Effect of the geographical origin on the presence of skeletal deformities and swimbladder anomaly in a cultured gilthead sea bream (*Sparus aurata* L) population

Estimation of genetic parameters

M. García-Celdrán[(1)], Z. Cutákova [(1)], G. Ramis [(2)], E. Armero[(1)]

(1) Department of Agricultural Science and Technology. Technical University of Cartagena, Paseo Alfonso XIII 48, 30203 Cartagena, España. martanietos@hotmail.com

(2) Departamento de Producción Animal. Facultad de Veterinaria. Universidad de Murcia. Campus de Espinardo. 3100 Murcia, España

ABSTRACT

The gilthead sea bream (Sparus aurata L.) is one of the most important Sparid farmed in Europe. However, abnormalities such us lordosis, lack of operculum and failure to inflate the swimbladder constitute a major problem for aquaculture industry by decreasing the final value of the fish. The use of stocks from different genetic origin to produce commercial fish usually cause great variability in the global quality of the end product. However, strategies that involve the development of selection schemes in sea bream for these traits of economic interest are scarce. In light of these circumstances, the effect of the geographical origin on the presence of anomalies as a measure of fish quality was analyzed. For this purpose a population of farmed gilthead sea bream obtained from broodstock from three differentiated geographical origin and reared under common conditions was analyzed from X-ray photograph for the mayor anomalies present in this species. A posteriori reconstruction of pedigree was carried out in order to determine genetic parameters for fish anomalies. An effect of the geographical origin on the presence of skeletal malformation and on the lack of functional swimbladder was found, being lordosis and lack of operculum the most common skeletal deformities. Heritabilities were medium for lack of operculum and lack of functional swimbladder and high for lordosis. A medium genetic correlation and a positive phenotypic correlation were observed between the lack of functional swimbladder and lordosis. All these findings could be relevant for the establishment of successful breeding programs in aquaculture of this species

Keywords: Heritabilities, genetic correlations, phenotypic correlations, lordosis and lack of operculum.

1. Introduction

The gilthead sea bream (*Sparus aurata* L.) is one of the most important Sparid farmed in Europe. The main producers of this species are Greece (39.6%), Turkey (22.5%) and Spain (11.2%). Five Spanish regions share the 100% of gilthead sea bream production; being Valencia (40.8%) and Murcia (20.5%) the most important producers [1]

Despite the growth and consolidation of the gilthead sea bream industry the high level of body abnormalities appearing in hatchery fish is an important problem for the development of this industry. This is often associated with growth depression, leading to high mortality rates [2]. Their appearance downgrades the biological performance, the marketing image, the production cost and the commercial value of the

reared fish since they are clearly evident, especially in those species such sea bream which are sold mainly as whole fish [3]. The main morphological abnormalities in fish can be grouped into five main categories depending on the aspect involved: shape, pigmentation, scales, skeleton, and swimbladder (SB) [4].

Several factors are believed to be the basis of them: Nutritional (vitamin A, C, D or tryptophan imbalances), environmental (water temperature, light, salinity, oxygen, density of eggs, heavy metal contamination), hydrodynamic conditions and genetic factor or their interaction [2,5].

Strategies that involve the development of selection schemes in gilthead sea bream for these traits of economic interest commented above are scarce especially due to the biological

characteristic of this species. Gilthead sea bream is a protandrous hermaphroditic mass-spawing species in which individuals are males during the firth two years of life and then gradually become females. Mass spawning prevents knowing the genealogy of fish under culture conditions which is essential to estimate genetic parameters and to introduce selection programs. The use of molecular markers such us microsatellites are a useful tool for addressing these matters [5].

The use of different rearing systems to produce commercial fish as well as the different genetic origin of the stocks usually cause great variability of the production i.e. in the growth rates and the global quality of the end product [6]**.** In light of these circumstances the goals of this research were to study the effect of the geographical origin on the presence of the major anomalies present in gilt head sea bream (spine and cranial deformities and lack of functional SB) as a measure of fish quality and to estimate the genetic basis of these anomalies in order to be included in future breeding program.

2. Materials and Methods

2.1 Broodstocks and offspring

Samples of sea bream were captured from wild populations from three geographically differentiated origins; Cantabrian Sea (CAN), the Atlantic Ocean (ATL), and Mediterranean Sea (MED). From these samples, three broodstock were established and maintained until their spawning season. Larvae were obtained from these mass-spawning. At 84 days post-hatching a random sample of 2.500 descendents were taken to the on-growing facilities of the Centro de Cultivos Marinos de la Región de Murcia (San Pedro del Pinatar, Murcia, Spain). In this sample was proportionally represented the original geographical origins. Fish were individually tagged in the abdominal cavity with a Passive Integrated Transporter (PIT; Trovan Daimler–Benz), and distributed in 12 tanks of 850-litres in which they were reared under communal conditions. At 185 days post-hatching a random sample of about 300 juveniles from each geographical origin was slaughter by immersion in ice cold water. In that way, near to 1.000 samples were obtained which were placed on their left side in PVC boxes (12.2 x 12.2 cm) and frozen at -20ºC until anomalies determination. Fish were radiographed and the X-ray pictures were used for the skeleton and SB examination. Skeletal abnormalities in the vertebral column were identified and classified according to a malformations catalog. The lack of left operculum was evaluated by visual inspection

2.2 PCR reaction Genotyping and Parental assignments

Animals were genetically characterized. For this purpose, DNA was extracted from the caudal fin (DNeasy kit QIAGEN®). DNA quality and quantity were determined using Nanodrop 2.000 spectrophotometer v.3.7 (Thermo Fisher Scientific, Wilmington, U.S.A.). We used the SMsa1 (Super Multiplex *Sparus aurata*) [7]. Electropherograms and genotypes were analysed using GeneMapper software (Applied Biosystem®). Parental assignments between breeder and their descendents were determined *a posteriori* using their genotypes in the exclusion method by Vitassing software.

2.3. Analysis data

Deformity and SB scores (0 or 1) for each individual and each type of deformity were analyzed by logistic regression, using the SPSS® software (SPSS, Chicago, IL, USA) to detect the effects of the origin of the fish. For the heritability calculation, malformation was considered as a threshold trait, and was first analysed on the observed scale (0=Normal, 1= Deformed) using VCE software with a univariate animal model:

$$Y = X\beta + Za + \varepsilon$$

where Y is the vector of observations (malformation), β is the vector of fixed effects (origin), a is the vector of random additive genetic effects and ε the vector of random residual effects. Then, heritability estimates were transformed to the underlying liability scale [8].

3. Results and Discussion

The presence of deformities is the second most economically important trait for the industrial production of sea bream [4]. The incidence of fish scored as deformed was lower in juveniles from CAN in which a 23.4% of examined fish had at least one or more kinds of deformities while

this incidence was higher (near to 40%) in fish from ATL and MED (Table 1).

Lordosis was the most common deformity, with frequencies around 20% in two of the studied origin, followed by vertebral shortening (Table 1). Several factors may cause deformities. An effect of the geographical origin was observed in the

present research as well. Individuals from CAN showed lower incidence of lordosis than those from ATL and MED. Other minor skeletal deformities such as vertebral fusion (Fig.1) and kyphosis were also detected although no effect of the geographical origin was found for them and their incidence was very low (<3%).

Lack of operculum was the cranial deformity most frequent in all origins although no statistical differences were detected (Table 1). The premaxilla was the only one cranial abnormality observed in the jaw (Fig. 1) and an effect of the geographical origin was found. Individuals from CAN showed the highest frequency followed by those from MED. Cranial skeletal deformities in the jaw were not observed in fish from ATL.

Individuals from ATL showed the highest frequency (>70%) of fish with an uninflated SB (Fig. 1) followed by those from MED and CAN.

Scarce and conflicting results have been found about genetic parameters in sea bream. Heritabilities (table 2) were found to be medium for lack of operculum and uninflated SB and high for lordosis in agreement with [2]. Genetic correlations among skeletal malformation in the column were high. The genetic correlations between malformation in the column and in the jaw were found to be very imprecise. The highest and positive phenotypic correlation was found between lordosis and lack of functional SB.

4. Conclusions

We observed an effect of the geographical origin on the presence of fish deformities which constitutes relevant information for the establishment of the broodstock to produce commercial fish. High heritability for lordosis and medium for lack of operculum and functional SB, which are the most common anormalities in this species, were observed. Moreover, a medium genetic correlation and positive phenotypic correlation was found between the lack of functional swimbladder and lordosis. All this findings could be relevant for the establishment of successful breeding programs in aquaculture of this species

5. Acknowledgments

6. References

[1] APROMAR, 2012. Fish marine aquaculture in Spain (http://www.apromar.es)

[2] Andrades, J.A., Becerra, J., Fernández-Llébrez, P., 1996. Skeletal deformities in larval, juvenile and adult stages of cultured gilthead sea bream (*Sparus aurata* L.).Aquacult 141, 1–11

[3] Koumoundouros, G., Oran, G., Divanach, P., Stefanakis, S. & Kentouri, M., 1997. The opercular complex deformity in intensive gilthead sea bream (*Sparus aurata* L.) larviculture. Moment of apparition and description. Aquacult 156, 165–177.

 Divanach, P., Boglione, C., Menu, B., Koumoundouros, G., Kentouri, M., Cataudella, S., 1996. Abnormalities in finfish mariculture: an overview of the problem, causes and solutions. In:International Workshop, Verona, Italy, October 16– 18, 1996. Eur. Aquacult. Soc., Oostende, Belgium, pp. 45– 66.

[5] Castro, J., Pino-Querido, A., Hermida, M., Chavarrias, D., Romero, R., Garcia-Cortes, L.A., Toro, M.A., Martinez, P., 2008. Heritability of skeleton abnormalities (lordosis, lack of operculum) in gilthead seabream (*Sparus aurata*) supported by microsatellite family data. Aquacult 279, 18–22.

[6] Ayala, M.D., Abdel, I., Santaella, M.,Martínez, C., Periago,M.J., Gil, F., 2010. Muscle tissue structural changes and texture development in sea bream, *Sparus aurata* L.,during posmortem storage. LWT-Food Sci.Technol. 43, 465-475

[7] Lee-Montero, I., Navarro, A., Borrell, Y., García-Celdrán, M., Armero, E., Berbel, C., Zamorano, M.J., Sánchez, J.J., Estévez, A., Ramis, G., Manchado, M., Afonso., 2013. Development of the first standardised panel of two new microsatellite multiplex PCRs for gilthead seabream (*Sparus aurata* L.). Anim. Genet, 44, 533-546

[8] Dempster, E.R., Lerner, I.M., 1950. Heritability of threshold characters. Genetics 35, 212–236

Table 1: Frequencies (%) of: deformed individuals, skeletal deformities in the vertebral column, cranial skeletal deformities and lack of an inflated swimbladder in gilthead sea bream from three differentiated geographical origins

Geographical origin[1]	CAN	ATL	MED
N	304	282	323
Deformed	23.4[a]	37.9[b]	38.7[b]
Lordosis	6.6[a]	21.6[b]	19.2[b]
Vertebral shortening	3.6[a]	7.8[b]	10.5[b]
Vertebral Fusion	1.3[a]	1.4[a]	1.5[a]
Kyphosis	0.7[a]	0.7[a]	2.5[a]
Lack of operculum	8.2[a]	9.2[a]	5.9[a]
Premaxillar	3.9[a]	0[b]	1.8[c]
Uninflated SB	6.9[a]	70.9[b]	32.5[c]

[1]Gepgraphicall origin: CAN=Cantabrian Sea, ATL=The Atlantic Ocean, MED= Mediterranean Sea

[ab]Different superscripts within each row indicate significant differences among geographical origins (P <0.05).
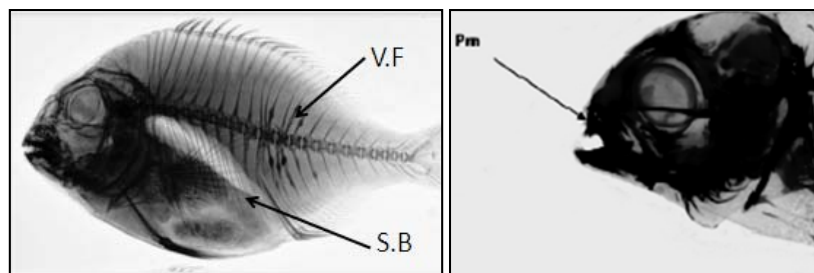

***Figure 1**: Column of sea bream showing V.F =vertebral fusion; S.B = Uninflated swimbladder. Head of gilthead sea bream showing Pm = premaxillar deformity.*

Table 2: Heritabilities ± standard errors (in bold on the diagonal), genetic (above diagonal) and phenotypic correlations (below diagonal) among skeletal deformities and uninflated SB in gilthead sea bream from three differentiated geographical origins

	Lordosis	V.Shortening	V.Fusion	Kyphosis	Operculum	Premaxillar	SB
Lordosis	**0.58±0.18**	-0.99±0.01	-0.99±0.01	0.99±0.01	-0.03±0.17	0.12±0.53	0.45±0.23
V.Shortening	-0.15	**0.03±0.06**	0.99±0.01	-0.98±0.01	0.99±0.01	-0.98±0.49	-0.69±0.20
V.Fusion	0.04	-0.03	**0.05±0.10**	0.98±0.01	-0.99±0.01	0.99±0.98	-0.99±0.01
Kyphosis	-0.06	-0.04	0.14	**0.07±0.14**	-0.99±0.01	0.59±0.97	-0.96±0.16
Operculum	-0.03	-0.02	0.02	-0.03	**0.19±0.13**	-0.09±0.03	-0.45±0.43
Premaxillar	-0.01	-0.03	-0.01	-0.02	-0.04	**0.12±0.22**	0.35±0.93
SB	0.31	0.07	0.04	0.06	0.10	-0.06	**0.24±0.11**