View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Aquatic Commons

Morphological malformations of the sea bass (*Dicentrarchus labrax*): Comparison between hormone injected and non-injected fish

Tan S.[1]; Bulut M.[1]; Bilen S.[2*]

Received: April 2013 Accepted: July 2014

Abstract

In this study, fertilization, hatching and deformation rate of the eggs, and larvae obtained from hormone injected (HI) and non-injected broodstock (NHI), were determined. In the experiment, no differences were observed in the groups' fertilization rates (p>0.05). Hatching rate was found higher in the HI group (p<0.05). During the experiment, malformations such as spinal fluid accumulation, saddleback, air-bladder deformation and general body deformations were observed in both groups. For the NHI group, saddleback rate was found 5.13±0.55 %, air-bladder deformation rate was found 1.43±0.13%, spinal fluid accumulation was found 2.33±1.33% and general body deformation rate was found 3.76±0.23 % (p<0.05). As a result, it was determined that the deformation rate increased with hormone injection (p<0.05).

Keywords: Sea bass, Egg, Embryonic development, Expansion rates, Deformation

1-Çanakkale Onsekiz Mart University, faculty of fisheries. Canakkale. Turkey.
2-Kastamonu University, Inebolu faculty of fisheries. Kastamonu. Turkey.
*Corresponding author's email: sbilen@kastamonu.edu.tr

Introduction

The variety of cultured finfish species (Karakaş and Türkoğlu, 2005) and their production rate have been increased in Turkey in the past twenty years (Tuik, 2012). In cultured finfish, especially in hatcheries, some parameters such as production rate, survival rate, fertilization rate and larval quality are important factors that influence the quality of production both in quantitative and qualitative terms. Morphological abnormalities are common problems for reared sea bass and sea bream (Divanach *et al*., 1996). Many malformations may concern different aspects of the morphology of the fish such as skeleton and swim bladder (Afonso and Roo, 2007).

There are many parameters influencing malformations such as environmental factors, diet components, genetic factors or their interaction, nutritional imbalances including tryptophan, essential fatty acids, vitamin C or vitamin D (Akiyama *et al*., 1987; Hinton *et al*., 1992; Divanach *et al*., 1996; Cahu *et al*., 2003; Sfakianakis *et al*., 2006). Malformation rates are also influenced by water temperature, density of eggs, heavy metals contamination or hydrodynamics during development (Faustino and Power, 1999; Kranenbarg *et al*., 2005; Sfakianakis *et al*., 2004, 2006).

Scoliosis, lordosis and lack of operculum are some of the mostly encountered problems in sea bass larvae and fingerling culture in Turkey. Beraldo *et al*. (2003) reported that lordosis and lack of operculum occurred in some reared populations at the rate of 80% of the individuals. As mentioned above, there are many studies which have already been done on the reasons of malformation in fishes except hormone injection. To the best of our knowledge there is no recorded data regarding the effects of hormone injection on larvae and juvenile malformation. Therefore, the aim of this study was to determine the effects of hormone induced spawning on the deformation of larvae and juveniles of sea bass.

Material and Methods

Broodstocks

All the broodstocks (wild caught) used in this study were obtained from different districts of the Marmara Sea. After capture the fishes were transferred in rearing tanks for acclimatization at a density of3 kg/m^3 and two females for one male.

Hormon injection, spawning and incubation

Two groups of broodstock fish were randomly collected. One group received LH-RH hormone at the dosage of 10 µg/ kg fish (Barnabé and Paris, 1984) and the other group was used as the control group. Incubators of plankton net (600 µ mesh size) were prepared and placed in a semi-cylindrical conical tank with4 m^3 capacity. Then 1200 g eggs from the both groups were weighed and equally divided and placed in three replicates of incubators in the hatchery tanks. During the incubation, the embryologic development, temperature, dissolved

oxygen (DO) concentration and the salinity were measured and, illumination was applied. Temperature, dissolved oxygen concentration and salinity were measured every 2 hours. The eggs were incubated at 17±1°C, and the DO concentration and the salinity were in the range of 5-7 mg/L and 38- 40‰, respectively. After hatching, larvae and juvenile fishes were sampled every 10 days for a period of 120 days and the malformation rate in the larvae and the juveniles were determined. The DO and temperature were measured by using probe (Handy Polaris). The protocol shown in Table 1 was used during the trial.

Table 1: Oxygen and temperature level of the tanks during experiment.

Days	Temperature (°C)	Oxygen (mg/L)
1-10	11.4 ± 1	7.2 ± 0.1
11-20	16 ± 1	8.1 ± 0.1
21-90	18 ± 1	7.3± 0.1
91-121	20 ± 1	7.4± 0.1

Tanks

During the experiment different types of water tanks were used according to the fish larval development progress. During the first 20 days of the study 6 semi-cylindrical fiberglass tanks with a capacity of 4 m^3 and set up with net of 600 µ mesh were used. In order to prevent larval stress the hatching was done in tanks that were black in color. After 21th day of the study the fishes were transferred to 6 cylindrical fiberglass tanks with a capacity of 12 m^3. On 55th day of the study the fishes were transferred to 20 m^3 tanks and kept there untill the end of the study.

Feeding

The wild caught broodstock fish were first fed a fresh wet food and were gradually acclimatized to pellet feed. After acclimatization and one month prior to the reproduction, fish were fed with nutritionally enriched feed. Seven days after hatching the larvae were fed with artemia and from the 35th day artemia with powdered compound feed were used. After the 50th day, the larvae were fed with only powdered compound feed.

Deformation

Determinationof deformation during the larval development period was done according to Boglione *et al*. (2001).

Statistical analysis

Analyses were conducted using one-way ANOVA. For the multiple comparison of the means, Duncan's multiple range was used (Zar, 1999). All results were regarded to be significant at the p>0.05 level.

Results

During the study the temperature and DO concentration were kept under control and were in the ranges as given in Table 2.

Table 2: Fertilization and survival rate of the experimental groups.

	Normal	Hormon Injected
Fertilization (%)	95.7 ± 0.02	91.3 ± 0.05
Survival (%)	60.3 ± 2.02*	51 ± 2.64

The fertilization rate for the hormone injection group (HI) was 91.3±0.05%, while for the non hormone injection group (NHI) was 95.7±0.02%, and there was no statistical differences between the groups (p>0.05). At the end of the trial the survival rate was found to be higher for the NHI group (p<0.05) (Table 3).

Table 3: Deformation rates in the experimental groups.

	% Malformation Rate	
Malformation Type	**Normal**	**Hormon Injected**
Scoliosis	2.4 ± 0.47 *	5.13 ± 0.55
Air bladder	0.53 ± 0.67 *	1.43 ± 0.13
Body Form	1.36 ± 0.67 *	3.76 ± 0.23
Vertebral Fluid	1.56 ± 1.33 *	2.33 ± 1.33
Total	6 ± 0.57 *	17.66 ± 1.45

The overall deformation rates in the larvae and the juveniles were found to be higher for the HI group. Considering the deformation types, deformations due to spinal deformities were higher in the HI group (p<0.05). Abnormalities such as air-bladder deformation, spinal fluid accumulation and general body deformation were found to be higher in the HI group (p<0.05).

Discussion

In this study, the embryologic development, hatching rate, survival rate and deformation rates during larval and juvenile developmental stages of the eggs obtained by natural fertilization and hormone injection of the sea bass broodstocks, fed under controlled conditions were compared and the observed deformation types were examined. At the end of the study, it was found out that the deformation rate in the eggs obtained by the hormone injection was higher and the hormone application negatively affected the eggs and larval quality.

In this study, deformations such as air-bladder deformation, saddleback deformation, body form deformation, fin erosion and mandible deformation were observed. The deformation rates were found to increase especially after the 50th day of hatching. Similar to our results, Afonso *et al*. (2000), reported that the rate of vertebral deformities increased in 56 days old larvae. In some studies, most abnormalities such as lordosis (V shape spinal formation), scoliosis (lateral deformity), and sometimes both of these deformities together were observed (Paperna, 1978). Boglione *et al*. (2001) suggested that there is a relation between the fish body deformation rates and improper environmental conditions at which the fish are cultured. According to Sfakianakis *et al*. (2006), developmental temperature has a significant effect on the incidence and severity of haemal lordosis in *D. labrax*. Nevertheless, in our study, the water quality was kept at optimum for both of the experimental groups. Thereby, the higher deformation rates in fish injected with hormone might be mainly due to the hormone application.

The assessment of fry quality has focused mainly on the appearance of malpigmentation in flatfish, a functional swim bladder or osteological and morphological deformities scoliosis, lordosis mainly in the sea bass or the sea bream (Planas and Cunha, 1999). Sea

bass and sea bream were affected mainly the absence of swim bladders (Paperna, 1978; Daoulas *et al*., 1991; Chatain, 1994). There is a direct relationship between skeletal deformities lordosis, scoliosis and uninflated swim bladders (Kitajima *et al*., 1981). However, in this study the air-bladder deformation rate was found to be quite low.

According to Divanach *et al*. (1996), skeletal deformities can be environmentally induced in two ways: a) by neuromuscular effects, which lead to deformities without a chemical change in the vertebral column, or b) by alteration of biological processes necessary for maintaining the biochemical integrity of bones. Hitherto, malformations due to hormone applications have not been studied in detail. According to the results of the present study, it can be suggested that the hormone induced spawning might be one of the reasons influencing the deformations in fish.

References

Afonso, J.M., Montero, D., Robaina, L., Astorga, N., Izquierdo, M.S. and Gines, R., 2000. Association of a lordosis–scoliosis–kyphosis deformity in gilthead seabream (*Sparus aurata*) with family structure. *Fish Physiology and Biochemistry*, 22, 159–163.

Afonso, J.M. and Roo, F.J., 2007. Anomalías morfológicas en peces cultivados: heredabilidad y selección P. Martínez, A. Figueras (Eds.), Genética y genómica en acuicultura, C.S.I.C., Madrid. pp. 213–240.

Akiyama, T., Ishida, J., Nakagawa, S., Ogawara, H., Watanabe, S., Itoh, N., Shibuya, M. and Fukami, Y., 1987. Genistein, a specific inhibitor of tyrosine-specific protein kinases. *The Journal of Biological Chemistry*, 262(**12**), 5592-5595.

Barnabe, G. and Paris, J., 1984. Ponte avancée et ponte normale du loup *Dicentrarchus labrax* (L.) à la Station de Biologie marine et lagunaire de Sète G. Barnabé, R. Billard (Eds.), L'Aquaculture du bar et des Sparides, INRA Publications, Paris, France. pp. 63–72

Beraldo, P., Pinosa, M., Tibaldi, E. and Canavese, B., 2003. Abnormalities of the operculum in gilthead sea bream (*Sparus aurata*): morphological description. *Aquaculture*, 220, 89–99.

Boglione, C., Gagliardi, F., Scardi, M. and Cataudella, S., 2001. Skeletal descriptors and quality assessment in larvae and post-larvae of wild-caught and hatchery-reared gilthead sea bream (*Sparus aurata* L. 1758). *Aquaculture*, 192(**1**), 1–22.

Cahu, C., Infante, J.Z. and Takeuchi, T., 2003. Nutritional components affecting skeletal development in fish larvae. *Aquaculture*, 227, 245–258.

Chatain, B., 1994. Abnormal swimbladder development and lordosis in sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus auratus*). *Aquaculture*, 119, 371–379.

Daoulas, C., Economou, A.N. and Bantavas, I., 1991. Osteological abnormalities in laboratory reared sea-bass (*Dicentrarchus labrax*)

fingerlings. *Aquaculture*, 97, 169–180.

Divanach, P., Boglione, C., Menu, B., Koumoudouros, G., Kentouri, M. and Cataudella, S., 1996. Abnormalities in finfish mariculture: an overview of the problem, causes and solutions. In: Chantain, B., Saroglia, M., Sweetman, J., Lavens, P. (Eds.). Seabass and seabream culture: problem and prospects. International Workshop. Verona, Italy. October 16-18, 1996. European Aquacultural Society, Oostende, Belgium, 21P.

Faustino, M. and Power, D.M., 1999. Development of the pectoral, pelvic, dorsal and anal fins in cultured sea bream. *Journal of Fish Biology*, 54(**5**), 1094–1110.

Hinton, D.E., Baumann, P.C., Gardner, G.R., Hawkins, W.E., Hendricks, J.D., Murchelano, R.A. and Okihiro, M.S. 1992. Histopathological biomarkers, In: Huggett, R.J., Kimerle, R.A., Mehrle, P.M., Jr Bergman, H.L., (Eds.), Biomarkers: Biochemical, physiological, and histological markers of anthropogenic stress, Lewis, USA. pp. 155–196.

Karakaş, H.H. and Türkoğlu, H., 2005. Su Ürünlerinin Dünyada ve Türkiye'deki Durumu. *Harran Üniversitesi Ziraat Fakültesi Dergisi*, 3, 21-28.

Kitajima, C., Tsukashima, Y., Fujita, S., Watanabe, T. and Yone, Y., 1981. Relationship between uninflated swimbladder and lordotic deformity in hatchery-reared red sea bream *Pagrus major* . *Bulletin of the Japanese Society for the Science of Fish*, 47(**10**), 1289–1294.

Kranenbarg, S., Waarsing, E., Muller, M., Weinans, H. and Leeuwen, L.J., 2005. Lordotic vertebrae in sea bass (*Dicentrarchus labrax* L.) are adapted to increased loads. *Journal of Biomechanics*, 38, 1239–1246.

Paperna, I., 1978. Swimbladder and skeletal deformations in hatchery bred *Sparus aurata*. *Journal of Fish Biology*, 12, 109–114.

Planas, M. and Cunha, I., 1999. Larviculture of marine fish: problems and perspectives. *Aquaculture*, 177, 171–190.

Sfakianakis, D.G., Koumoundouros, G., Divanach, P. and Kentouri, M., 2004. Osteological development of the vertebral column and of the fins in *Pagellus erythrinus* (L. 1758). Temperature effect on the developmental plasticity and morpho-anatomical abnormalities. *Aquaculture*, 232, 407–424.

Sfakianakis, D.G., Georgakopoulou, E., Papadakis, I.E., Divanach, P., Kentouri, M. and Koumoundouros, G., 2006. Environmental determinants of haemal lordosis in European sea bass, *Dicentrarchus labrax* (Linnaeus, 1758). *Aquaculture*, 254(**1–4**), 54–64.

Tuik, 2012. Fishery statistics. Turkish Statistical Institude. Ankara. 71P.

Zar, H.J., 1999. Biostatistical analysis. Prentice Hall (Upper Saddle River, N.J.) 4th Edition. 929P.