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Inheritance of skeletal deformities in gilthead seabream (*Sparus aurata*) – lack of operculum, lordosis, vertebral fusion and LSK complex[1]

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ABSTRACT: Morphological abnormalities in farmed gilthead seabream (*Sparus aurata*) are a major problem as it entails significant economic losses. In this study, 3 large scale experiments under different conditions of spawning, offspring handling and breeders phenotype were performed to analyze the inheritance of 4 types of deformities in this species: lack of operculum, lordosis, vertebral fusion, which are 3 of the most important skeletal deformities, and LSK, which is a consecutive repetition of lordosis/scoliosis/kyphosis. In Exp. [1] (mass spawning and fingerling sorting), 900 fish were analyzed at 509 d post-hatching: 846 fish that had been on-grown in a farm and 54 LSK-deformed fish that had been reared separately after being selected during the fingerling sorting process. A total of 89 families were represented. A statistically significant association between 5 of these families (from 6 breeders) and LSK-deformed fish was found. In Exp. [2] (mass spawning and no fingerling sorting), 810 fish were analyzed at 2 ages: 179 and 689 d post-hatching. Significant relationships between 2 of the breeders and 2 of the families with the lack of operculum prevalence of their descendants were found at 689 d but not at 179 d. Heritabilities: 0.09 ± 0.09 at 179 d and 0.17 ± 0.08 at 689 d. Column deformities prevalence was low and no association with family was observed. Family relationships were determined by microsatellites multiplex PCR in both experiments. In Exp. [3] (designed mating), sires suffering from lordosis or lack of operculum or vertebral fusion deformities were mated with non-deformed dams and a mass-spawning mating was considered as a control. After analyzing 11,503 offspring at 159 d post-hatching, a significant relationship between each deformity prevalence and the mating of breeders suffering from the same deformity was observed. In addition, a significant prevalence of lack of operculum in offspring from lordotic matings was observed. Heritabilities ranged from 0.34 to 0.46 for the 3 deformities. The results of the present study suggest that these deformities have a genetic origin. They also suggest that the sorting process is not recommended and that producers should consider these deformities in genetic breeding programs to significantly improve their fish morphological quality and to minimize farmed fish deformities incidence.

Key words: deformity, genetic improvement, kyphosis, scoliosis

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INTRODUCTION

The presence of morphological abnormalities in farmed gilthead seabream (*Sparus aurata*) is a major problem for current aquaculture as it entails significant economic losses (Afonso and Roo, 2007; Bardon et al., 2009). Skeletal deformities are the most relevant deformities and they include head and vertebral column anomalies. Lack of operculum in head, and lordosis, scoliosis, kyphosis and vertebral fusion

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in column, are the most frequent skeletal anomalies in this species (Galeotti et al., 2000; Beraldo and Canavese, 2011; Boglione et al., 2013). They lead to physiological alterations which affect fish appearance, growth, viability, welfare, stress response, and disease resistance (Gjerde, 2005; Karahan et al., 2013).

A high number of studies has determined that environmental factors are linked to fish deformities (Bardon et al., 2009), but only a few of them have contributed to these deformities genetic determination. In gilthead seabream, a significant family association for a triple column deformity (lordosis-scoliosis-kyphosis [**LSK**] complex) was found by Afonso et al. (2000) and a high heritability for presence/absence of any kind of deformity was estimated by Astorga et al. (2004). I. Lee-Montero (personal communication) estimated the average value of heritabilities for deformity traits in fish reared in 4 Spanish regions (PROGENSA®).

The main objective of the present study was to analyze the prevalence of 4 skeletal deformities (lack of operculum, lordosis, vertebral fusion, and LSK complex) in gilthead seabream, and their association with the family. This analysis considers different breeding conditions, breeders phenotype, and offspring handling to determine the influence of breeders genetic predisposition and management on the emergence of these deformities in offspring and to provide aquaculture producers with knowledge-based genetic advice.

MATERIAL AND METHODS

Three experiments to assess the prevalence of deformity in offspring from different broodstocks were performed at the facilities of the Marine Science and Technology Park of the University of Las Palmas de Gran Canaria (**PCTM-ULPGC**, Gran Canaria, Spain). It belongs to the Foundation of Science and Technology Park of ULPGC (**FCPCT-ULPGC**).

Experiment [1]: Mass Spawning and Fingerling Sorting

Breeders and mating structure. An egg batch was collected by using mass spawning from an industrial hatchery broodstock. This broodstock consisted of 66 nondeformed breeders and the sex ratio of which was 1♀: 2♂.

Offspring and rearing conditions. Eggs and larvae were reared at PCTM-ULPGC under the conditions described by Roo et al. (2009). Fingerlings were sorted by presence versus absence of deformity at 111 d post-hatching (1.9 ± 0.02 g; mean ± SE). The sorting process was developed as performed by companies visually detecting and slaughtering skeletal deformed fish and fish showing non-inflated swim bladder by flotation (Boglione et al., 2013). With respect to fish showing LSK deformity, in the whole batch of fingerlings (about 98,000), 216 LSK-deformed fish (0.22%) were found and selected during the sorting process but not slaughtered. Instead, they were reared separately in a 1,000-L fiberglass tank at PCTM-ULPGC. Water temperature ranged from 19.3 ± 0.1°C in March to 25.0 ± 0.1°C in September, and values for dissolved oxygen and water flow were 6.0 ± 0.1 $mg \cdot l^{-1}$ and 21 $l \cdot min^{-1}$, respectively. Commercial feed was provided through self-feeders. With respect to fish considered as normal during the sorting process, they were taken to a cage of Playa de Vargas 2001 S.L. Company (PLV2001, Gran Canaria, Spain) at 130 d post-hatching (4.8 ± 0.1 g), and reared under industrial conditions and fed with commercial feed (Skretting®, Burgos, Spain) provided by an air feeding system. Dissolved oxygen in the water had an average value of 7.4 $mg \cdot l^{-1}$. The on-growing period lasted up to 509 d post-hatching, when fish weight was 419.7 ± 3.1 g.

Visual assessment. At the end of the experiment, a sample of fish from PLV2001 was slaughtered and visually analyzed to determine the presence or absence of deformities according to AquaExcel-ATOL (AquaExcel Project, 2013; ATOL: 0000087). Vertebral deformities were assessed, after fish filleting, by direct observation of the axial skeleton.

Parental assignment. Family relationships between breeders and offspring (fish from PLV2001 and LSK-deformed fish, from PCTM-ULPGC) were determined by the exclusion method by using the software VITASSING (v8.2.1; Vandeputte et al., 2006), after DNA analysis and genetic characterization by using the multiplex PCR SMsa-1 (SuperMultiplex *Sparus aurata*), as described by Lee-Montero et al. (2013).

Experiment [2]: Mass Spawning and No Fingerling Sorting

Breeders and mating structure. An egg batch was collected by using mass spawning of an industrial broodstock of PCTM-ULPGC within the context of the PROGENSA breeding program (Afonso et al., 2012). This broodstock consisted of 59 non-deformed breeders and the sex ratio of which was 1♀: 1.81♂.

Offspring and rearing conditions. Eggs and larvae were cultured at the PCTM-ULPGC facilities as described in Exp. [1]. At d 179 post-hatching (17.2 ± 0.2 g), fingerlings were individually tagged with passive integrated transponder (**PIT**; Trovan Daimler-Benz) by following the tagging protocol described by Navarro et al. (2006) and transported to a cage of CANEXMAR S.L. company (Gran Canaria, Spain).

They were reared under industrial conditions and fed with commercial feed provided by an air feeding system. The average value of dissolved oxygen in the water was 7.4 $mg \cdot l^{-1}$ and water temperature ranged from 20.2°C to 24.2°C. No sorting or culling processes were performed during larval rearing or on-growing periods, which lasted up to 689 d post-hatching, when fish weight was 524.4 ± 12.6 g.

Visual assessment. During fish tagging (179 d post-hatching), fish were observed to determine the presence or absence of deformities (initial analysis; ATOL: 0000087). At the end of the experiment (689 d post-hatching), a sample of 810 fish was slaughtered and visually analyzed (final analysis) to determine the presence or absence of deformities (ATOL: 0000087). Vertebral deformities were assessed, after fish filleting, by direct observation of the axial skeleton (like in Exp. [1]).

Parental assignment. Family relationships between breeders and offspring (810 fish) were determined as described in Exp. [1].

Experiment [3]: Designed Mating

Breeders and mating structure. Different directed matings were established by using gilthead seabream breeders from PCTM-ULPGC facilities, more concretely, 18 deformed sires and 9 non deformed (**ND**) dams. Deformed fish were classified into 3 groups: Lack of operculum (**OP**), Lordosis (**LO**) and Vertebral Fusion (**VF**). Three matings for each deformity were constituted: N × O, N × L, and N × VF. The sex ratio in each tank was 1 normal dam to 2 deformed sires ($1♀_{ND}$: $2♂_{D}$). Additionally, a broodstock of 60 non-deformed breeders ($1♀_{N}$: $2♂_{N}$) was used to constitute a Control mating (**CM**) by mass spawning.

Offspring and rearing conditions. Eggs from each mating were separately cultured in PCTM-ULPGC facilities as described in Exp. [1]. Fingerlings from each mating were separately reared in 500-L fiberglass tanks until the end of the experiment (2 tanks/mating). The rearing conditions were as follows: commercial feed was provided by automatic feeders, water flow was 0.5 $l \cdot min^{-1}$, dissolved oxygen concentration was 5.9 ± 0.1 $mg \cdot L^{1}$, and water temperature ranged from 19.3 ± 0.1°C at the beginning of the experiment to 23 ± 0.1°C at the end of the experiment. The experiment ended at 129-d post-hatching (fish weight: 9.6 ± 0.1 g).

Visual assessment. At the end of the experiment, fingerlings from all the matings, including those from mass spawning, (11,503 fish) were visually analyzed to determine the presence or absence of deformities (ATOL: 0000087).

Data Analysis

The identified deformities were LD, VF, OP, and LSK complex. Fish without any of these deformities were considered N. The prevalence rate of deformities was calculated as a percentage of deformed descendants with respect to the total of analyzed fish in each group (breeder, family or mating).

The association between any factor (breeder, family or mating) and deformity within experiment was analyzed by a log linear model by using the statistical software SPSS (PASW Statistics v18). Deformity factor was measured in each fish as presence (1) or absence (0) of the deformity. Log linear model gives the significance of any deformity factor (its prevalence [*i*]) against any biological or functional factor (depending of data [*j*]), organized under a two-way contingency table, through the normalized values or *Z*-values. Normalized *Z*-values for statistical significance of deformity in any family, breeder or mating ranged from > +1.96 (excess) to < −1.96 (deficit).

$$lnf_{ij} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij}$$

where lnf_{ij} is the expected frequency from observed frequency of each deformity (*i*) in each family, breeder or mating considered (*ij*); μ is the average value of expected frequencies logarithms, α_i is the effect of the deformity factor (*i*), β_j is the effect of the family, breeder, mating or experiment (*j*) and $\alpha\beta_{ij}$ is the effect due to these factors interaction.

In Exps. [1] and [2], the relationship of each deformity with each family, sire, and dam was analyzed. In Exp. 3, the relationship of each deformity with each mating, family, and tank within family was analyzed. Sex factor was not considered because all the analyzed fish were males, as gilthead seabreams can change sex after reaching their commercial size.

In Exps. [1] and [2], the association between genotype (heterozygote vs homozygote) and phenotype (deformed vs normal), within experiment and within the families where any deformed fish were found was analyzed. This analysis consisted of a log linear model by using the statistical software SPSS (PASW Statistics v18**,** SPSS, Chicago, IL) for each microsatellite marker.

Variance components of deformity traits to obtain heritabilities on the observed scale were estimated by the REML method by using the VCE (v 6.0) software (Neumaier and Groeneveld, 1998).

RESULTS

Deformities prevalence (%) in total offspring in each experiment is shown in Table 1.

Table 1. Deformities prevalence (%) in total offspring in each experiment[1]

Experiment	OP	LO	VF	LSK	ND	*n*
Exp. 1	0.44	5.67	0.44	6	87.44	900
Exp. 2	5.80	1.73	0.12	0	92.35	810
Exp. 3	4.84	0.64	0.63	0.03	93.85	10650

[1]Non deformed = ND; OP = operculum; LO = lordosis; VF = vertebral fusion; *n* = number of descendants from each experiment; Experiment 1 = mass spawning with sorting; Experiment 2 = mass spawning without sorting; Experiment 3 = only designed mating with deformed breeders.

Experiment 1

In the parental assignment of the whole offspring analyzed, 100% success was obtained (i.e., 54 LSK-deformed fish survived until the end of the experiment [75% of mortality]), as well as 846 individuals from PLV2001. Twenty-eight breeders (17 dams and 11 sires) contributed to the spawn and 89 full-sibling families were represented. Only the prevalence of LSK deformity showed a statistically significant association ($Z > +1.96$, $P < 0.05$) with sires, with dams, and with the families formed by these breeders. All the LSK individuals were included in 6 of the 89 families. These families (named msF1 to msF6) were composed of 6 breeders: 4 dams (named ms♀1 to ms♀4) and 2 sires (named ms♂1 and ms♂2). Deformities prevalence (and its significance) in descendants (LSK and PLV2001 fish) of these dams and sires compared with the other breeders (mean value of the prevalence), and with each sire, dam, and family, are shown in Table 2. The 2 sires, the 3 dams, and 5 of the families showed a statistically significant relationship with deformity due to their high LSK-deformed fish prevalence ($Z_{sire/dam/family,\ LSK} > +1.96$; $P < 0.05$). These families had from 48.15 to 66.67% of LSK-deformed descendants. It is remarkable that ms♂1 and ms♂2 were also responsible for 75% of VF-deformed fish, while ms♀2 was responsible for 51.85% of LSK-deformed fish and for 25% of lack-of-operculum fish. None of the dams was responsible for any VF-deformed fish.

The mean heterozygosity of LSK-deformed fish and normal fish from families msF1 through msF6 were very similar (0.66 ± 0.27 and 0.65 ± 0.31, respectively) and there were no statistically significant differences between these groups with respect to any microsatellite marker or family ($P > 0.05$). Heritability could not be estimated because LSK-deformed fish prevalence in the final sample was modified, since LSK-deformed fish had been selected at the beginning of the experiment.

Experiment 2

A 100% success rate was also obtained in the parental assignment (i.e., all the offspring [810 individuals] were assigned to 45 breeders [19 dams and 26 sires], and 66 full-sibling families were formed). In the initial analysis, at 179 d post-hatching, 7% of offspring showed lack of operculum, and neither L nor VF deformities were observed. Deformity prevalence was breeder-independent and family-independent ($-1.96 < Z_{breeder/family,deformity} < +1.96$; $P > 0.05$). At 689 d post-hatching, at the final analysis, the total prevalence of deformities was 7.65%. Only the O deformity prevalence showed a statistically significant association ($Z > +1.96$, $P < 0.05$) with sires, with dams and with the families formed by these breeders. 48.94% of O-deformed individuals were descendants of one sire (named mw♂1), which represents a significant prevalence. Only 2 families, those formed by mw♂1 and 2 dams (named mw♀1 and mw♀3), showed a significant prevalence of O deformity, as they represented 12.77 and 21.28% of all the individuals, respectively. Deformities prevalence (%) in offspring from mw♂1 at the final analysis is shown in Table 3.

The mean heterozygosity of O-deformed fish and normal fish from families mwF1 through mwF6 were very similar (0.64 ± 0.20 and 0.63 ± 0.21, respectively) and there were no statistically significant differences between these groups with respect to any microsatellite marker ($P > 0.05$).

Table 2. Deformities prevalence (%) in total descendants (LSK and PLV2001 fish) of breeders responsible for LSK-deformed fish with respect to each sire, dam and family of the Exp. [1][1]

	LSK fish	PLV2001 fish				Total
	LSK	L	VF	O	N	*n*
Sire						
ms♂1	22.58[a]	4.52	0.65	0	72.26	155
ms♂2	17.92[a]	4.72	1.89	0.94	74.53	106
Mean ms♂[R]	0	6.77	0.10	0.34	92.79	639
Dam						
ms♀1	11.81[a]	3.15	0	0	85.04	127
ms♀2	33.73[a]	3.61	0	1.20	61.45[b]	83
ms♀3	5.00	7.50	0	0	87.50	40
ms♀4	27.27[a]	0	0	0	72.73[b]	33
Mean ms♀[R]	0	13.29	0.43	0.21	86.07	617
Family						
msF1 (♀1♂1)	48.15[a]	0	0	0	51.85[b]	27
msF2 (♀1♂2)	28.57	0	0	0	71.43	7
msF3 (♀2♂1)	66.67[a]	3.33	0	0	30.00[b]	30
msF4 (♀2♂2)	50.00[a]	6.25	0	0	43.75[b]	16
msF5 (♀3♂1)	66.67[a]	0	0	0	33.33	3
msF6 (♀4♂2)	56.25[a]	0	0	0	43.75[b]	16
Mean msF[R]	0	6.75	0.64	0.95	91.66	801

[1]LSK = lordosis-scoliosis-kyphosis complex; OP = operculum; LO = lordosis; VF = vertebral fusion; ND = non deformed; *n* = number of descendants. [R] = mean of prevalence from the rest of males, females and families.

[a]Significant associations when $Z \geq +1.96$.

[b]Significant associations when $Z \leq -1.96$.

Table 3. Deformities prevalence (%) in offspring from mw♂1 of the Exp. [2][1]

	OP	LO	VF	ND	*n*
Sire					
mw♂1	14.84[a]	0	0	85.16	155
Mean mw♂[R]	6.15	3.13	0.02	90.70	655
Dam					
mw♀1	17.54[a]	1.75	0	80.70	57
mw♀2	3.66	0	0	96.34	82
mw♀3	4.62	1.54	0.31	93.54	325
mw♀4	4.69	1.56	0	93.75	64
mw♀5	2.08	2.08	0	95.83	48
mw♀6	4.59	2.75	0	92.66	109
Mean mw♀[R]	10.97	4.14	0	84.89	125
Family					
mwF1(♀1♂1)	46.15[a]	0	0	53.85	13
mwF2(♀2♂1)	20	0	0	80	5
mwF3(♀3♂1)	11.63[a]	0	0	88.37	86
mwF4(♀4♂1)	7.69	0	0	92.31	13
mwF5(♀5♂1)	12.50	0	0	87.50	8
mwF6(♀6♂1)	13.33	0	0	86.67	30
Mean mwF[R]	6.23	2.40	0.01	91.35	655

[1]ND= non deformed; OP = operculum; LO = lordosis; VF = vertebral fusion; N = normal; *n* = number of descendants. [R] = mean of prevalence from the rest of males, females and families.

[a]Significant associations when $Z \geq +1.96$.

Table 4. Deformities prevalence (%) in offspring with respect to different matings of the Exp. [3][1]

	Type of deformity					
Mating	O	L	VF	LSK	N	*n*
N × O	4.47[a]	0.18[b]	0.1 [a]	0.03	95.22	6803
N × L	11.63 [b]	2.47 [b]	0.22 [a]	0.05	85.63 [a]	1823
N × VF	1.78 [a]	0.69	2.52 [b]	0.05	94.96	2024
Control	0.59 [a]	0.35	1.17	0	97.89 [b]	853

[1]LSK = lordosis-scoliosis-kyphosis complex; O = operculum; L = lordosis; VF = vertebral fusion; N = normal; *n* = numbers of descendants from each mating; N × O = O mating. N × L = L mating. N × VF = VF mating. Control = control mating.

[a]Significant associations when $Z \geq +1.96$.

[b]Significant associations when $Z \leq -1.96$.

Heritability values for O deformity were 0.09 ± 0.09 at 179 d and 0.17 ± 0.08 at 689 d.

Experiment 3

Three matings for each type of deformity were conducted and the 3 N × O, 2 N × L and only one N × VF were viable. All the offspring from 1 N × O mating and 1 N × L mating died, so only offspring from 2 N × O matings, 1 N × L mating, and 1 N × VF mating could be analyzed. A total of 11,503 fish were analyzed: 6.47% of offspring from directed matings and 2.11% of offspring from Control mating showed any of the studied deformities. The prevalence (and its significance) of each type of deformity in offspring from different matings is shown in Table 4. As it can be observed, the type of deformity was strongly associated to the type of mating: N × O matings showed an excess of O-deformed fish, the N × L mating showed an excess of O-deformed fish and L-deformed fish, the N × VF mating showed an excess of VF-deformed fish, and the Control mating showed an excess of normal fish ($Z_{\text{mating, deformity}} > +5$; $P < 0.01$). The family effect (half-sibling), which only could be analyzed for N × O mating, was statistically significant. One of the families showed an excess of O-deformed fish (9.98%) and a deficit of normal fish (89.54%), differently from what was observed in the other family (0.98% and 99.69%, respectively; $Z_{\text{family, deformity}} > +3.6$; $P < 0.01$). Indeed, when matings were analyzed with respect to the family, only 1 N × O family was responsible for the association between O deformity and N × O mating. However, no tank effect on prevalence of deformities was observed in each mating (during the rearing period; $P > 0.05$).

Half-sibling family heritabilities were 0.46 ± 0.11 for O deformity, 0.34 ± 0.03 for L deformity, and 0.40 ± 0.07 for VF deformity.

DISCUSSION

Growth and deformities are the most economically important traits for the industrial production of gilthead seabream (Georgakopoulou et al., 2010). The most relevant deformities are those affecting opercular complex, neurocranium and vertebral column (Afonso and Roo, 2007; Izquierdo et al., 2010; Boglione et al., 2013; Prestinicola et al., 2013). There are numerous studies on environmental effects on fish deformities in both freshwater and marine aquaculture. Many of them have demonstrated that alterations on biotic and abiotic, physiological, xenobiotic, nutritional, and rearing factors can be responsible for the development of fish deformities at an early stage (Boglione et al., 2001; Bardon et al., 2009; Boglione and Costa, 2011; Prestinicola et al., 2013) or during their on-growing period (I. Lee-Montero, personal communication). Additionally, genetic factors are also possibly responsible for the prevalence of different skeletal deformities in different species: Atlantic salmon (*Salmo salar*; Gjerde, 2005), rainbow trout (*Oncorhynchus mykiss*; Kause et al., 2005; Gislason et al., 2010), Atlantic cod (*Gadus morhua*; Kolstad et al., 2006) and European seabass (*Dicentrarchus labrax*; Bardon et al., 2009, Karahan et al., 2013). However, in gilthead seabream, only a few studies have proposed a genetic origin for some types of deformities (Afonso et al., 2000; Astorga et al., 2004; I. Lee-Montero, personal communication). In this study, 4 skeletal deformities genetic analyses have been conducted, for the first time in gilthead

sebream, to determine these deformities association with the family and its effect on their prevalence in offspring.

The presence of morphological abnormalities in farmed fish entails significant economic losses which are difficult to be accurately determined, the minimum annual loss estimated for European aquaculture is higher than 50 M€/yr, so a 50% reduction of deformities could save 25 M€/yr (Hough, 2009). One of the reason by which deformities entail economic losses is the fact that companies have to perform manual sorting to select and eliminate deformed fingerlings at the end of the hatchery phase, which is a slow and expensive method (2,000 to 4,000 fish/h/person; Divanach et al., 1996; Boglione and Costa, 2011). In Exp. [1], a commercial batch of 846 fish (PLV2001 fish) was analyzed to determine the prevalence of 3 of the most frequent deformities in gilthead seabream production (O, L, and VF) at commercial size. Fish rearing was performed under industrial conditions, and fish were sorted in term of deformities before being sent to an ongrowing company. Differently from the other deformed fish, LSK-deformed fish were not excluded during the sorting process, they were selected and reared separately. LSK complex was described for the first time in gilthead seabream as a triple column deformity consisting of a consecutive repetition of LSK from head to tail (Afonso et al., 2000). These authors designed a crossbreeding scheme including separate rearing of 31 full-sibling families and found a statistically significant interaction between the prevalence of this deformity and one of the families. In their study, LSK-deformed fish represented 0.2% of all offspring; similarly to what was obtained in the present study: 0.22% of all the fish from the commercial batch. In this study, a statistically significant family correlation was also observed in LSK-deformed fish. Moreover, the offspring was obtained by mass spawning and fingerlings of all families were reared under de same conditions. From a genetic point of view, this has the advantage that common environmental sources are reduced (Herbinger et al., 1999). The results from this experiment support the hypothesis of a genetic origin for LSK complex deformity, previously proposed by Afonso et al. (2000).

Since there were no statistically significant differences between LSK-deformed fish and normal fish from families msF1-msF6 for any microsatellite marker, this deformity seems to be not determined by recessive alleles.

Breeders that had LSK-deformed descendants were also responsible for the 11.22% of L-deformed fish and for the 75% of VF-deformed fish. These data suggest a possible relationship between these 3 deformities. However, no statistically significant relationship was observed between L, VF, or O deformities and any of the families or breeders. As expected from the fact that fish had been previously culled in terms of deformities (during their fingerling stage), thus, making it difficult to determine the genetic origin of this trait. In this sense, Castro et al. (2008) also found no-different-from-zero heritability for lack of operculum and lordosis (0.02 ± 0.02 and 0.03 ± 0.02, respectively) in sorting gilthead seabream.

In Exp. [2], a batch of 810 gilthead seabreams were analyzed and the 4 deformities were evaluated, but no sorting or culling process during the fish rearing were conducted. At 179 d post-hatching (initial analysis), no deformity was detected with the exception of O. This could be due to the fact that vertebral deformities are difficult to be detected at small sizes. As in other studies in gilthead seabream (I. Lee-Montero, personal communication) and in European seabass (Bardon et al., 2009) in which individual fish monitoring at different ages was performed, vertebral deformities were detected more easily over time as the fish grow. The initial analysis of this experiment showed no relationship between O deformity prevalence and any family or breeder. However, at 689 d post-hatching, a significant relationship between a sire or a dam or 2 of the families and O deformity prevalence in their offspring was observed. Additionally, in other families including this sire, the O deformity prevalence was much higher than the average of the experiment, although it was nonsignificant. Indeed, the heritability estimated in this population was very low at 179 d post-hatching but it reached a value of 0.17 at 689 d post-hatching. I. Lee-Montero (personal communication) also indicated a higher genetic determination for this deformity at commercial size (300 to 800 g) than at small size (15 g), as the estimation of heritability was negligible at the initial point but it increased until medium values (0.11) at commercial size. Additionally, these authors monitored individually tagged gilthead seabreams and demonstrated that some fish showing O deformity at small size recovered during their development. Indeed, in this experiment, O deformity prevalence at final size is lower than at initial size. Taken together, these results suggest that the genetic determination of O deformity in small size fish is hardly noticeable, whereas at larger sizes, after a percentage of O recovering, it is more significant. In this sense, the appearance of this deformity at small size has been widely related with culture or environmental factors (Koumoundouros et al., 1997; Beraldo et al., 2003; Roo et al., 2005; Boglione et al., 2013). Since there were no statistically significant differences between O-deformed fish and normal fish for any microsatellite marker, this deformity seems to be not determined by recessive alleles.

With respect to vertebral deformities, their prevalence was breeder/family-independent. Nevertheless, a genetic origin of these deformities cannot be discarded as their prevalence was very low. Deformity is

a binary trait, so that when its prevalence is very low, its genetic additive value is difficult to be accurately estimated (Falconer and Mackay, 1996). Accordingly, Kause et al. (2007), in farmed salmonids, estimated close-to-zero values of heritability for skeletal deformities when their prevalence was low and elevated values when their prevalence was high. In this regard, Evans and Neff (2009) did not found a significant relationship between spinal deformity prevalence and additive genetic effect in 2 different populations of Chinook salmon (*Oncorhynchus tshawytscha*) larvae, whose average prevalence for these deformities was close to zero in both populations (0.69% and 0.05%).

Considering all the above, an efficient strategy to increase the prevalence of deformities and to reveal their possible genetic origin would be an intentional mating by using deformed fish to evaluate their effective predisposition to produce deformed descendants (Bardon et al., 2009). This statement led us to Exp. [3], in which directed matings formed by 2 deformed sires (O, L, and VF) and 1 nondeformed dam (N) were conducted. A mass spawning by nondeformed breeders was considered as control mating.

A statistically significant relationship between the prevalence of a deformity and the mating of breeders suffering from the same deformity was obtained after evaluating all the offspring (11,503 individuals). However, a statistically significant prevalence of O deformity was observed in N × O and N × L matings. Additionally, the family effect in the N × O mating was statistically significant, just 1 family was responsible for the high prevalence of operculum deformity in this mating. These results suggest that breeders genetic predisposition to produce O-deformed offspring is independent of the breeder phenotype, with respect to this deformity. This could be reason by which this is the most common deformity, even when all industrial breeders are non-deformed. Therefore, a genetic criteria-based selection of breeders would be recommended to minimize the prevalence of this deformity.

On the other hand, no significant prevalence of L or VF deformities was observed in the other matings. It is also remarkable the fact that the number of normal individuals was significantly higher in the control mating. Moreover, no tank effect within family was observed, as expected from the fact that the rearing conditions were similar in all the matings. The family effect could not be analyzed in matings N × L and N × VF because of their high larvae mortality, presumably due to a lower viability of matings in which one of the breeders shows these deformities. This is similar to the results reported by Astorga (2005), in whose study the total production of eggs per female/d and kilogram was significantly lower in deformed-breeders matings than in normal-breeders matings. All these results support the idea of an important genetic determination of these 3 skeletal deformities. Accordingly, their heritabilities were medium-high. However, they can be possibly overestimated as each family was reared into the same tank and, in consequence, was influenced by common environmental conditions (Herbinger et al., 1999).

It is remarkable that, being three experiments very different, all their results support the genetic origin of all the studied deformities. Moreover, broodstocks of Exps. [1] and [2], rearing conditions and deformity assessment of 3 experiments were always industrial. The differences among batches, as also happens industrially (Boglione et al., 2013), support the robustness of the results. When comparing the deformities prevalence among the experiments (Table 1), it can be observed that LSK deformity shows the lowest prevalence in all the offspring (0 to 0.2%), thus revealing that it is an extremely rare deformity. That could be the reason by which it has been rarely included in deformities studies (Afonso et al., 2000; Ebrahimnezhad et al., 2009), despite its severity. In addition, as it has been observed in this study, mortality of these individuals is high even when they are separated from nondeformed fish, so, it can be concluded that they do not reach their final ongrowing stage. This indicates that the sorting process for this deformity is neither relevant nor cost-effective. In Exp. [1], by using nondeformed breeders and culling deformed individuals, the prevalence of deformities at the final point was 12.55%, more concretely, column deformities prevalences were higher than O deformities prevalence. Similarly, Oliva (2008) observed, in an industrial batch of 430 g gilthead seabreams, 9.80% of fish showing vertebral deformity and 0.8% of head- and O-deformed fish. Contrastly, in Exp. [2], despite no sorting process was performed, column deformity prevalence was lower than in Exp. [1]. It could be due to differences among batches, which have already been described by several authors (Witten et al., 2009; Boglione et al., 2013). This highlights that the sorting process is not an efficient method to cull deformed fish during the production. Accordingly, I. Lee-Montero (personal communication) found a low phenotypic correlation (0.38) between fingerling stage and commercial size for any type of deformity in gilthead seabream. With respect to Exp. [3], the deformities prevalence of descendants from deformed breeders was significantly higher than in control mating descendants than in Exp. [2] (at the initial size). Indeed, in Exp. [3], the presence of fish showing column deformity was easily detected even at early ages, possibly due to a higher intensity or severity of that deformity (Bardon et al., 2009). Differently, in Exps. [1] and [2], deformed individuals were not detected until they reached a higher size. In summary, the sorting process is not recommended, as it increases the production costs and given its low

efficiency, the O deformity recovery rate and the high mortality of LSK-deformed fish. However, the results of this study suggest that the inclusion of deformities as a selection objective in a breeding program may decrease the prevalence of skeletal deformities in this species. As selection criteria it would be recommended the inclusion of O deformity at commercial size, as its genetic determination is more significant at this age. With respect to vertebral deformities, L and VF may be also included at commercial size, as they are hardly detected at early ages. Nevertheless, including the LSK complex deformity at fingerling age may be a better strategy, if considering its possible relationship with L and VF deformities and its ease of diagnosis at early ages.

Conclusion

The results of this study show that prevalences of the studied deformities (lack of O, L, VF, and LSK complex) significantly correlates with the family in the 3 studied populations. Moreover, they suggest that genetic improvement would be more effective than the sorting process at significantly improving fish morphological quality. Once the breeders genetic predisposition to produce deformed descendants is known. In addition, this study represents a first step toward the knowledge of the etiology of skeletal deformities, as it provides 3 interesting populations to locate and identify QTLs for these deformities, which could increase the efficiency of gilthead seabream breeding programs.

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