THE INFLUENCE OF STOCKING DENSITY ON GROWTH AND FEED UTILIZATION IN GILTHEAD SEABREAM (*Sparus aurata*)

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SUMMARY

Stocking density is considered a priority topic in aquaculture research due to its bearing on the welfare of farmed fish and the need for future recommendations governing stocking density management on fish farms. The aim of this study was to evaluate the influence of stocking density (10, 20, 30 and 40 fry/L^3) on growth performance, Feed utilization and survival rate of fry gilthead seabream (*Sparus aurata*). The results showed that the group of fry stocked at density 30 fry/ L^3 was significantly the highest final weight, weight gain, daily gain, SGR and insignificant differences in survival rate. Growth performance parameters were the highest with stocking density 30 fry/ L^3 compared with stocking density (10, 20 and 40 fry/ L^3), respectively. The results indicated that the best FCR, PER and FE group of fry stocked at 30 fry/ L^3 than the rest of experimental groups. Stocking density also did not affect the crude protein and moisture content in body composition. But ash content was significantly highest at density 30 fry/ L^3 than the rest of other stocking density (10. 20 and 40 fry/L^3) groups. In contrast, total lipid was significantly highest at density 10 fry/L^3 than the rest of experimental groups (20, 30 and 40 fry/L^3). From the aforementioned results, it could be concluded that stocking density 30 fry/L^3 had the best growth performance and feed utilization under experimental conditions.

Keywords: Seabream (Sparus aurata), stocking density, growth performance and feed utilization.

INTRODUCTION

Gilthead seabream (*Sparus aurata* L.) is a species of high commercial value, especially in the Mediterranean region, where it was one of the first species to be intensively cultivated. In the last few years, the drop in the gilthead seabream market price due to overproduction is forcing the aquaculture industry to reduce production costs and enhance fish quality. It prefers warm coastal euryhaline waters and its life-cycle is determined by protandrous hermaphroditism (FEAP, 2017).

Stocking density has become widely recognized as an important husbandry factor in intensive fish culture due to it representing a potential source of chronic stress, which may have adverse effects on the physiological, health and/or behavioral status of the individual fish involved, for example reductions in reproductive output and changes in disease resistance (Ashley, 2007). These indicators can in turn be used as signs of compromised welfare (Huntingford *et al*., 2006). However, highly intensive recirculation systems of densities up to 100 kg/m^3 are being developed (Blancheton, 2000). It has been demonstrated that rearing stocking densities may impair the growth, reduce immune competence and induce abnormal behavior (Kristiansen *et al*., 2004; Schram *et al*., 2006). Tan *et al*.,(2018) indicate that a greater stocking density increase stress and significantly affected the growth of eel. Recently, research on stocking density is receiving a great attention (Turnbull *et al*., 2005) since this study is necessary to provide information on a better aquaculture management in relation to the fish welfare. Although some studies on stocking density have been published, it is still difficult to obtain information on better densities for each species, because the best densities are affected by different culture systems, fish species and fish age (Ellis *et al*., 2002). In aquaculture system, mostly aquaculturists cultivate their fish in high stocking density in order to maximize productivity (Iguchi *et al*., 2003). Effect of stocking density on growth performance was illustrated by many authors different in densities used/m^3 but all of them used recirculation intensive system (Turnbull *et al*., 2005, North *et al*., 2006;Ashley, 2007). No references are available about production of Gilthead seabream under different stocking densities.

Many studies have demonstrated that stocking fish at a high density may have a negative effect on farmed fish. The growth, survival, and food utilization rates have all been shown to decrease with an increase in stocking density (Gang *et al*., 2010 and Zhu *et al*., 2011). A high stocking density not only increases direct competition among fish for space and food but it may also increase the incidence and mortality of cultured fish (Salas-Leiton *et al.*, 2010). Generally high density is considered as a potential source of stress, with a negative effect on fish growth rate (Lefrançois *et al*., 2001) and survival and feeding rates (Rowland *et al*., 2006). Daily feed intake and specific growth rate decreases were observed with increasing density levels on several species such as Atlantic cod (*Gadus morhua L.*) (Lambert and Dutil, 2001), brook charr (*Salvelinus fontinalis*) (Vijayan and Leatherland, 1988), gilthead sea-bream (*Sparus aurata*) (Canario *et al*., 1998) and largemouth bass (*Micropterus salmoides*) (Petit *et al*., 2001).

Stocking density directly influences survival, growth, behavior, water quality and feeding. In aquaculture, stocking density is the concentration which fish are stocked into a system (Gomes *et al*., 2006 De Oliveira *et al*., 2012). Consequently, identifying the optimum stocking density for a species is a critical factor not only to enable efficient management and to maximize production and profitability, but also for optimum husbandry practices (Kristiansen *et al*., 2004; Rowland *et al*., 2006).

The goal of this study was to evaluate the influence of stocking density on growth performance and feed utilization for sea bream fry .

MATERIALS AND METHODS

Experimental fish:

Fry gilthead sea bream (*Sparus aurata*), used in this study with mean average initial weight of 0.30 ± 0.01g were obtained from Private fish farm – Elkantra- Ismailia Governorate- Egypt. Fish were acclimatized to laboratory conditions for 2 weeks before beginning the experiment. Fish were homogenous in body weights and apparently healthy, unhealthy fish were removed from tanks, and replaced with others healthy.

Experimental unit:

The fry rearing trials were carried out at the intensive hatchery Unit Private Fish hatchery in Elkantra– Ismailia Governorate.

Gilthead sea bream larvae will stocked in nine white circular fiberglass tanks (each of 100 L volume) in black greenhouse at a density four density 10, 20, 30 and 40 larvae/ Liter (3 replicate/ treatment).

Water quality:

Water temperature was maintained at 25ºC by a 250 watt immersion heater with thermostat. Water temperature were recorded daily by mercury thermometer, and dissolved oxygen by metteler Toledo, model 128.s/No1242. Where the average range of dissolved oxygen was above 6.10 mg/l. Other water quality parameters including pH was measured every two days by pH meter (Extech pH / temp pen model pH 60), where the average range of pH was in 7.7 ± 0.7 throughout the experiment. Water salinity was measured, using temperature compensated refractmeter. Water exchange was approximately 15% per day. Temperature was monitored at 8:00 h daily, while salinity, ammonia, pH and dissolved oxygen were measured every day, in tanks of each treatment. Averages water quality parameters are presented in Table (2).

Experimental diets:

The experimental diet was formulated from practical ingredients (Table 1). They were based on fish meal and soybean meal as the protein source. These diets formulated to containing four protein levels 50% crude protein. The experimental diet was prepared by individually weighing of each component and by thoroughly mixing the mineral, vitamins and additives with corn. This mixture was added to the components together with oil. Water was added until themixture became suitable for making granules. The wet mixture was passed through CBM granule machine with fin mash. The produced mash were dried at room temperature and kept frozen until experimental start. Diets were analyzed for crude protein, crude lipid, crude fiber and ash according standard AOAC (2004) as shown in Table (1).

Table (1): Composition and proximate analysis of the experimental diets.

Ingredient	(%)
Fishmeal (70 % CP)	60
Soybean meal (44 % CP)	18
Yellow Corn	10
Fish oil	5
sunflower oil	5
Minerals mix1	1
Vitamins mix2	1
Total	100
Proximate analysis (%)	
Moisture	7.80
Crude protein	50.70
Ether extract	15.30
Crude fibre	2.10
Ash	11.10
Nitrogen free extract	13.00
Metabolizable energy (Cal / Kg)3	398.21
Protein energy ratio (mg p/ K Cal)	127.31

1, 2 . NRC (1993).

3. Metabolizable energy (ME):- calculated using values of 4.50, 8.15 and 3.49 Kcal for protein, fat and carbohydrate, respectively.

The nitrogen free-extract (NFE %) was calculated by differences. The fish were hand-fed to apparent satiation within half an hour, twice/day (11 and 3 pm) for 60 days. Five fish from each treatment was taken for body composition without viscera analyzed for crude protein, crude lipid, crude fiber and ash according standard AOAC (2004)**.**

Fish performance:

Samples for total length and wet weight were taken during the 60 days to identify potential differences on the growth between treatments. For the total lenth (TL), 10 fishes per tank every 2nd day were measured under a stereo microscope Leica type MZ 125 or Olympus Optical co., Ltd., type SZH-ILLK. Deformed specimens were omitted from the data. Fry were weighted using a precision electronic balance Mettler AT201. Measurements mentioned were calculated according to the following formula:

Fish survival rate (%) = 100 (FN / IN) Where: FN: number of fish at the end of the experiment IN: number of fish at the beginning of the experiment,.

Larval survival rate was calculated after 60 days through counting the total number of the produced fish.

Statistical analysis:

All of the data were analyzed by one-way analysis of variance (ANOVA) using the general linear models procedure of statistical analysis system (SAS, 1999) version 8.02. Duncan's multiple range test (Duncan, 1955) was used to resolve differences among treatment means at 5% significant level.

RESULTS AND DISCUSSION

Water quality:

Mean values (mean ± SD) and ranges of water quality parameters measured between treatments over the experimental period are summarized in Table (2). There was no significant difference ($P < 0.05$) in the mean temperature, pH, dissolved oxygen, ammonia and salinity between all treatments.

Table (2): Averages water quality parameters are presented in the experimental tanks.

Parameter	Means ±SD
Temperature	25.00 ±1.000
Oxygen (mg/L)	6.00 ±1.000
Ammonia NH3 (mg/L)	0.02±0.001
pH	7.70±1.000
Salinity ppt	35.00±1.000

Mean±SE of means

Growth performance and survival rate of gilthead Sea bream fry:

The growth performance of gilthead seabream (*Sparus aurata*) fry which stocked at different density are shown in Table (3). Average body weight (g) of fry seabream fed experimental diets at the start did not differ, indicating that groups were homogenous.

Table (3): Effect of stocking density on growth   performance and feed utilization of Sea bream fry at the end of experimental period (60 days).

Parameter	Density (fry/L^3)			
	10	20	30	40
Initial weight (g)	0.30±0.01	0.30±0.01	0.30±0.01	0.30±0.01
Final body weight (mg)	2.40±0.02^d	3.50±0.01^b	4.10±0.02^a	3.0±0.01^c
Weight gain (mg)	2.10±0.02^d	2.70±0.01^b	3.8±0.02^a	2.70±0.01^c
Daily weight gain (g)	0.035±0.02^d	0.045±0.01^b	0.063±0.01^a	0.045±0.01^c
Specific Growth Rate	1.31±0.10^d	1.99±0.20^b	2.23±0.20^a	1.65±0.10^c
Feed intake	3.57±1.10^c	4.86±1.10^b	6.08±1.10^a	6.48±1.10^a
Feed conversion Ratio	1.70±0.10^c	1.80±0.20^b	1.60±0.20^a	2.4±0.10^d
Protein intake	1.80±0.10^d	2.46±0.20^b	3.44±0.20^a	3.28±0.10^c
Protein efficiency ratio	1.16±0.10^d	1.09±0.20^b	1.10±0.20^a	0.82±0.10^c
Feed Efficiency	0.59±0.10^d	0.55±0.10^b	0.63±0.10^a	0.42±0.10^c
Survival %	70±2.10^d	65±2.10^b	60.00±2.10^a	50±2.10^c

Mean ± SE of three replicates. Means in the same row with different superscripts are significantly different (P<0.05).
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The results showed that the group of fish stocked at 30 fry/L^3 was significantly (P<0.05) had the highest final weight, weight gain, daily gain, SGR and insignificant differences (P>0.05) in survival rate than t he rest of experimental groups.

The results indicated that Stocking density of 30 fry/L^3 improved the growth performance compared with other stocking densities (10, 20 and 40 fry/L^3). these results agreed with the resulted obtained by Biswas *et al*. (2007). This effect was as result of increased voluntary activity at higher stocking densities, which might be dissipated as energy, resulting in lower weight gain. Moreover, higher stocking densities were reported to cause chronic stress, which in turn reduced the growth rate because of the reallocation of energy towards activities aimed at restoring homeostasis such as; respiration, locomotion, hydromineral regulation, and tissue repair (Biswas *et al*., 2007). Similar trends were observed in white sea bream where a high stocking density led to reduced growth performance (lower final body weight and total length, SGR, weight gain) (Karakatsouli *et al*., 2007) and low survival. According to Cuvin-Aralar *et al*., (2007) better growth of the giant fresh water prawn (*Macrobrachium rosenbergii*) was achieved at low stocking density (15 prawns m^2) compared to those stocked at higher stocking density (90 prawns m^2). Some studies showed the increase in stocking densities improved growth performance parameter in gilthead sea bream (Yilmaz and Arabaci, 2010) in sea bass (Sammouth *et al*., 2009) this improved related to the immunological responses and physiological processes, mainly those related to metabolism and behavior (Barcellos *et al*., 2004; Kristiansen *et al*., 2004; Schram *et al*., 2006 and Tan *et al*.,2018). This was suggested to be as a result of increased metabolic cost caused by aggressive behavior at intermediate stocking density, and it was further suggested that aggressive behavior could be suppressed by a further increasing the stocking density. Petit *et al.*, (2001) recorded decreasing SGR with increasing stocking density hence declined growth with increasing stocking density when fish stocked at 5, 10, 15 fish per aquaria. Similar trends were also observed in catfish (*Clarias batrachus*) where there was decreased growth, SGR and survival of larvae stocked at higher densities (3000–5000 m^2) and the decreased growth

was attributed to crowding, which resulted to difficulties in movement of the fish to reach the food, thereby depressing the feeding rate (Sahoo *et al*., 2004).

The result probably due to overcrowding effect for limited living spaces, oxygen depletion, limited surfaces for proper feeding which might cause serious feed competition and nutritional deficits, more energy expenditure and finally increased stress, stunted growth and overwhelming of fish (Chakraborty *et al*., 2010; Moniruzzaman *et al*., 2015)

Mortality is an important indicator of adaptation of fish to the environment. In several studies, high stocking density resulted in injury or death of fish (Ellis, 2002;and Ashley, 2007), In seabream fry survival was strongly affected by stocking density. Results revealed that survival significantly (P<0.05) improved at the densities of 10 and 20 fry/L[3]. Cannibalism was identified as the most important factor inducing mortality during this stage. Sea bass post-larvae exhibited two types of cannibalism: (I) attack and ingestion from the tail and (II) attack and whole ingestion from head. At the beginning of the experiment, most of the cannibalized fish were damaged in the caudal fin and a few days later, they were half eaten from the tail to the body. In the last week of the experiment, as the differences in size increased, prey were caught from the head and completely ingested and digested by the largest fish of each population. The shift from the first to the second type was achieved when the predators reached a capable size and capture ability to consume their prey's head. Once begun, cannibalism continued even after food was given. Two types of cannibalism were also distinguished during the early life stage of African catfish *Clarias gariepinus* (Baras *et al*., 1999). The high survival observed at lower densities in other studies were linked to availability of more space and food (Narejo *et al*., 2005), optimal water quality and less competition whereas incidence of mortality at higher densities were attributed to behavioural changes, rapid spread of virulent pathogens (Cruz and Ridha, 1991), stress, as a result of competition for food and space (Akinwole *et al*., 2014), decreased water quality due to increased biomass (Ronald *et al*., 2014), poor handling (Vera Cruz and Mair, 1994) and deterioration of health due to positive interaction of stressful factors (Barton and Iwama, 1991).

Feed utilization:

Results of feed utilization parameters are shown in Table (4). The results showed insignificant (P< 0.05) were obtained in feed intake, FCR, PER, and FE. Results of feed utilization parameters were highest with stocking density 30 fry/L[3] compared with other stocking density10. 20 and 40 fry/L[3]. Results showed that feed intake (g/fish) increase with increasing stocking densities. The FCR was 1.70, 1.80 1.60 and 2.4 for stocking density 10, 20, 30 and 40 fry/L[3], respectively. These results indicated that the best FCR was recorded with stocking density 30 fry/m^{3}. This result indicates probably low density stocked fishes might have high efficiency to convert given feed to flesh than fish stocked with high density in terms of growth, (Abou *et al*.,2007 and Moniruzzaman *et al*.,2015)**.**

Body composition:

At the end of the experiment, body moisture content was not different between the three stocking studies revealed that differences in stocking density may cause differences in body composition. Stocking density also did not affect the crude protein and moisture content (P <0.05). But ash content was

Table (4): Effect of stocking density on body composition of Sea bream fry at the end of experimental period (60 days).

Stocking densities fry/L[3]	10	20	30	40
Moisture (%)	73.35±0.20	73.94 ±0.20	73.90±0.20	74.21 ±0.20
Total lipid (%)	7.17 ±0.20[a]	5.48 ±0.20[b]	5.40±0.20[b]	5.79 ±0.20[b]
Ash (%)	2.62±0.20[c]	3.56 ±0.20[b]	4.50±0.20[a]	3.60±0.20[b]
Crude protein(%)	16.86 ±0.20	16.66 ±0.20	16.20±0.20	16.40 ±0.20

Mean plus SEM In each row, values with the different letters are significantly different (P < 0.05).

significantly highest (P<0.05) in density 30 fry/L[3] than the rest of other stocking density (10. 20 and 40Fry/L[3]) groups. In contrast, total lipid was significantly highest (P<0.05) in density 10 fry/L[3] than the rest of experimental groups (20, 30 and 40 fry/L[3]) (Table 4). Piccolo, *et al*., (2008) found that the moisture, crude protein and ash contents of sole muscle were not influenced by stocking densities while the lipid content was higher in the low density group. In gilthead sea bream (*Sparus aurata*), lipid contents was significantly influenced by stocking density, the total lipid was significantly (P<0.05) lower in 20, 30and 40 fry/L[3] than that density 10 fry/L[3] group. The possible reason might be due to over

expenditure of body energy for maintaining normal metabolic activity by the fish during the experimental period (Moniruzzaman *et al*., 2015).This reduction seems to be related to an increasing lipid mobilization to cope with the increasing energy demand caused by the crowding environment.

CONCLUSION

It could be concluded that stocking density 30fry/L^3 was the best growth performance and feed utilization under experimental conditions.

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تاثير كثافة التخزين على النمو و تحويل الغذاء فى اسماك الدنيس

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يعتبر كثافة التخزين موضوعًا ذا أولوية في بحوث الاستزراع المائي بسبب تأثيره على حيوية الأسماك المستزرعة والحاجة إلى توصيات مستقبلية تحكم إدارة كثافة المخزون في المزارع السمكية. كان الهدف من هذه الدراسة هو تقييم تأثير كثافة التخزين (10 ، 20 ، 30 ، 40 L / fry) على أداء النمو ، وتحويل الغذاء ومعدل البقاء على قيد الحياة لزريعة الدنيس Seabream (*Sparus aurata*). اظهرت النتائج أن مجموعة الزريعة المخزنة بكثافة 30 زريعة / L3 اعطت أعلى وزن نهائي ، وزن مكتسب ، وزن يومى مكتسب ، معدل نمو نوعى واختلافات ضئيلة في معدل البقاء. وكانت معايير أداء النمو هي الأعلى مع كثافة التخزين 30 زريعة / L3 مقارنة بكثافة التخزين (10 و 20 و 40 زريعة / لتر 3) ، على التوالي. اشارت النتائج إلى أن أفضل مجموعة من معدل التحويل الغذائى ، معدل الاستفادة من البروتين و الاستفادة من الغذاء كانت فى الكثافة 30 زريعة / L3 عن بقية المجموعات التجريبية. كذلك لم تؤثر كثافة المخزون على البروتين الخام ومحتوى الرطوبة في تركيبة الجسم. لكن محتوى الرماد كان أعلى بكثير في كثافة 30 زريعة / L3 عن بقية المجموعات الأخرى ذات الكثافة السمكية (10و20 و 40 L3 / fry). في المقابل ، كان محتوى الدهون أعلى بكثير في كثافة 10 زريعة / L3 عن بقية المجموعات التجريبية (20 و 30 و 40 زريعة / L3). من النتائج المذكورة أعلاه ، يمكن الاستنتاج أن كثافة التخزين 30 زريعة / L3 اعطت أفضل أداء نمو و تحويل غذاء في ظل ظروف التجربة.