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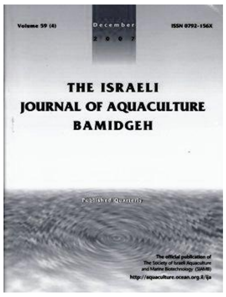

Effects of Intensive and Semi-Intensive Rearing on Growth, Survival, and V-Shaped (Lordotic) Skeletal Deformities in Juvenile Gilthead Sea Bream (*Sparus aurata*)

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Key words: *Sparus aurata*, rearing method, growth, survival, lordosis

Abstract

Intensive production can reduce production costs and improve efficiency of larvae culture. This study compared the effects of intensive and semi-intensive production on growth, survival, and V-shaped skeletal deformities in juvenile gilthead sea bream (*Sparus aurata*). At the end of the 50-day study, survival was 68.01±0.8%, mean weight was 1.32±0.03 g, and lordosis was 32% among juveniles raised in the intensive system. Survival and mean weight were significantly higher, 92.06±1.1% and 1.61±0.02 g, respectively, among juveniles raised in the semi-intensive system, and none had lordosis (p<0.05). Results suggest that semi-intensive systems are more advantageous than intensive for rearing sea bream juveniles.

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Introduction

Gilthead sea bream (*Sparus aurata*) were one of the first intensively cultivated species in the Mediterranean area (FAO, 2005). However, the market price of gilthead sea bream is dropping due to overproduction, forcing the aquaculture industry to reduce production costs and improve larvae rearing efficiency.

In a wide range of marine species, the use of semi-intensive technology for larvae rearing results in higher survival and growth performance than intensive technology (Roo et al., 2005, 2010a). Semi-intensive larvae rearing is used in the Mediterranean area to improve the quality of juvenile gilthead sea bream (Divanach and Kentouri, 2000; Boglione et al., 2003; Roo et al., 2010b; Russo et al., 2010). However, skeletal malformations and their incidence affect the fish farmer's production costs (Russo et al., 2010). In some cases, 15-50% of a gilthead sea bream juvenile population are culled at the end of the hatchery phase because of deformities (Boglione et al., 2001).

Among vertebral column disorders, V-shaped deformation (lordosis) is one of the most severe (Eissa et al., 2009). Identification of single causative factors for the onset of skeletal anomalies is difficult as there are many possible factors: forced swimming, stress from stocking density, bacteria load, predation, lack of food, competition, environmental conditions, inadequate food quality, lack of space, and handling practices (Russo et al., 2010). The fact that hatchery-reared fish show a higher incidence of these abnormalities than wild-caught fish strongly suggests an environmental component related to rearing conditions (Mana and Kawamura, 2002).

In some cases, intensification of the rearing system appears to increase the incidence of skeletal deformities (Boglione et al., 2001; Koumoundouros et al., 2001; Roo et al., 2005; Roo et al., 2010a). Thus, study of the type of rearing system seems to be a good method for integrating many and different types of information and obtaining complete, clear, and rapid results from data analysis (Russo et al., 2010). The hypothesis in the present study is that growth, survival, V-shaped skeletal deformation (lordosis), and welfare of gilthead sea bream juveniles may be improved by using semi-intensive rather than intensive methods.

Materials and Methods

Experimental conditions. The study was carried out in the commercial hatchery of Hunkar Sea Products Inc., Turkey, in 2007. All specimens originated from the commercial sea bream hatchery at Akvatek Sea Products Inc., Izmir. No hormonal treatment was applied to the broodstock. Maturation and spawning occurred spontaneously under the natural photoperiod and temperature.

Eggs were distributed (100 eggs/l) into 12-m^3 cylindrical tanks connected to a recirculation unit (Carbo et al., 2003). During the larvae stages, water conditions were 18-19°C, 40 ppt salinity, pH 7.7-8.3, and 20% daily water exchange, with gentle aeration and oxygenation. The photoperiod was 12L:12D and the light intensity at the water surface was 500 lx. The surface was kept free of lipid film, as required for good swim bladder inflation, using a skimmer (Chatain and Ounais-Guschemann, 1990) and normal swim bladder inflation was achieved in 83% of the larvae. Larvae were fed enriched rotifers, *Brachionus plicatilis* (Selco, INVE S.A., Belgium), three times per day from day 4 post-hatch (dph) to 20 dph. Rotifer density was progressively increased from 5 to 10 rotifers/ml. *Artemia* nauplii (EG, INVE, Belgium) were offered four times per day from 16 to 22 dph in increasing density from 0.5 to 2 nauplii/ml, and enriched metanauplii were offered from 20 to 40 dph at 1 to 5 metanauplii/ml.

The nursery phases were conducted in the same tanks with open sea water circulation, under intensive conditions, and with progressive transition from live to inert food (Proton, INVE, Belgium) that was manually distributed *ad libitum* from 36 to 60 dph. At weaning (60 dph), fish were divided into two groups of 60,000 fish by a fish counter (Type Micro, Impex Agency, Hoerning, Denmark). One group was raised in intensive conditions in three replicate 12-m^3 outdoor tanks at an initial density of 5 fish/l; the second group was raised in semi-intensive conditions in three replicate 400-m^3 outdoor tanks at an initial density of 0.15 fish/l. Both groups were raised in water with 40%

salinity, 80-100% oxygen saturation, and the natural photoperiod. Temperature in the intensive treatment was 18.5±0.4°C and in the semi-intensive 18.8±0.9°C.

Growth, survival, and radiographic analysis. At the beginning and end of the experiment, 1000 specimens from each group were measured to determine mean total length (cm±SE) and mean wet weight (g±SE). In this way, growth rates were calculated. Condition factor was calculated as CF = 100(L × W^3) and length-weight relationships were calculated by the exponential regression equation W = aL^b, where W is the total weight and L is the total length (Ricker, 1975).

Daily mortality was estimated by counting dead fish removed from the rearing units during surface and bottom cleaning (Hatziathanasiou et al., 2002). The initial and final numbers of fish were determined by weighing (Ohaus, Pa 214 C Model) and the formula: total fish wet wt/mean fish wt. In addition, they were counted by a TPS Fish Counter. Survival was calculated as 100(initial no. of fish - final no. fish/initial no. fish).

Every week during the juvenile stage (61-110 dph), at least 30 individuals from every tank were sampled with a dip net and anesthetized with 0.2-0.5/ml phenoxyethanol (ethylenglycol-monophenyl ether, Merck). The juveniles were radiographed by X-ray (GE, Silhouette VR 500 MA 150 KV, Fuji, 40 KV, 1.25 mA/s, 50 mA, 25 ms) to assess the degree of lordosis (Koumoundouros et al., 2001). Lordosis was categorized into two groups: (a) light lordosis where fish showed 4-8 deformed shortened vertebrae (especially on the dorsal side), sometimes fused, with twisted neural and hemal spines, and (b) severe lordosis where fish were heavily affected with 5-8 deformed, shortened, and often fused vertebrae with twisted neural and hemal spines (adapted from Divanach et al., 1997). In cases of morphological abnormality or lesions indicating cannibalism, photos were taken of the live or dead fish under an Olympus CX21 stereoscope with an Olympus C-500 camera (adapted from Hatziathanasiou et al., 2002). In addition, the specimens were measured to determine the length-weight relationship.

Statistical analysis. Results are given as means±standard error of the mean (SEM). Data on survival and incidence of skeletal deformities are expressed as percentages and were compared by Fisher's chi-square test. Statistical comparisons of mean total lengths and wet weights were made by *t* test with SPSS (Norusis, 1993). In all statistical analyses, the level of significant difference was set at $p<0.05$.

Results

Growth. Optimal environmental conditions were maintained throughout the larval, post-larval, and juvenile stages; there were no differences between treatment groups in terms of environmental parameters. At the end of the experiment, mean wet weight and survival were significantly higher in fish reared in the semi-intensive conditions than in those raised in the intensive conditions (Table 1). The increase in mean body length relative to weight is shown in Fig. 1.

Survival and frequency of lordosis. At the end of day 110, the intensively-reared population consisted of 47,200±384 fish, of which 32% had lordotic vertebrae (Fig. 2). The semi-intensively-reared population contained 56,824±495 juveniles of which none had lordotic vertebrae. The number of lordotic individuals increased by day for unknown reasons as did mortality of lordotic fish (Fig. 3).

Table 1. Growth, condition factor, and survival of gilthead sea bream juveniles raised in intensive and semi-intensive conditions.

	Intensive (5 fish/l)		*Semi-intensive (0.15 fish/l)*	
	Initial (61 days)	*Final (110 days)*	*Initial (61 days)*	*Final (110 days)*
Total length (cm)	1.722±0.005	3.544±0.075	1.722±0.004	3.847±0.089
Wet wt (g)	0.13±0.01	1.32±0.03	0.13±0.01	1.61±0.02**
Condition factor	2.543±0.156	2.977±0.229	2.545±0.181	2.847±0.277
Survival (%)	-	68.01±0.8	-	92.06±1.1*

Final values in a row marked by asterisks significantly differ: * $p<0.05$, ** $p<0.01$.

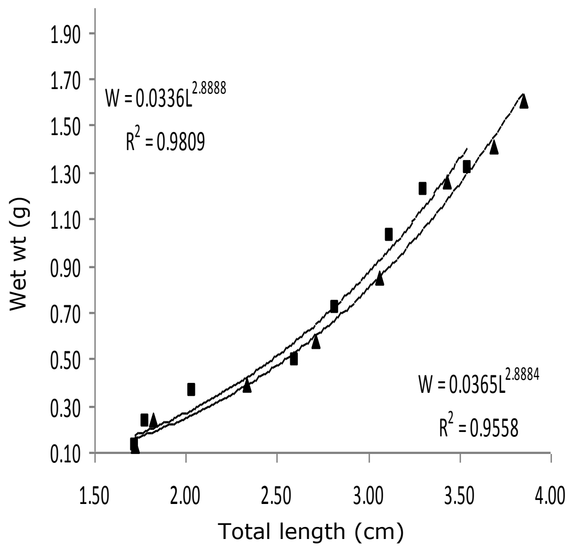

Fig. 1. The length-weight relationship of gilthead sea bream juveniles raised in intensive (■) and semi-intensive (▲) conditions.

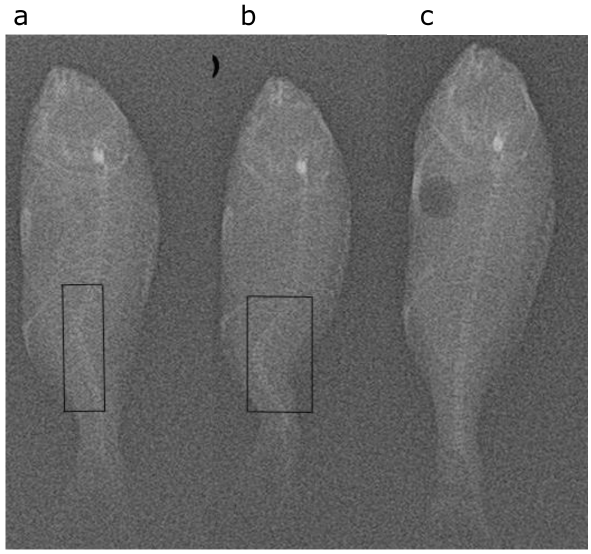

Fig. 2. Gilthead sea bream juveniles with (a) light and (b) severe lordosis, and (c) a normal juvenile.

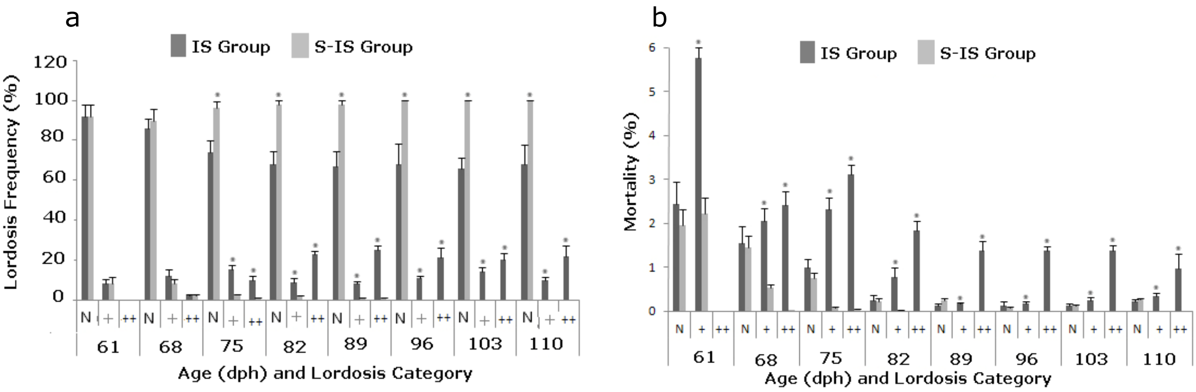

Fig. 3. Weekly percent (a) lordosis and (b) mortality in gilthead sea bream juveniles raised in intensive (IS) and semi-intensive (S-IS) conditions (dph = days post hatching, N = normal, + = light lordosis, ++ = severe lordosis).

Discussion

At the end of the experiment, the difference in body weight between the two groups was significant but the difference in mean total length was insignificant. Survival was significantly better in the semi-intensive group. The high mortalities recorded during the study in the intensive system could be related to other causes, such as unsuccessful use of nutrients under the more stressful rearing conditions or changes in the bacterial community structure of the rearing water (Nakase et al., 2007; Roo et al., 2010b). Higher densities can cause this situation because of higher bacterial bloom (Queiroz and Boyd, 1998; Can et al., 2010). Cannibalism was the most important factor inducing mortality during the juvenile phase in similar studies in other Mediterranean species, i.e., sea bass and red porgy (Kayim et al., 2010; Roo et al., 2010b). Our observations showed that all dead fish removed from rearing units throughout the experimental period were cannibalized, but there was no evidence that fish with skeletal deformities were predated upon by healthy fish before or after they died. On the other hand, the increased mortality during the first days of the experiment might have been due to a lack of adaptation of some individuals to artificial dry food or to stress caused by manipulation of the fish on 61 dph (handling at the beginning of the experiment). Survival was much higher in the semi-intensive group than in the intensive, similar to findings of Boglione et al. (2009)

and Roo et al. (2010b) who reported that survival rates were modulated by rearing conditions and reached the highest value in larger volumes.

The rearing systems were associated with osteological development, in agreement with studies of gilthead sea bream (Faustino, 2002) and red porgy (Roo et al., 2010a). Induction of lordosis has been attributed to increased muscle activity (Chatain and Ounais-Gushumann, 1990), but the mechanistic coupling muscle activity and abnormal vertebral curvature is unclear (Divanach et al., 1997). Increased tail beat frequency and amplitude require increased muscle power, causing an increase in the magnitude of the bending moment and a compressive load on the axis of animals that become lordotic (Kranenbarg et al., 2005). It can therefore be suggested that the direct action of muscles on bones is the causative factor of swimming-induced lordosis (Sfakianakis et al., 2006). Muscle and swimming activity increased with density in captive rainbow trout, *Oncorhynchus mykiss* (Cooke et al., 2000). Under this hypothesis, tank hydrodynamics and conditions are the most important causative factors for hemal lordosis of common type V-shaped skeletal deformities in commercial production farms (Andrades et al., 1996; Divanach et al., 1997). Further, tank hydrodynamics can change as the rearing method regarding fish density changes, and other production conditions such as feeding, lighting, and aeration can impact fish behavior and swimming activity (Lunger et al., 2004).

Food consumption and growth rates fell significantly while individual swimming activity rose as the density increased in Atlantic halibut *(Hippoglossus hippoglossus* L.; Kristiansen et al., 2004). Increased swimming activity (personal observations) might be the cause of social interactions (e.g., competition for food, cannibalism, depending on the rearing method) as reported by Sfakianakis et al. (2006). In the present study, the frequency of skeletal abnormalities was higher in the intensive system and similar to those reported for *S. aurata* (Boglione et al., 2001), *Diplodus puntazzo* and *Pagellus erythinus* (Boglione et al., 2003), and sea bass (Sfakianakis et al., 2006). Deformities in semi-intensive reared fish were rare in comparison with other species produced by this system (Roo et al., 2005, 2010a), perhaps because the rearing density in our study was lower.

In general, high density conditions may increase swimming activity and behavioral interactions between fish, leading to a rise in energetic expenditure to levels that could be detrimental to physiological processes. Particularly, higher swimming activity can result in higher anaerobic metabolism, which represents reserve energy used in stress situations (Lembo et al., 2007). Abnormal calcification of lordotic vertebrae can be an adaptive response to increased swimming activity or to local changes in curvature due to buckling (Kranenbarg et al., 2005). The starter factor may be stressful rearing conditions and all factors mentioned above are related. Likewise, growth and survival may have been influenced by stressful environmental conditions involved in intensification in the present study.

We compared semi-intensive and intensive rearing of juvenile sea bream in terms of growth, survival, and skeletal deformities. Semi-intensive conditions are probably closer to or even more feasible than those in the natural environment. The severe deformities were the main discriminating characteristic between fish reared in the two rearing methods, as shown by Boglione et al. (2009).

In conclusion, the rearing method may affect growth, survival, and lordosis in commercial production of *Sparus aurata*. The juvenile stages are crucial phases during which major efforts in intensification should be made to increase growth and prevent mortality and occurrence of V-shaped skeletal deformities. Further research is needed in other species.

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