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## What is the heritable component of spinal deformities in the European sea bass (*Dicentrarchus labrax*)?

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### ABSTRACT

Malformations in farmed fish are a prime research topic as it has a major impact on fish welfare and on the economical value of the product. In this study, the genetic component of spine deformities was estimated in European sea bass, a major species of Mediterranean aquaculture, with the use of *a posteriori* reconstruction of pedigree with microsatellites. Our population exhibited a very high malformation rate (81%), probably due to intense swimming of fish during pre-growing. The heritability of global spine deformities was evaluated to be  $0.21 \pm 0.04$  on the underlying scale ( $0.33 \pm 0.06$  for lordosis,  $0.13 \pm 0.04$  for scoliosis). Deformities scored by an internal or external examination turned out to be genetically the same trait (genetic correlations  $> 0.9$ ), although phenotypic correlations were moderate to high ( $0.45 \pm 0.04$  to  $0.92 \pm 0.01$ ) due to difficulties in scoring the less severe deformities. Along time (from 100 to 800 g mean weight), lordosis had a constant heritability, with very high genetic correlations between stages ( $> 0.9$ ). Its incidence increased from 39 to 53% indicating that the malformation was probably present from the beginning of the rearing period but that its expression increased over time. A positive linkage between spine deformities and growth rate was demonstrated both by the higher early weight of affected fish and by the positive genetic correlation between weight and malformation (from  $0.21 \pm 0.15$  to  $0.40 \pm 0.14$ ), although deformities have a negative phenotypic impact on growth. However, heritabilities of malformation traits calculated here are only estimates that may indirectly describe the sensitivity of fish to the particular pre-growing environment as a function of different body weight genotypes. A quantification of realised heritability would therefore be necessary before concluding on the existence of a genetic basis for spinal malformations such as lordosis and scoliosis. Nevertheless and by precaution, selection for fast growth rate needs careful monitoring of deformities, and selection against malformations may counteract impacts of selection for fast growth.

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### 1. Introduction

The study of malformations in farmed fish is a research topic of prime importance for the aquaculture sector. It has received an increasing attention over the last 20 years, and progress has been made towards causative factors and improved rearing procedures. The problem nevertheless persists, and remains significant for the industry as it reduces the marketing value of fish throughout the value chain of almost all aquaculture species. These anomalies also have important implications on the sustainable development of aquaculture, consumer perception, and animal welfare (Komen et al., 2002). Important external deformities negatively impact the product image, and fish with small deformities that may externally look normal, also represent a great problem for the hatcheries that sell their fry X-rayed to

attest their quality. Additionally, it may cause technical problems to automated filleting equipment (Gjerde et al., 2005). Deformed fish increase production costs, as they require manual sorting, and lower performances (*i.e.* swimming ability, conversion index, growth rate, survival, and susceptibility to stress, pathogens and bacteria; Boglione et al., 2001).

Fish species may develop different types of abnormalities that have been widely described (Andrades et al., 1996; Divanach et al., 1996; Afonso et al., 2000; Gavaia et al., 2002; Fraser et al., 2004; Kranenborg et al., 2005a; Boglione et al., 2006; Fjellidal et al., 2007). The most cited are malformations of the spine comprising lordosis (V-shaped curvature in the sagittal plan), scoliosis (lateral curvature), and sometimes a combination of both. In the European sea bass (*Dicentrarchus labrax*), most of the malformations occur during the larval period and average 10% in most batches, while without any reason presently understood, some batches may reach 80 to 100% deformed fish.

Several physiological, environmental, xenobiotic, nutritional and genetic factors have been linked to this problem during larval and

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juvenile development stages of cultured freshwater and marine fish (Divanach et al., 1996; Boglione et al., 2001; Gorman and Breden, 2007), e.g.:

- Hypoxia during somitogenesis (Sawada et al., 2006a,b).
- Brutal variations of temperature during incubation or larval rearing (Abdel et al., 2004; Sfakianakis et al., 2004; Georgakopoulou et al., 2007).
- Abnormal swimbladder development causing spinal curvature affecting vertebrae located behind the digestive mass and aggravated by muscle pressure during forced swimming (Chatain, 1994a; Divanach et al., 1997; Kihara et al., 2002; Kranenborg et al., 2005b; Sfakianakis et al., 2006).
- Dysfunctions in osteogenesis due to inadequate amounts or interactions of diverse nutritional components such as calcium, phosphorus, vitamins A, C, D, E and K (Beaverfjord and Asgard, 1998; Madsen and Dalsgaard, 1999; Olivia-Teles and Pimentel-Rodrigues, 2004; Sugiura et al., 2004; Villeneuve et al., 2006; Cahu et al., 2003; Lall and Lewis-McCrea, 2007). The physiological process involved in the normal bone development in fish was only recently investigated, but the recently demonstrated mineralization rearrangement during growing indicates its complexity (Deschamps et al., 2008).

Genetics is usually considered marginal although it appears to be significant in several cases. For example, the level of morphological aberrations of pectoral fins that varied between 0 to 75% in crosses of two strains of African catfish (*Clarias gariepinus* and *Heterobranchus longifilis*) indicated the involvement of the genotype rather than the influence of environment (Aluko et al., 2001). The heritability of fin deformities was also shown to be significant but low ( $0.09 \pm 0.04$ , estimated by animal model on the observed scale) in the common carp (*Cyprinus carpio*, Kocour et al., 2006). In Atlantic salmon (*Salmo salar*), the susceptibility to a specific spinal deformity was estimated heritable (0.25 and 0.14, based respectively on the sire and dam component of variance, McKay and Gjerde, 1986), and the vertebral deformity was shown to be determined by a substantial additive genetic component (from  $0.00 \pm 0.00$  to  $0.20 \pm 0.08$  on the observed scale in four year-classes of Atlantic salmon; Gjerde et al., 2005). Moreover, genetic correlations established between deformed skeletal structures, recorded in different cohorts of salmonids and rearing environments, showed that improved management conditions could decrease the deformity incidence, and that the genetic architecture of the “deformity” trait depends on the environment and on the incidence of deformities (Kause et al., 2007). On the other side, no evidence of genetic component for skeleton abnormality has been found in the gilthead sea bream (Castro et al., 2008). In the European sea bass, a major species for Mediterranean aquaculture, the genetic component of spine deformities was never evaluated.

One of the most important constraints for estimating genetic parameters in fish is the inability to tag hatchlings, and consequently the need to separately rear the families until tagging size, which may bias or reduce the precision of the estimated parameters. In the present study, we used an interesting alternative to separate rearing of families, which is the use of mixed rearing of progenies with *a posteriori* reconstruction of pedigrees using highly variable markers such as microsatellites. The major benefits of this methodology are the absence of between-family environmental effects, the possibility to work in industry conditions with good experimental power, and to use highly informative factorial designs (Vandeputte et al., 2001). In the present experiment, we took advantage of the high incidence of inadvertent spine deformities in a large set of mixed families of sea bass to examine the genetic component of spine deformities. Internal deformities were visually evaluated on filleted fish about 800 g mean weight, and related to external records at this stage. The evolution over time of externally recorded deformities was also tracked, thanks to the fact that fish were individually tagged and photographed at different ages. These data were

used to study the genetic variation of deformities and its interactions with growth traits.

## 2. Materials and methods

### 2.1. Animals

Selection of breeders, reproduction process and larval rearing were previously described in details in Dupont-Nivet et al. (2008). Briefly, the parents of the studied animals were wild sea bass of Atlantic origin collected by Panittica Pugliese S.A. (Torre Canne di Fasano, Italy) on the Northern coast of Brittany (France). Reproduction of these fish was performed in this farm, i.e. an expected 253 full-sib families issued from 33 sires and 23 dams produced according to a partly factorial mating design in three sets of 11 sires mated with 9, 7, and 7 dams respectively. Within each set, a full factorial crossing was accomplished. Sperm was cryopreserved (Fauvel et al., 1998) and eggs were obtained by manual stripping following hormonal induction of ovulation. For all parents a fin clip was collected and kept in 90% ethanol for DNA analyses and later parentage assignment. All fish were reared in the same tank until the age of 134 day-old post hatching (D134; 3.6 g), then a random sample of 16,000 fish was sent to the Ifremer station in Palavas (France) to be pregrown in a 5 m<sup>3</sup> tank in a recirculated system. At D156, the batch was split at random into four 5 m<sup>3</sup> tanks to lower the density. These tanks are referred to as “initial tanks” in the further statistical analyses. At D370 (35 g), 1753 fish were randomly selected from these tanks, individually PIT-tagged, fin-clipped (kept in 90% ethanol for further DNA analyses so as the parent were) and gathered in a unique tank of the same circuit. The rearing density then was maintained below 30 kg/m<sup>3</sup>, and the temperature between 20 and 22 °C during the rest of the experiment. At three occasions, because fish density reached 33 to 35 kg/m<sup>3</sup>, each batch was randomly split in two: at D504, from one tank to two, at D594 from two tanks to four and at D714 from four tanks to eight. These eight tanks are referred to as “final tanks” in the further statistical analyses.

### 2.2. Data collection

The fish were anaesthetized (0.3 ml/l phenoxy-2-ethanol), and their weight and length individually recorded at D370 (mean weight 36 g), D504 (101 g), D714 (398 g), and D986 (824 g). To simplify the writing, these stages are referred as stages 35 g, 100 g, 400 g and 800 g, respectively. Each fish was photographed at the stages 100, 400 and 800 g. At this last record the surviving fish ( $n = 1,466$ ) were killed, their sex was determined by visual examination of the gonads, and their internal deformities were recorded by the observation of the spine after removal of the left fillet. Spine deformities that could be seen externally were also visually recorded on the photographs according to individual shape characteristics. Only 1328 fish were kept for the analysis that responded to the following criteria: (i) their photographs could be exploited at the three stages 100, 400 and 800 g, (ii) they were checked for internal deformities at 800 g and (iii) they were correctly assigned to a single parental pair. Two types of deformities were assessed, i.e. lordosis (L) when spinal column had a V-shaped curvature in the sagittal plan, and scoliosis (S) when it presented an abnormal lateral curvature. Each deformity was coded e (external score) or i (internal score at stage 800 g), and deformed or normal fish scored one or zero, respectively. Whatever the type of malformation, all deformed fish were pooled to create the trait referred to as malformation (M) while the non-affected fish were scored as normal (N).

### 2.3. Parentage assignment

Parentage assignment was performed by Landcatch Natural Selection (Alloa, Scotland) using six microsatellite markers organised

in a single PCR multiplex. Parentage allocation was performed using a proprietary software for pedigree analysis. The software used two separate algorithms for pedigree assignment: a Bayesian probabilistic calculation computed the most likely parents; and a simple text matching algorithm compared parental and offspring genotypes at each locus sequentially, and excluded mismatches in turn. The two sets of results were then compared. There was almost perfect concordance between the two sets of assignments. Out of the 1753 genotyped fish, 1743 (99.4%) could be assigned to a single parental pair, of which 1328 had complete data (see before). The number of fish per family is given in Table 1.

2.4. Statistical analyses

The individual deformity scores (0 or 1 for each deformity trait) were first analysed by logistic regression based on a factorial design, using SAS® Logistic procedure to determine the potential significant fixed effects.

For the heritability calculation, malformation was considered as a threshold trait, and was first analysed on the observed scale (0 = N, 1 = M) for all data using VCE5.1.2 software (Kovac and Groeneveld, 2003), with a univariate animal model:

$$Y = X\beta + Za + \varepsilon \tag{Model 1}$$

where *Y* is the vector of observations, *β* is the vector of fixed effects (overall mean, sex, initial and final tank), *a* is the vector of random additive genetic effects and *ε* the vector of random residual effects. *X* and *Z* are known incidence matrices. Initial tank fixed effects were used to model deformities at all stages, while final tank effects were used only for deformities at stage 800 g. Maternal effect was not significant for the

malformations and therefore was not included in this model. Then, heritability estimates were transformed to the underlying liability scale using the Dempster and Lerner (1950) correction, which has been shown to yield unbiased estimates of heritability on the underlying scale in most cases (Mantysaari et al., 1991). Additionally, restricted maximum likelihood estimates of variance components for animal additive genetic and error effects were obtained directly on the underlying liability scale (LOGIT modelling) using the ASREML software (Gilmour et al., 2002). In this case, a sire and dam model with the same fixed effects was used, and heritability was estimated from the sire component of variance using the formulae from Becker (1984). The error variance for the LOGIT model was the variance of the logit link function (3.29 or π<sup>2</sup>/3).

Genetic correlations between malformations were estimated with model 1 from bivariate analyses, using the VCE5.1.2 software (Kovac and Groeneveld, 2003). Analyses were made first between internal and external malformations recorded at D986, then between types of deformities (lordosis, scoliosis and malformation) at each stage, and finally between the different stages to precise the evolution of lordosis over time. The observed scale was used to estimate the genetic correlations because genetic correlations between binary traits have been shown to be equal on the observed and on the underlying scale (Gianola et al., 1982), and because genetic correlations between one binary trait on the observed scale and a normally distributed trait are very similar to the true correlations as long as the threshold trait does not have both low heritability and low incidence (Olausson and Ronningen, 1975; Mercer and Hill, 1984). Phenotypic correlations between deformity traits were calculated as tetrachoric correlations, and their standard errors evaluated with SAS-FREQ.

A general linear model (SAS® GLM) was used to estimate least squares means of weight and length in deformed and normal fish. In

**Table 1**  
Number of offspring per family for the analysed sample of 1328 sea bass.

Sire ID	Dam ID																							Total	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23		
1	5	4	6	8	5	7	10	8	13															66	
2	3	5	4	5	16	2	7	12	8															62	
3	0	4	7	5	4	7	11	10	10															58	
4	2	2	3	4	2	5	13	5	4															40	
5	4	5	4	3	3	6	5	6	4															40	
6	2	1	8	1	4	6	7	6	6															41	
7	2	3	6	4	10	10	11	3	3															52	
8	1	5	1	4	4	3	6	2	2															28	
9	0	0	2	3	15	9	7	3	3															42	
10	6	4	3	4	9	5	7	5	7															50	
11	7	6	6	10	7	14	13	9	10															82	
12										10	3	5	5	2	5	2								32	
13										3	6	7	12	6	8	6								48	
14										6	8	3	5	10	8	3								43	
15										5	8	3	1	6	2	3								28	
16										7	5	5	8	16	9	4								54	
17										7	6	5	7	7	6	9								47	
18										2	11	7	5	11	6	1								43	
19										6	4	2	3	5	6	3								29	
20										4	7	0	9	7	10	2								39	
21										7	6	4	5	6	5	6								39	
22										8	4	7	6	7	8	3								43	
23																	0	0	0	0	0	0	0	0	0
24																	2	0	0	1	1	2	5	11	
25																	2	4	4	4	9	2	13	38	
26																	10	0	1	4	0	4	4	23	
27																	6	6	5	8	6	0	4	35	
28																	12	3	2	5	4	0	5	31	
29																	6	4	1	2	3	0	2	18	
30																	13	7	3	8	8	2	6	47	
31																	9	5	7	7	8	3	6	45	
32																	7	12	4	5	7	0	10	45	
33																	5	5	3	3	5	1	7	29	
Total	32	39	50	51	79	74	97	69	70	65	68	48	66	83	73	42	72	46	30	47	51	14	62	1328	

**Table 2**

Number and proportion of sea bass scored with and without malformation according to two types of measurement and incidence of malformation.

Record	External						Internal					
	100 g			400 g			800 g			800 g		
Stage	Lordosis	Scoliosis	Malformation	Lordosis	Scoliosis	Malformation	Lordosis	Scoliosis	Malformation	Lordosis	Scoliosis	Malformation
N affected	524	70	544	628	103	681	700	408	867	640	975	1077
N unaffected	804	1258	784	700	1225	647	328	920	461	688	353	251
Incidence (%)	39	5	41	47	8	51	53	31	65	48	73	81
Initial tank	0.0007	0.0324	0.0008	0.0196	0.1946	0.0230	0.0047	0.1646	0.0659	0.0001	0.1553	0.0337
Final tank							0.0001	0.0001	0.0001	0.0082	0.8537	0.0723
Sex	0.0150	0.4943	0.0158	0.0003	0.6953	0.0020	0.0317	0.0016	0.0240	0.2015	0.1108	0.2688
$h^2_o$	0.19 ± 0.03	0.06 ± 0.02	0.19 ± 0.04	0.19 ± 0.03	0.07 ± 0.02	0.20 ± 0.03	0.17 ± 0.03	0.11 ± 0.03	0.15 ± 0.03	0.21 ± 0.04	0.07 ± 0.02	0.10 ± 0.02
$h^2_{ASReml}$	0.27 ± 0.10	0.39 ± 0.20	0.27 ± 0.10	0.19 ± 0.08	0.33 ± 0.17	0.24 ± 0.09	0.22 ± 0.09	0.15 ± 0.08	0.13 ± 0.08	0.26 ± 0.09	0.08 ± 0.06	0.15 ± 0.08
$h^2_u$	0.31 ± 0.05	0.27 ± 0.09	0.30 ± 0.06	0.30 ± 0.05	0.23 ± 0.07	0.31 ± 0.05	0.27 ± 0.05	0.19 ± 0.05	0.25 ± 0.05	0.33 ± 0.06	0.13 ± 0.04	0.21 ± 0.04

Three stages of measurement were realised, i.e. 100 g, 400 g and 800 g. Significance levels of the effects were estimated by logistic regression. Heritability estimates ± standard errors (SE) from an animal model in VCE on the observed scale ( $h^2_o$ ), and the underlying scale using Dempster and Lerner transformation ( $h^2_u$ ), and from a sire–dam model in ASReml ( $h^2_{ASReml}$ ).

this model, the malformation effect (lordosis, scoliosis and malformation) was considered as a fixed effect, as well as sex, initial tank and final tank. Sire and dam effects were included as random effects. The least squares means were compared with a Tukey–Kramer test when the malformation effect was significant.

To estimate the genetic correlations between malformations and growth-related traits (weight and length), we used a bivariate animal model, including a non-genetic maternal random effect for the modelling of growth traits:

$$Y = X\beta + Z_1a + Z_2m + \varepsilon \tag{Model 2}$$

where Y is the vector of observations (malformation and weight or length),  $\beta$  is the vector of fixed effects (overall mean, sex, initial tank and final tank at stage 800 g), a is the vector of random additive genetic effects, m is the vector of random non-genetic maternal effects and  $\varepsilon$  the vector of random residual effects. X, Z<sub>1</sub> and Z<sub>2</sub> are known incidence matrices. Maternal random effects were applied to the modelling of growth traits (but not malformation traits), as they were shown to have an impact on the additive variance of growth traits in this experiment (Dupont-Nivet et al., 2008).

**3. Results**

*3.1. Incidence of deformities and significance of effects*

In the fish population studied, the global incidence of malformations was high (>40%, Table 2). The incidence of internal spine deformities at stage 800 g attained 81% (Mi), the proportion of lordosis (Li) and scoliosis (Si) respectively 48% and 73%, and 40% of the fish suffered both from lordosis and scoliosis. The rate of external deformities increased with age (e.g. from 41 to 65% for Me), lordosis being the main deformity scored in this way (>75% of the observed external deformities at all stages). External scoliosis had a very low

incidence level at stages 100 g (5%) and 400 g (8%) but soared to 31% at stage 800 g, even if such external determination largely underestimated the real incidence of scoliosis at this stage.

At slaughter (stage 800 g) incidence of internal and external deformities could be compared. If we refer to the internal deformity globally, among the 81% fish being internally deformed (Table 2), only 77% were externally spotted; on the contrary, among the 19% internally normal fish, 15% were scored wrongly as externally deformed. If we refer to the internal deformity type by type, among the 48% and 73% of fish respectively scored with at least one internal lordosis or scoliosis, 91% and 37% were adequately identified externally as lordotic or scoliotic, respectively.

*3.2. Heritabilities and correlations between deformities*

According to the SAS® logistic procedure, sex influenced malformations except on internal scoring, and only when their incidence was higher than 10% (P<0.05, Table 2). The initial tank effect affected significantly all deformity types at stage 100 g (P<0.05). This factor had no effect on scoliosis at stages 400 and 800 g (external and internal records), or on external malformation trait at stage 800 g. The final tank effect was significantly linked to external deformities at slaughter and to internal lordosis (P<0.05).

As expected, heritability estimates on the liability scale following the Dempster and Lerner correction ( $h^2_u$ ) were higher than heritabilities on the observed scale ( $h^2_o$ ). Heritabilities estimated directly on the liability scale with the sire component of variance from ASREML ( $h^2_{ASReml}$ ) were quite close to the estimates obtained with the Dempster and Lerner corrections, although generally lower. Heritability estimates on the liability scale ( $h^2_u$ ) had lower standard errors than  $h^2_{ASReml}$ , which could be expected as they were from an animal model, therefore taking into account the whole additive genetic relationship matrix instead of only the variation between sires. Therefore, only heritability

**Table 3**

Phenotypic (below diagonal) and genetic correlations (above diagonal) between the malformations types for two types of measurement at stage 800 g.

Record	Deformity	Internal			External		
		Malformation	Lordosis	Scoliosis	Malformation	Lordosis	Scoliosis
Internal	Malformation	<b>0.21 ± 0.04</b>	0.92 ± 0.05	0.96 ± 0.03	0.96 ± 0.04	0.93 ± 0.05	0.62 ± 0.12
	Lordosis	N/A	<b>0.33 ± 0.06</b>	0.69 ± 0.10	0.84 ± 0.05	0.99 ± 0.01	0.06 ± 0.15
	Scoliosis	N/A	0.39 ± 0.04	<b>0.13 ± 0.04</b>	0.84 ± 0.08	0.70 ± 0.10	0.90 ± 0.10
External	Malformation	0.79 ± 0.03	0.85 ± 0.02	0.54 ± 0.04	<b>0.25 ± 0.05</b>	0.92 ± 0.03	0.53 ± 0.11
	Lordosis	0.77 ± 0.03	0.92 ± 0.01	0.43 ± 0.04	N/A	<b>0.27 ± 0.05</b>	0.21 ± 0.15
	Scoliosis	0.46 ± 0.05	0.19 ± 0.03	0.45 ± 0.04	N/A	0.14 ± 0.04	<b>0.19 ± 0.05</b>

Heritabilities on the liability scale ± standard errors ( $h^2_u$  ± SE) are given in bold on the diagonal. N/A: these phenotypic correlations are meaningless as lordosis and scoliosis are included in the malformation trait.



**Table 4**  
Phenotypic (below diagonal) and genetic correlations (above diagonal) of lordosis at the different stages.

Record Stage	External			Internal
	100 g	400 g	800 g	800 g
External	100 g	<b>0.31 ± 0.05</b>	0.97 ± 0.02	0.92 ± 0.03
	400 g	0.92 ± 0.01	<b>0.30 ± 0.05</b>	0.94 ± 0.02
	800 g	0.83 ± 0.02	0.93 ± 0.01	<b>0.27 ± 0.05</b>
Internal	800 g	0.84 ± 0.02	0.93 ± 0.01	<b>0.33 ± 0.06</b>

Heritabilities on the liability scale ± standard errors ( $h_u^2 \pm SE$ ) are given in bold on the diagonal.

estimates on the liability scale ( $h_u^2$ ) will be considered in the rest of the paper. They were low to moderate for the different traits, ranging from  $0.13 \pm 0.04$  to  $0.33 \pm 0.06$ . For internal deformities recorded at stage 800 g, heritability estimates were  $0.21 \pm 0.04$  for the malformation trait, and  $0.33 \pm 0.06$  and  $0.13 \pm 0.04$  for lordosis and scoliosis, respectively.

The heritability estimates for the internal and external malformation trait were almost the same ( $0.21 \pm 0.04$  and  $0.25 \pm 0.05$  respectively, Table 3), and the genetic correlation between both was very high ( $0.96 \pm 0.04$ ) while the phenotypic correlation was moderately high ( $0.79 \pm 0.03$ ) probably due to external scoring errors. The heritabilities for internal and external lordosis were also similar ( $0.33 \pm 0.06$  and  $0.27 \pm 0.05$  respectively), and the genetic correlation between them was very close to 1 ( $0.99 \pm 0.01$ ) with a high phenotypic correlation ( $0.92 \pm 0.01$ ). Finally, although similar, the heritability estimates of internal and external scoliosis were lower than for the other deformities ( $0.13 \pm 0.04$  and  $0.19 \pm 0.05$  respectively) so as the value of their genetic correlation, even if it still remained high ( $0.90 \pm 0.10$ ). The phenotypic correlation between these both traits was low ( $0.45 \pm 0.04$ ), the limited reliability of external scored scoliosis being explained by the fact that only 37% of internal scoliosis was externally visible.

To precisely estimate the link between the different types of deformities, only internal malformations were compared to limit the bias induced by scoring errors. The moderately high genetic correlation ( $0.69 \pm 0.10$ ), and the low phenotypic correlation ( $0.39 \pm 0.04$ ) between lordosis and scoliosis could indicate they only had a partial common genetic basis, and perhaps different patterns of expression.

### 3.3. Evolution of malformations over time

Because scoliosis was difficult to evaluate externally and the malformation trait was a combination of all deformities, the most precise trait to study the malformations evolution over time was lordosis. Its heritability was around 0.30 at all stages (Table 4), and genetic correlations between measurements were high ( $0.92 \pm 0.03$  to  $0.99 \pm 0.01$ ), meaning that lordosis evaluated from stage 100 g had the same genetic basis than as those evaluated at stage 800 g, although the incidence was different (39% vs. 53%). On the contrary, phenotypic

correlations slightly decreased over time from  $0.93 \pm 0.01$  to  $0.83 \pm 0.02$ , probably due to this variation in incidence.

### 3.4. Deformities and growth

Least squares means of weight and length at the different stages are given in Table 5, for fish affected or not by an internal malformation (global malformation, lordosis or scoliosis). Deformed fish were significantly heavier at stages 35 and 100 g but shorter at stages 400 and 800 g. Those affected by lordosis were shorter at all stages and lighter at stages 400 and 800 g. Those affected by scoliosis were heavier and longer at stages 35 and 100 g but shorter at stages 400 and 800 g.

The genetic correlations between weight, length and the different types of deformities are shown in Table 6. Malformation trait and scoliosis were positively correlated with weight ( $0.30 \pm 0.15$  and  $0.41 \pm 0.19$  on average, respectively), but less with length ( $0.17 \pm 0.15$  and  $0.28 \pm 0.19$ , respectively). On the contrary, lordosis was weakly but negatively correlated with weight and length, and more related to length than weight ( $-0.11 \pm 0.11$  and  $-0.28 \pm 0.13$ , respectively). Globally, the standard errors were relatively high (around 0.15), meaning that the genetic correlations were not different from zero in many cases. The only significant ones were positive correlations between weight and malformations at 100 and 400 g, between weight and scoliosis at 35 and 800 g and negative correlations between length and lordosis at 800 g.

## 4. Discussion

### 4.1. Incidence and heritability

First of all, it is important to point out that our experimental population was unusual as it exhibits a very high malformation rate (81%). According to Kause et al. (2007) such a high incidence would suggest that deformities were induced by a single major stressor. Indeed, we sensed that the likeliest cause lied in the hydrodynamics conditions the post-larvae were subjected to during pre-growing, i.e. the juveniles were maintained, from 3.6 g to the end of the experiment, in  $5 \text{ m}^3$  circular tanks the main characteristic of which is the self-cleaning by a circular water current. Besides, it is known that intense swimming provoked by such a water current is not suitable for juveniles that have not completed their bone calcification, because fast swimming increases muscle mechanical constraints on vertebrae (Chatain, 1994b). This hypothesis would be supported by the fact that another batch of same fish kept in "soft swimming" conditions in a raceway did not suffer from such deformities, and does not exclude that hydrodynamics might only reveal the fish sensitivity to spinal deformities, the skeletal development remaining under genetic control (Wagner and Karsenty, 2001).

The first finding of this study was that the spine deformities in the European sea bass could have a heritable component. The heritability of the internal malformation trait was indeed estimated to be  $0.21 \pm 0.04$  on the underlying scale using Dempster and Lerner correction, which is

**Table 5**  
Least squares means of fish affected or not by internal deformities at 800 g for weight (W) and length (L) at all stages adjusted for internal deformities effect;  $P$  = probability value of the  $F$  test for difference between affected and non-affected fish.

Stage	Malformation			Lordosis			Scoliosis			
	Affected	Unaffected	$P$	Affected	Unaffected	$P$	Affected	Unaffected	$P$	
W	35 g	38.77 ± 0.43	36.52 ± 0.72	0.0019	38.41 ± 0.51	38.32 ± 0.51	0.8751	39.10 ± 0.44	36.21 ± 0.62	≤0.0001
	100 g	108.36 ± 1.03	102.32 ± 1.72	0.0005	107.32 ± 1.21	107.21 ± 1.22	0.9375	109.30 ± 1.05	101.34 ± 1.49	≤0.0001
	400 g	416.76 ± 3.76	413.97 ± 6.24	0.6548	404.14 ± 4.33	428.63 ± 4.36	≤0.0001	420.35 ± 3.83	404.31 ± 5.41	0.0033
	800 g	864.86 ± 7.64	885.87 ± 12.70	0.0982	827.66 ± 8.68	910.58 ± 8.74	≤0.0001	873.89 ± 7.82	853.44 ± 11.03	0.0663
L	35 g	121.62 ± 0.43	121.31 ± 0.72	0.6728	120.27 ± 0.50	122.88 ± 0.50	≤0.0001	121.92 ± 0.44	120.50 ± 0.62	0.0241
	100 g	166.26 ± 0.51	165.85 ± 0.85	0.6343	164.57 ± 0.59	167.83 ± 0.59	≤0.0001	166.68 ± 0.52	164.72 ± 0.73	0.0082
	400 g	251.49 ± 0.81	259.39 ± 1.34	≤0.0001	245.35 ± 0.88	260.66 ± 0.89	≤0.0001	252.30 ± 0.84	254.72 ± 1.18	0.0430
	800 g	312.62 ± 1.16	329.45 ± 1.92	≤0.0001	301.54 ± 1.20	330.12 ± 1.21	≤0.0001	314.08 ± 1.21	320.33 ± 1.71	0.0003

**Table 6**

Genetic correlations and standard errors between weight, length at different stages and internal deformities at 800 g.

Stages	Weight				Length			
	35 g	100 g	400 g	800 g	35 g	100 g	400 g	800 g
Malformation	0.21 ± 0.15	0.38 ± 0.15	0.40 ± 0.14	0.21 ± 0.16	0.13 ± 0.14	0.32 ± 0.16	0.25 ± 0.15	−0.01 ± 0.16
Lordosis	−0.10 ± 0.11	−0.03 ± 0.12	−0.08 ± 0.07	−0.25 ± 0.13	−0.23 ± 0.12	−0.13 ± 0.14	−0.30 ± 0.14	−0.45 ± 0.12
Scoliosis	0.40 ± 0.17	N/A	N/A	0.43 ± 0.21	0.37 ± 0.19	N/A	N/A	0.19 ± 0.19

N/A: not available due to lack of model convergence.

the range of heritability estimates for vertebral deformity found in other species, *i.e.* 0 to  $0.36 \pm 0.14$  in four year-classes of farmed Atlantic salmon *S. salar* (Gjerde et al., 2005), 0.64 for the same species estimated on the liability scale based on the sire component of variance (McKay and Gjerde, 1986), and 0.27 in Atlantic cod, *Gadus morhua* using a mixed linear animal model (Kolstad et al., 2006a).

#### 4.2. Correlations between malformations

The correspondence between internal and external malformations is clearly established here according to the high genetic correlations found ( $>0.90$ ). The heritability estimates are almost the same for all malformations, and the phenotypic correlations are relatively high ( $0.77 \pm 0.03$ – $0.92 \pm 0.01$ ) with an exception for scoliosis ( $<0.54 \pm 0.04$ ). Indeed, the difference between internal lordosis and scoliosis was expressed by a low phenotypic correlation ( $0.39 \pm 0.04$ ), and a moderate genetic correlation ( $0.69 \pm 0.10$ ). Scoliosis seems to be less heritable than lordosis, and fish may have a differential genetic sensitivity to lordosis and scoliosis whereas the malformation trait can be approached like a standard measure of both lordosis and scoliosis. Even though Afonso et al. (2000) suggested that a triple column abnormality in sea bream consisting of a consecutive repetition of lordosis–scoliosis–kyphosis (LSK) from head to tail, had polygenic origin, here, scoliosis and lordosis, although significantly linked, cannot be considered strictly as the same trait.

This study also points out that, in sea bass, external recording of malformations underestimates the true deformity rate, as less severe deformities like scoliosis may remain unseen. This was already shown in other species, for example, in Atlantic cod, the proportion of malformations determined by visual examination of shape, varied from 28 to 74% according to rearing sites (Kolstad et al., 2006b). In the Atlantic salmon, Fjellidal et al. (2007) found a prevalence of vertebral deformities of 12.4% when evaluated on radiographs, and 1.3% when externally recorded. On the same species, Witten et al. (2006) and McKay and Gjerde (1986) found prevalences of vertebral deformities of 12% both on radiological and/or external examinations, but in the four-year classes, malformations rates varying between 1.5 and 25.2% when estimated by external examinations (Gjerde et al., 2005). The low phenotypic correlation between external and internal scoliosis found in this study confirms the difficulty to distinguish by external examination animals suffering from scoliosis. Therefore, the validity of heritability and genetic correlations for the “external scoliosis” trait in the present experiment should be taken with caution. In the framework of a breeding programme, external recording of deformities would therefore be relevant in a family-based scheme, as it would allow effective ranking of families thanks to the high genetic correlation between external deformities and the “real” (internal) deformity trait. However, in the case of individual selection, this might not be as effective, especially for detecting scoliosis, the X-ray remaining the only method to clearly perceive them without killing the fish.

#### 4.3. Evolution of malformations over time

Even if the severity of deformities may increase with age and the early visual evaluation may be more difficult than at a later stage, the homogenous heritabilities estimated at the different stages for

lordosis indicate that the deformity was present from the beginning of rearing period, but not pronounced enough to be externally detected. This is supported by the high genetic correlations between deformities at different stages ( $>0.92 \pm 0.03$ ). Indeed, the number of deformed fish progressively increased in each class of malformation and no fish classified as deformed precociously became normal later. This observation supports the hypothesis that prematurely deformed fish remained deformed and that normal fish could become deformed. The ontogenesis of deformities can then be explained by both genetic and environmental factors, the prevalence of the latter depending of its intensity and the development stages during which it impacts.

#### 4.4. Malformation and growth

The fish that were affected by scoliosis at slaughter were found equivalent in weight and length to those that did not present such a deformity. They were nevertheless shown to have been larger (both length and weight) than non-affected fish at 35 and 100 g. For lordosis and malformation trait, the picture was less clear. However, it is highly unlikely that spine deformities could have a positive impact on growth, as they mechanically shorten the fish, and probably reduce their access to food by preventing correct swimming as suggested in different species where malformation often related to reduce growth rate (McKay and Gjerde, 1986; Kause et al., 2005; Kocour et al., 2006). Still, fast growth was also recognized to be a risk factor for spinal malformations (Rauw et al., 1998) a phenomenon that fits well to our study, and that would occur as follows: shortly after the onset of malformations, more deformities would affect fast-growing fish but with little effect, then as the fish grow, their deformities progressively aggravate (as described in salmon; Witten et al., 2006), and have a negative impact on growth. This would be confirmed by the positive genetic correlation found between scoliosis or malformation and weight at the different ages, even though the standard errors are large, and these estimates have to be taken cautiously, as the only significant correlations were with weight and malformations at 100 and 400 g, and with weight and scoliosis at 35 and 800 g. It would also confirm previously suspected links between fast growth rate and sensitivity to spine deformities in cod (Kolstad et al., 2006a), and sea bass (Vandeputte et al., 2009). Furthermore, the fish used for this analysis have grown in particular conditions, which are known to induce spinal deformities, here at a very high incidence. At high levels of deformity rate, families may show different but consistent sensitivities to the stressor, leading to high genetic variation and moderate heritability, while the genetic architecture depends on the existing expression of trait and deformities at different generations may not share common genetic determination (Kause et al., 2007).

However, heritabilities of malformation traits calculated here are only estimates that may indirectly describe a sensitivity to the particular pre-growing environment endured by fish characterised by different body weight. Indeed, if the occurrence of malformations in a batch depends on a limiting factor affecting the body weight at a certain age, then big or small fish would be differently affected by the malformation according to their weight. This would be supported by the highest genetic correlation with growth observed at the younger ages, and by the instability of the genetic basis of such type of traits according to year-classes (Gjerde et al., 2005; Kause et al., 2007). It

would be strongly recommended to proceed to the quantification of realised heritability before concluding to the existence of a genetic basis of spinal malformations such as lordosis and/or scoliosis.

Nevertheless, as in this study, the heritability of malformations was significant, it can be safely recommended, by precaution, to eliminate deformed fish from a breeding nucleus. However, this trait has to be monitored carefully in breeding programmes directed to fast growth because eliminating deformed fish while major risks of malformations are suspected to lie on early fast-growing individuals, might not permit to fully exploit the genetic potential for growth. In another experiment, selection for growth in sea bass did not result in a higher percentage of deformed fish in the offspring of selected males (Vandeputte et al., 2009). However, the fact that deformed males were removed from the selection process in this latter experiment could alter the results.

Finally, to clearly demonstrate the genetic basis of such malformations, we would recommend to proceed to intentional crossing between malformed fish to evaluate their effective predisposition to produce malformed progenies. Moreover, because environmental variations were clearly shown to impact the expression of deformities (Kause et al., 2007; Kolstad et al., 2006b; Witten et al., 2006), the future experiments should also include the determination of the interactions between environment and malformations.

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