Only large fish in mixed groups were tagged with plastic numbers with suture materials. Each tag consisted of a needle with an attached length of thread (braided polyester-silicone coated). Fish were sutured from dorsal area with gently. No fish died during tagging and no tags were lost during the experiment.

Calculations

Performances of the fish were evaluated by calculating the following parameters from the data collected: Weight gain (g); ($W_{\text{final}} - W_{\text{initial}}$). Daily weight gain ($\text{g} \cdot \text{day}^{-1}$); [$(W_{\text{final}} - W_{\text{initial}}) \cdot \text{day}^{-1}$]. Specific growth rate (SGR % body weight day^{-1}); [$(\ln w_1 - \ln w_0) (t_1 - t_0)^{-1}$] 100, where w_1 and w_0 are wet weight at times t_1 and t_0 . Feed conversion ratio (FCR); consumed feed / ($W_{\text{final}} - W_{\text{initial}}$), where W_{final} and W_{initial} are live weights (g) of the fish at day final and initial, respectively and a coefficient of variation [$\text{CV} = (\text{SD}/\text{mean})$] for individual fish within each tank was compared among treatments. The mean and standard deviation (\pm SD) was calculated for all parameters in each group and the differences on growth performance were examined by comparison of mean weights of fish.

Statistical analyses

Data are reported as mean \pm S.D (n=20) throughout the text. Data were analyzed by one-way analysis of variance (ANOVA) at a significance level of 0.05%, after confirmation of normality and homogeneity of variance. Where significant differences were detected, data were subjected to Duncan post hoc test for identifying homogeneous subset SPSS11.5 (SPSS Inc., Chicago, IL, USA).

RESULTS

During the experiment, survival ranged from 95.0% to 100% with no statistical difference among the experimental groups and unaffected by treatments ($P > 0.05$). Overall growth performance is reported in Table 1. Some statistically significant differences were noted in overall performance of the treatments, including final weight, weight gain, FCR and $\text{CV}_{\text{final}}/\text{CV}_{\text{initial}}$, however, no difference were observed in daily weight gain and SGR. In general, S25 (16.2 g) group had significantly higher growth than that of S100 (13.3 g) (Figure 1).

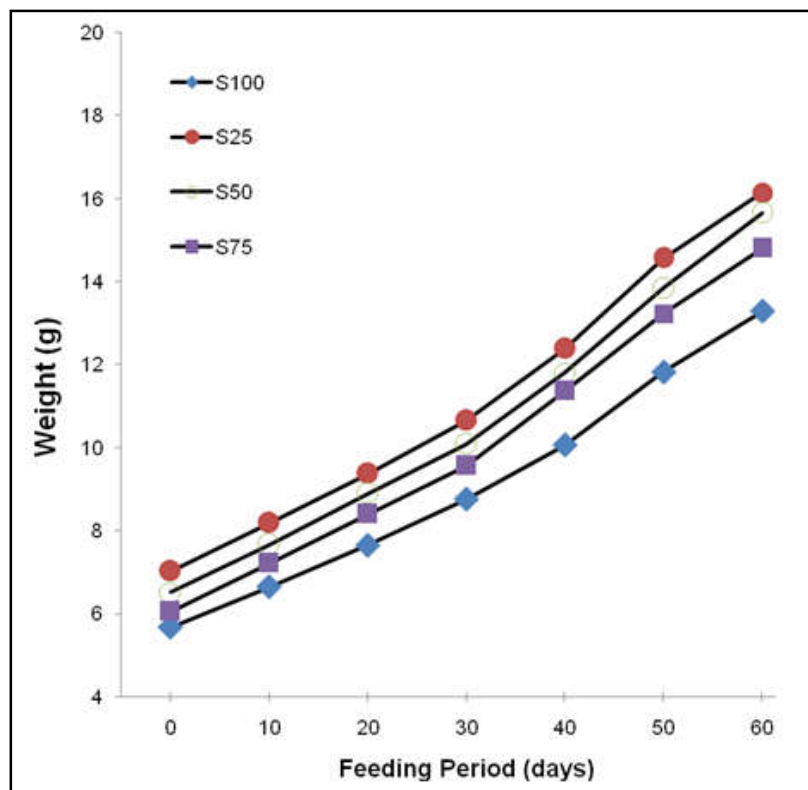
Table 1. Growth performance and feed utilization of white sea bream reared in four different groups for 60 days

	S25	S50	S75	S100
<i>Overall Fish Performance</i>				
Initial Weight (g)	7.0 ± 0.39	6.55 ± 0.04	6.1 ± 0.09	5.7 ± 0.07
Final Weight (g)	16.2 ± 0.80 ^a	15.65 ± 0.21 ^b	14.8 ± 0.18 ^b	13.3 ± 0.29 ^c
Weight gain (g)	9.2 ± 0.49 ^a	9.10 ± 1.17 ^a	8.7 ± 0.19 ^a	7.6 ± 0.34 ^b
Daily Weight gain (g/day)	0.153 ± 0.01 ^a	0.150 ± 0.02 ^a	0.145 ± 0.00 ^a	0.126 ± 0.01 ^a
FCR	1.6 ± 0.06 ^b	1.7 ± 0.24 ^b	1.6 ± 0.19 ^b	1.9 ± 0.26 ^a
SGR	1.4 ± 0.06 ^a	1.5 ± 0.12 ^a	1.5 ± 0.03 ^a	1.4 ± 0.05 ^a
CV _{final} /CV _{initial}	1.2 ± 0.09 ^{ab}	0.8 ± 0.14 ^b	1.1 ± 0.19 ^{ab}	1.5 ± 0.51 ^a
<i>Small Fish Performance</i>				
Initial Weight (g)	5.5 ± 0.26	5.7 ± 0.04	5.7 ± 0.06	5.7 ± 0.07
Final Weight (g)	12.1 ± 0.85 ^c	14.6 ± 0.53 ^a	13.8 ± 0.40 ^{ab}	13.3 ± 0.29 ^b
Weight gain (g)	6.6 ± 0.59 ^c	8.9 ± 0.57 ^a	8.1 ± 0.43 ^b	7.6 ± 0.34 ^b
Daily Weight gain (g/day)	0.0.110 ± 0.01 ^b	0.148 ± 0.02 ^a	0.135 ± 0.01 ^{ab}	0.126 ± 0.01 ^b
SGR	1.3 ± 0.04 ^c	1.6 ± 0.07 ^a	1.5 ± 0.06 ^{ab}	1.4 ± 0.05 ^{bc}
CV _{final}	0.15 ± 0.04 ^a	0.10 ± 0.02 ^a	0.13 ± 0.02 ^a	0.15 ± 0.02 ^a
<i>Large Fish Performance</i>				
Initial Weight (g)	7.6 ± 0.43	7.4 ± 0.12	7.2 ± 0.48	
Final Weight (g)	17.5 ± 0.82 ^a	16.7 ± 1.83 ^a	17.6 ± 0.64 ^a	
Weight gain (g)	9.9 ± 0.70 ^a	9.3 ± 1.71 ^a	10.4 ± 0.40 ^a	
Daily Weight gain (g/day)	0.165 ± 0.01 ^a	0.155 ± 0.03 ^a	0.173 ± 0.01 ^a	
SGR	1.4 ± 0.08 ^a	1.4 ± 0.16 ^a	1.5 ± 0.07 ^a	
CV _{final}	0.15 ± 0.03 ^a	0.10 ± 0.02 ^{ab}	0.07 ± 0.03 ^b	

Values in the same row with different superscripts are significantly different ($P < 0.05$) as determined by ANOVA. Values are means ± standard deviation ($n=3$)

Table 2. Slope, intercept, and regression coefficient (R^2) of growth linear model $W = axt + b$ where W is weight in grams and t is time in days. Values not sharing same letter are significantly different ($P < 0.05$)

Treatment	Slope (a)	Intercept (b)	R^2
S25	1.54 ^a	5.03	0.99
S50	1.52 ^a	4.55	0.99
S75	1.47 ^b	4.20	0.99
S100	1.27 ^c	4.05	0.99

**Figure 1. Growth of size graded juvenile white sea bream over 60 days. Data represent mean ± SEM ($n=3$)**

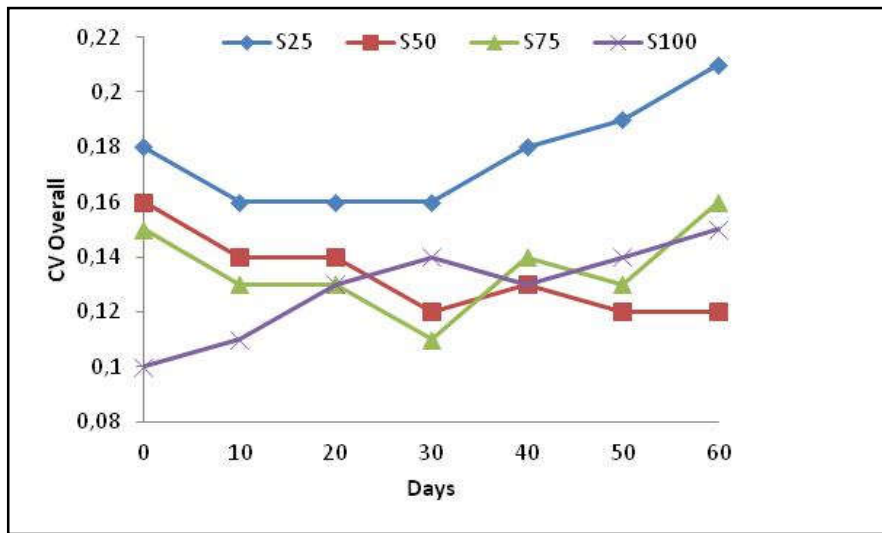


Figure 2. Coefficient of variation (CV) in overall fish weight within each treatment for juvenile white sea bream over 60 days. Data represent mean± SEM (n=3)

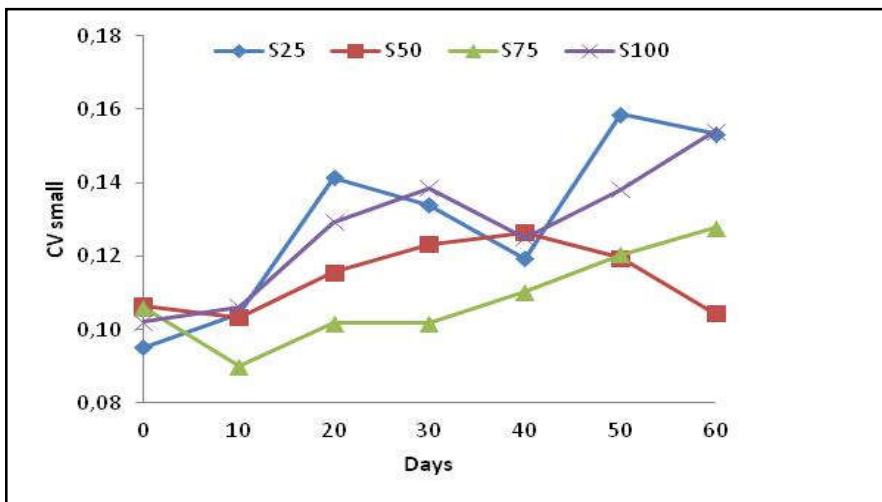


Figure 3. Coefficient of variation (CV) in small fish weight within each treatment for juvenile white sea bream over 60 days. Data represent mean± SEM (n=3)

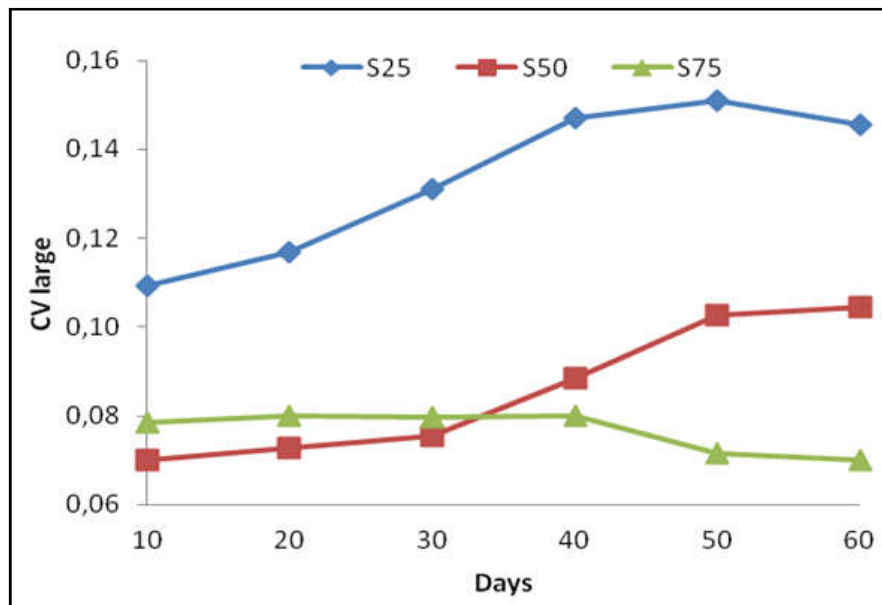


Figure 4. Coefficient of variation (CV) in large fish weight within each treatment for juvenile white sea bream over 60 days. Data represent mean± SEM (n=3)

The slope of growth curve (weight vs. time) for fish in S25 treatment was significantly greater than those in all other treatments. The slope of growth for S100 treatment was significantly lesser than that of S50 or S75 treatment (Table 2). FCR for fish in S100 (1.9) was significantly greater than those for S25 (1.6), S50 (1.7 g), or S75 (1.6) treatments (Table 1). It is noteworthy that the $CV_{\text{final}} / CV_{\text{initial}}$ of S100 group was higher than their respective $CV_{\text{final}} / CV_{\text{initial}}$ (Table 1). Coefficient of variation (CV) in overall fish weight within each treatment for juvenile white sea bream over 60 days is shown in Figure 2.

By the end of the feeding trial, small fish final weight, average weight gain (8.9 g) and SGR (1.6 % body weight day⁻¹) of the S50 group was higher than the others ($P < 0.05$). Graded small individuals (S100) grew slower compared to the others. As clearly depicted, the most remarkable growth performance was obtained when number of small fish and large fish were balanced (S50). Small fish of S50 had highest SGR (1.6%) among all other treatments. CV_{final} for small fish was ranged from 0.10 to 0.15. A relatively large variability in the data was responsible for a lack of statistically significant results for CV for small fish despite evident numerical differences throughout the experimental period (Figure 3). No significant differences were recorded for final weight, weight gain, daily weight gain and specific growth rate in large fish whereas there were significant differences in CV large final values among treatments (Figure 4).

Briefly, the major affects of large fish in the treatments were as follow: 1) absence of large fish had significant effect on the growth performance of small fish. 2) The presence of small fish has no impact on the growth of large fish. 3) Different proportions of large fish in mixed size groups have shown similarly growth performance.

DISCUSSION

The results of present study demonstrated that the presence of large fingerlings of white sea bream had positive impacts on the growth performance of smaller individuals when kept together at equal numbers. It is generally accepted that intraspecific competition and agonistic interaction are found to be greater when fish of the similar size are reared together in fishes like Arctic charr *Salvelinus alpinus* L. (Baardvik and Jobling, 1990), turbot *Scophthalmus maximus* (Sunde *et al.*, 1998) and Atlantic halibut *Hippoglossus hippoglossus* (Stefánsson *et al.*, 2000). However, some recent studies have produced findings different from the above-mentioned general assumption (Wallace and Kolbeinshavn, 1988; Baardvik and Jobling 1990; Jobling *et al.*, 1991; Kamstra, 1993; Strand and Øiestad, 1997; Sunde *et al.*, 1998; Dou *et al.*, 2004). Some mechanisms have been proposed to explain how size hierarchies influence the growth, e.g., physiological stress (Jobling, 1985; Abbott and Dill, 1989; Huntingford *et al.*, 1993; Griffiths and Armstrong, 2002), disproportional food acquisition (Koebele, 1985; Grant, 1997), and activity differences (Adams *et al.*, 1998; Sloman and Armstrong, 2002). These mechanisms are all based on the general assumption that relative size is the most important determinant of fighting ability in aggressive behavior as well as attacking

opponents and obtaining preferential access to food (Dou *et al.*, 2004). Differential access to food or disproportional food acquisition is often considered to be an important factor responsible for the retardation of growth of the subordinates in fish populations (Magnuson, 1962; Jobling and Wandsvik, 1983; Koebele, 1985; Abbott and Dill, 1989). The mechanism underlying this social interaction is that dominants defend the point of sources of food or areas with high prey concentration, thus behaviorally preventing the subordinates accessing food (Dou *et al.*, 2004). In present study, provision of excessive food might have made it unworthy for the large white sea bream juveniles to defend food for all the time. As a result, the large juveniles might have lost their competitive advantage over the small ones and therefore the small juveniles could get equal overall access to food. In line with these results, food intake of the juvenile medaka and rainbow trout is highly correlated with dominance, but that relationship tends to become unstable if food is offered to excess (Magnuson, 1962; McCarthy *et al.*, 1992). Besides, FCR for graded small white sea bream was higher than FCR's for the other groups and this is thought to be due to poor competition for food among the graded fish. The mixed groups (predominantly small group and large group) had lowest FCR values. These findings suggest that FCR's of white sea bream were affected from proportion of size fraction in the stock. To our understanding, it is likely that dominance hierarchy would be further enlarged when food supply is limited in white sea bream juveniles.

Monopolization and defense of food resources have consequences in the social environment of groups of juvenile white sea bream, generating the establishment of dominance hierarchies (Castro and Caballero, 1998) as a consequence of agonistic interactions and asymmetry in fighting ability (Maynard Smith and Parker, 1976). Lutnesky and Szypper (1991) and Ryer and Olla (1995) observed that agonistic behavior increases when the distribution of food is spatially clumped; however, the results of Castro and Santiago (1998) indicate that the levels of aggression were greater in groups of juvenile white sea bream given spatially variable food than in groups given spatially fixed food. These authors claim that feeding regime of juvenile white sea bream is affected by food distribution to the culture media. As also suggested by Castro and Santiago (1998), it appeared in our study that small juvenile white sea bream tended to consume more food under variable food supply compared to dominant individuals. In the present experiment, the coefficients of variation in weight in S100, S25 and S75 groups were similar ranging between 1.1 and 1.5. However, CV of S50 was markedly smaller (0.8) than all the other groups. Growth differences among fish of the same generation could be due to environmental factors, associated physiological differences, and/or to the formation of social hierarchies, or due to genetic differences (Brown, 1946). In a population in which the CV increases with time, hierarchy effects might be responsible for the suppression in growth of certain individuals (Purdum, 1974). Dominance hierarchies lead to trade off between increased resource acquisition and energy losses associated with agonistic encounters. In the study, the growth rates of subordinate fish can be depressed as a consequence of dominant aggression but aggression may also involve energetic costs and loss of feeding opportunities during time spent in territory.

Jobling and Baardvik (1994) reported that increased CV was caused by hierarchical effect and some members of the population were dominant because of agonistic behaviors. Agonistic behaviors are instinctual acts performed by animals for the sake of surviving, continuing their bloodline, attacking, defending, or simply providing access to food sources. The researchers concluded that dominant members of the population caused level of CV increasing in time. In another similar study, growth was reported to slow down because of the hierarchy caused by large fishes on small ones. Another important reason generally reported in various studies is the genetic differences between fishes, which are thought to produce an agonism arising from the nature of the fish (Ghanawi et al., 2010). In the present study, when we generally evaluated the performances of individual members, we observed that weight gain in the S100 group was lower than those displayed by S25, S50 and S75 groups. When CV results are taken into account, the highest value was seen to be present in S100 group, in which weight gain was lowest. Other similar studies also reveal that a continuous competition of surviving takes place and highest levels of CV values, hierarchical relations and dominance are observed in groups, which consist of the same-sized members. Our results suggest that highest level of hierarchical interactions (high CV) and dominance were present in the S100 group, whilst these were almost nil in the S50 group, in which growth was highest and CV (0.8) lowest. This is consistent with studies by Jobling and Reinsnes (1986) and Jobling (1990) who also found out that hierarchical relation did not develop and length grading was not needed during the fingerling stage in mixed groups consisting of small and large sized members with a proportion of half-to-half.

In conclusion, we demonstrated that size grading in white sea bream juveniles culture did neither lead to improved growth nor survival and also the presence of larger individuals did not suppress smaller individuals. Therefore, it appears that size grading is unnecessary to improve growth and survival of white sea bream at size of around 5.6-7.5 g. Furthermore, based on our results, culturing small and large fish together at the same tank for a purpose of achieving better growth is also not recommended. These current findings contribute to the better understanding of the hatchery management of this species at fingerling stage, but further studies are clearly needed to deepen our insight about behavior of large fish under commercial stocking densities in white sea bream hatcheries.

Acknowledgements

The authors would like to thank the Resource Fund of the University of Cukurova, (Turkey) for their financial support (project no: SÜF2008BAP10). We also would like to thanks Dr. Metin Kumlu for his valuable comments.

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