



# The Effect of the Deformity Genetic Background of the Breeders on the Spawning Quality of Gilthead Seabream (*Sparus aurata* L.)

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Fish egg quality is strongly related with the ability of the egg to be fertilized and develop a normal embryo with good survival and a lack of abnormalities. Large variations in the spawning quantity or quality impact directly in the competitiveness and sustainability of hatcheries, which create an overly large broodstock in order to satisfy the on-growing companies' demand for undeformed fry. The present study reports, for the first time in relation to gilthead seabream, the effect of the genetic background of breeders for presence or absence of deformity on their spawning quality and the importance of considering this when creating broodstock. The spawning quality of crosses of breeders with genetic background for presence or absence of deformity (EBV<sub>def</sub>), were evaluated during a whole spawning season, through study of the following traits: oocyte yield, fertilization rate, viability rate, hatching rate, larval survival rate, fertilized eggs, viable eggs, hatched eggs, and number of alive larvae. Breeders with a genetic background for deformity and a normal phenotype had shorter spawning periods, lower oocyte yield and, consequently, produced a lower number of alive larvae. In these two traits, the genetic background of breeders was of greater importance during intermediate spawning periods, when spawning is generally considered optimal for the industry, while environmental factors were more important at the beginning and end of the spawning season. In conclusion, these results demonstrate the importance of controlling the breeders' genetics when creating broodstock.

**Keywords:** gilthead seabream, estimated breeding value, skeletal deformity, spawning quality, oocyte yield, viability rate, number of alive larvae

## INTRODUCTION

The gilthead seabream (*Sparus aurata* L.) is a demersal and eurythermal species with a large reproductive capacity. It is found in subtropical coastal areas, from the Eastern Atlantic to the Mediterranean (Froese and Pauly, 2019). In the wild spawning starts in October in the Gulf of Cadiz or December-January in eastern Mediterranean and lasts for 3–4 months (Arias, 1980; Kissil et al., 2001). Every female releases over two million eggs per kilogram they weigh (APROMAR, 2017). In aquaculture, larval rearing of the gilthead seabream is carried out at 16–24°C (Tandler et al., 1989; Koumoundouros et al., 1997a,b; Shields, 2001), an optimal

temperature for the *hatching rate* and *larval survival rate* at the yolk sac resorption stage (Polo et al., 1991). The wide natural distribution and environmental conditions experienced by the gilthead seabream have made it one of the most important species in the European marine fish farming and production is still expanding worldwide. In a European context, gilthead seabream was fifth in terms of annual production, at 91,964 tons, and fourth in terms of economic value (APROMAR, 2020).

Breeding programs are essential tools that contribute to the long-term development of aquaculture in order to improve the quality of the farmed strains. Aquaculture production based on genetic selection schemes has increased from 1% in 1997 to 80–83% in 2017 (Gjedrem, 1997, 2004, 2012; Janssen et al., 2017). In gilthead seabream, genetically selected seed production only accounts 31–44% of total fry in the European market (Chavanne et al., 2016), with an estimated increase in growth of 5–29% (Knibb, 2000; Brown, 2003; Thorland et al., 2015). However, in this species quality of morphology and growth traits are deemed to have the same level of importance in the industry (Janssen et al., 2017) as the fish are sold whole and due to the loss of market value throughout the value chain (Bardon et al., 2009; Boglione and Costa, 2011; Boglione et al., 2013).

Skeletal deformity is the most important trait when defining the morphological quality of the fish, reducing the physiological ability of fish for correct development i.e., reducing their growth rate, increasing their mortality rate and significantly affecting their welfare (Andrades et al., 1996; Karahan et al., 2013; García-Celdrán et al., 2016). The presence of deformities can affect up to 30% of production, causing an annual loss of more than €50M in the European aquaculture industry (Castro et al., 2008; Fernández et al., 2008; Haga et al., 2011). In gilthead seabream, deformity appears early in development and its prevalence increases with age (Lee-Montero et al., 2015).

Abiotic, biotic, xenobiotic, nutritional and environmental factors have been studied as the causes of deformities (Afonso and Roo, 2007), as well as genetic factors or interactions (Andrades et al., 1996; Afonso et al., 2000; Fernández et al., 2008). Although environment has a large effect on deformity, different families or cohorts have shown a variable response to specific environmental stressors, suggesting additive genetic variation in these traits (Kause et al., 2007; Lee-Montero et al., 2015; García-Celdrán et al., 2016), the presence of QTLs (Negrín-Báez et al., 2015a,b) or inbreeding depression as a consequence of the consanguinity level (Aulstad and Kittelsen, 1971; Kincaid, 1983). In gilthead seabream, segregation of a major gene (Astorga et al., 2004) and polygenic inheritance (Afonso et al., 2000; Lee-Montero et al., 2015) have been suggested as explanations for the incidence of skeletal deformities. Negrín-Báez et al. (2015b) reported a significant excess of descendants with severe deformities from directed crosses involving parents with the same deformities, suggesting the elimination of deformed fish from a breeding nucleus or the inclusion of this trait in breeding programs.

In sparid species, evidence of mineralization can be discerned from 30 DPH (Socorro, 2006), where the temperature-dependent differential expression of genes associated with bone formation suggests the long-term regulation of osteogenesis (Riera-Heredia et al., 2018). In gilthead seabream, jaw and mouth bones are

formed after the onset of exogenous feeding (Faustino and Power, 2005). In fact, additive components already appear from the larvae (Fragkoulis et al., 2018, 2020) and fingerling stages (García-Celdrán et al., 2016).

Therefore, a major issue in the aquaculture industry is the production of a large number of eggs through controlling gamete quality in order to satisfy market demand for seeds through breeding programs (Bobe and Labbé, 2010; Migaud et al., 2013). Fish egg quality, measured through traits such as *fertilization rate*, *oocyte yield* and *alive offspring number*, is considered very important in accordance with European scientific criteria (AQUAEXCEL, 2013), including good survival and lack of abnormalities (Kjørsvik et al., 1990; Brooks et al., 1997). Gametes, both in terms of their quantity and quality, are closely associated with the competitiveness and sustainability of hatcheries and farms (Bromage et al., 1992; Migaud et al., 2013; Theodorou et al., 2016).

The spawning quality of breeders depends of many factors related with nutrition, genetics, environmental conditions and any stress (Brooks et al., 1997; Fernández-Palacios et al., 1997; Almansa et al., 1999; Bobe and Labbé, 2010; Scabini et al., 2011; Jerez et al., 2012; Migaud et al., 2013; Bobe, 2015). Many traits related to reproduction, survival, morphology and growth (Kjørsvik et al., 1990, 2003; Bromage et al., 1992; Fernández-Palacios et al., 1995; Brooks et al., 1997; Shields et al., 1997; Lahnsteiner and Patarnello, 2004, 2005; Lahnsteiner et al., 2008; Bobe and Labbé, 2010; Migaud et al., 2013; Bobe, 2015) have been proposed for the characterization of spawning quality in different species. At the industrial level it is important to start fattening a suitable quantity and quality of animals. Spawning quality can affect the success of juveniles in the completion of internal metamorphosis (Kjørsvik et al., 2003), survival, and the appearance of deformities (Bonnet et al., 2007).

The aims of this study were to research, for the first time in gilthead seabream, the additive genetic component effect of skeletal deformities on spawning quality and to establish the main traits for evaluating reproductive ability at an industrial level. For this purpose, estimated breeding values for the presence or absence of deformity when the breeders were a commercial size ( $EBV_{def}$ ) was calculated and their reproductive ability traced throughout an entire spawning season.

## MATERIALS AND METHODS

All animal experiments were conducted in accordance with the European Union Directive (2010/63/EU) on the protection of animals for scientific purposes, at the Fundación Canaria Parque Científico Tecnológico (FCPCT) of the Universidad de Las Palmas de Gran Canaria (Canary Islands, Spain).

### Biological Material and Experimental Design

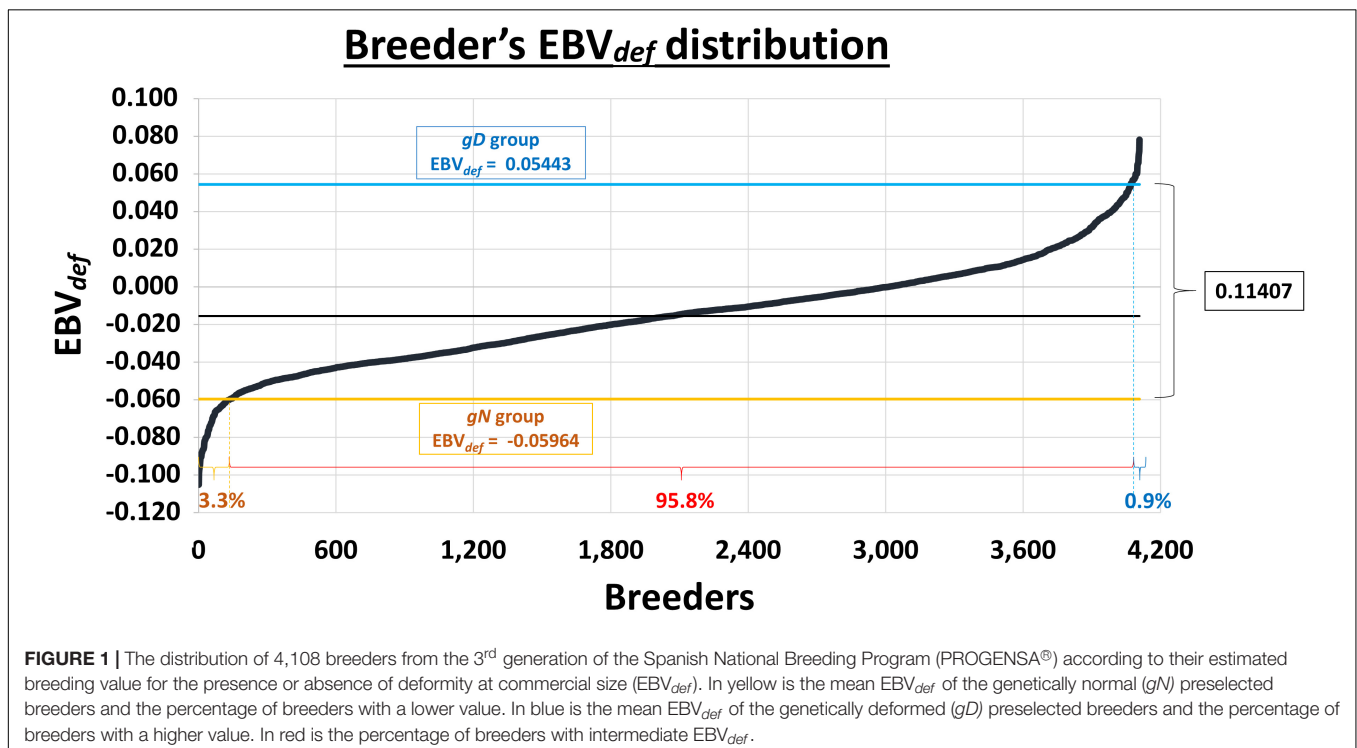
A total of 4,108 gilthead seabream adults from the Canary Islands region and the 3<sup>rd</sup> generation of the Spanish National Breeding Program (PROGENSA®) (Lee-Montero et al., 2015) at AQUANARIA S.L. were visually assessed for the presence

or absence of deformity at commercial size (ATOL:0000087), in accordance with AquaExcel-ATOL (AQUAEXCEL, 2013). The genotyping of all animals evaluated for morphology was also established through the molecular characterization of microsatellite markers (SMSa1), with PCR methodology, following the instructions of its authors (Lee-Montero et al., 2013). The genetic relationship between descendants (study population) and their parents (parental assignment) was reconstructed, comparing breeders' and descendants' genotypes by using VITASSIGN software (Vandeputte et al., 2006). Genetic parameters were estimated for both data matrix (phenotypic and genotypic information) by using the restricted maximum likelihood method with VCE 6.0 software (Neumaier and Groeneveld, 1998; Groeneveld et al., 2010). A stock of breeders was then preselected on the basis of its  $EBV_{def}$  for conditioning and sexing before the spawning season, and moved to the FCPCT-ULPGC facilities. Fish with a deformed phenotype were discarded.

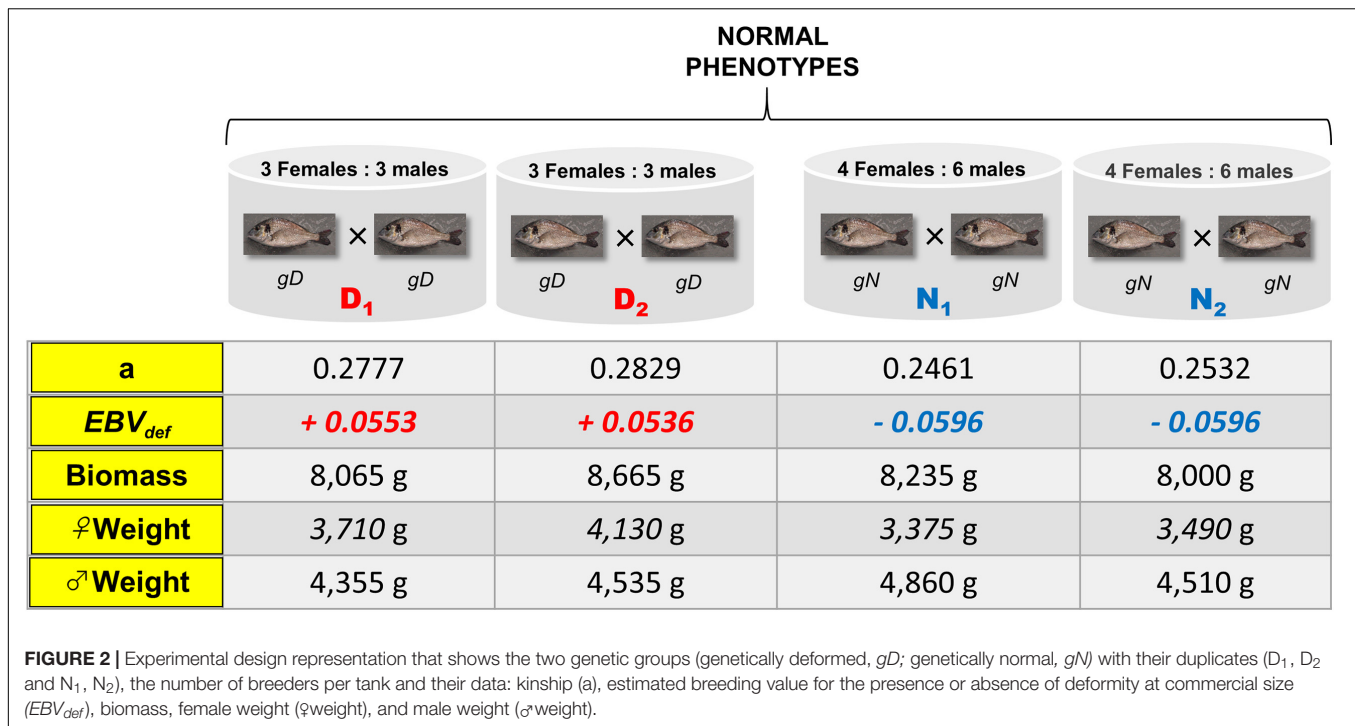
The  $EBV_{def}$  of the 4,108 gilthead seabream stock ranges between  $-0.10515$  for normality and  $+0.07821$  for deformity (Figure 1), with an average value of  $-0.01551$  and a standard deviation value of  $0.02681$ . After discarding the dead and misshapen, a total of 32 breeders were selected on the basis of their  $EBV_{def}$ , gender, weight and relationship coefficient. In this way, it was possible to establish two groups of breeders with opposite values in their  $EBV_{def}$ , which corresponds to a value of  $+0.05443$  in the group of genetically deformed (*gD*) breeders, and  $-0.05964$  in the group of genetically normal (*gN*) breeders. Between  $EBV_{def}$  values of *gD* and *gN* selected breeders, was contained almost 96% of the evaluated population (Figure 1).

Two different types of crosses, with different  $EBV_{def}$ , were established in  $2\text{ m}^3$  cylindroconical tanks, with duplicates to minimize the environmental factors. The first was a cross of *gN* fish (N1 and N2) and the second a cross of *gD* fish (D1 and D2), with the same intensity of selection but reverse  $EBV_{def}$  ( $EBV_{def} = -0.05$  vs.  $EBV_{def} = +0.05$ , respectively). The crosses were established by taking into account the  $EBV_{def}$ , relationship coefficient, biomass, and the weight ratio of males and females, so that the duplicates were similar and the differences between crosses were exclusively due to the  $EBV_{def}$ . The biomass sex-ratio (kg male/kg female) was similar between tanks; 1.43, 1.3, 1.18, and 1.1 for N1, N2, D1, and D2, respectively, with a mean of 1.28 kg male/kg female, as recommended by Fernández-Palacios et al. (1995, 1997). All breeders had normal phenotypes and the same age, between and within crosses, aiming to avoid the impact of females age on spawning quality (Jerez et al., 2012), by keeping the biomass sex-ratio constant. Thus, breeders' maturation and spawning occurred spontaneously under natural conditions. The coefficient of relationship,  $EBV_{def}$ , total biomass, male and female biomass, number of males and females were measured in breeders in the four experimental tanks (Figure 2). Tanks were supplied with 16 L/min seawater at  $7.98 \pm 0.10$  pH and kept under natural light photoperiod (11–13 h light). Breeders were fed a commercial diet (Skretting ARC, Stavanger, Norway).

During the spawning season, the monthly average temperature decreased between December and February from  $20.9$  to  $19.9^\circ\text{C}$ , followed by an increase to  $22.8^\circ\text{C}$  in June. Food intake, expressed as a percentage of biomass, remained constant throughout the experiment with a range from 0.1 to 1.1% and an average value of 0.4% in all tanks.



**FIGURE 1 |** The distribution of 4,108 breeders from the 3<sup>rd</sup> generation of the Spanish National Breeding Program (PROGENSA<sup>®</sup>) according to their estimated breeding value for the presence or absence of deformity at commercial size ( $EBV_{def}$ ). In yellow is the mean  $EBV_{def}$  of the genetically normal (*gN*) preselected breeders and the percentage of breeders with a lower value. In blue is the mean  $EBV_{def}$  of the genetically deformed (*gD*) preselected breeders and the percentage of breeders with a higher value. In red is the percentage of breeders with intermediate  $EBV_{def}$ .



## Spawning Quality Traits

During the spawning season, eggs were collected daily (20 h after release and fertilization) from each tank overflow in semi-submerged 500- $\mu\text{m}$  mesh net egg collectors placed in a nearby incubation tank. The *oocyte yield* (ATOL:0001723), *fertilization rate* (ATOL:0001775), *viability rate* (ATOL:0001531), *hatching rate* (ATOL:0001531), *larvae survival rate* at yolk sac resorption (ATOL:0001531) were measured daily in accordance with AquaExcel-ATOL (Kjørsvik et al., 1990; Fernández-Palacios et al., 1995, 1997; AQUAEXCEL, 2013).

Samples of eggs were collected and observed under a binocular stereo microscope. The *oocyte yield*, *fertilization rate* and *viability rate* were estimated volumetrically after samples were counted. In addition, two 96-well plates were used to hold 192 floating eggs (1 per well) with 200  $\mu\text{l}$  filtered seawater and incubated at 19°C. After 24 and 96 h, the *number of alive larvae* was counted in order to calculate the *hatching rate* (*number of hatched larvae/number of fertilized eggs*) and the *survival larval rate* (%).

The *number of fertilized eggs*, *viable eggs*, *hatched eggs* and the *number of alive larvae* were also calculated as associated traits, since they represent the ultimate aim of the industry, a collection of quantitative and qualitative data that determines the final number of larvae available for rearing.

## Statistical Analysis

The data were tested for normality using the one sample Kolmogorov–Smirnov test, as well as for homogeneity of variance using Levene's test. When a normal distribution and/or homogeneity of variance was not achieved, data were subjected to the Kruskal–Wallis non-parametric test (Zar, 1984). To study the variation of spawning quality traits throughout the whole

spawning season, comparisons between types of crosses (*gD* vs. *gN*) and between experimental tanks ( $D_1$ ,  $D_2$ ,  $N_1$ ,  $N_2$ ) were carried out, using the day as a unit of measurement (Table 1). The variation of spawning quality traits was also studied in short time periods, structuring the whole spawning season into twelve fortnights. Each spawning quality trait was compared between types of crosses (*gD* vs. *gN*) within each fortnight. Additionally, each trait was compared within types of crosses (*gD* or *gN*) between fortnights (Table 2). The following general linear model was used:

$$Y_{ijk} = \mu + \alpha_i + \beta_{ij} + \varepsilon_{ijk}$$

Where,  $\mu$  is the mean of the population,  $\alpha_i$  is the effect of the genetic factor (types of crosses),  $\beta_{ij}$  is the effect of the tank factor within genetic factor, and  $\varepsilon_{ijk}$  is the residual error.

For the *oocyte yield* and *number of alive larvae* traits, within fortnights, the influence of genetic and tank factors was measured by the following statistical algorithm (SA): execution of a standard regression analysis, and estimation of variance components of principal factors. Within fortnights, the same statistical procedure SA was also applied to explain the *number of alive larvae* trait using as predictors the *oocyte yield*, *fertilization rate*, *viability rate*, *hatching rate* and *larvae survival rate* traits. Statistical analysis was performed using the SPSS package, version 22.0 (SPSS Inc, Chicago, United States). All statistically significant comparisons of this work, Tables 1, 2, include the correction factor of Bonferroni's to minimize the Type-I error (Gordon et al., 2007; see **Supplementary Material** for differences between Bonferroni and Benjamini-Hochberg tests). Thus, significant differences were considered from 0.05, as confident level.

**TABLE 1** | The mean values of spawning quality traits throughout the spawning season (*oocyte yield, fertilization rate, viability rate, hatching rate, larvae survival rate, fertilized eggs, viable eggs, hatched eggs, number of alive larvae*) per Tank (D1, D2, N1, N2) and per Genetic group (*gD, gN*).

Trait	Tank				Genetic group	
	D1	D2	N1	N2	gD	gN
<i>Oocyte yield</i> ( $10^3$ )	4.7 ± 0.4	8.3 ± 0.6	38.2 ± 1.0 <sup>a</sup>	30.4 ± 0.9 <sup>b</sup>	6.5 ± 0.4 <sup>B</sup>	34.3 ± 0.7 <sup>A</sup>
<i>Fertilization rate</i> (%)	98.4 ± 0.3 <sup>a</sup>	90.8 ± 1.2 <sup>b</sup>	96.1 ± 0.4	95.4 ± 0.6	94.1 ± 0.8	95.8 ± 0.3
<i>Viability rate</i> (%)	44.9 ± 3.5	39.9 ± 3.0	49.9 ± 1.2 <sup>a</sup>	40.9 ± 1.7 <sup>b</sup>	42.0 ± 2.3	45.3 ± 1.1
<i>Hatching rate</i> (%)	96.1 ± 0.3 <sup>a</sup>	84.9 ± 1.1 <sup>b</sup>	89.2 ± 1.2 <sup>a</sup>	87.6 ± 0.8 <sup>b</sup>	89.8 ± 0.8	88.4 ± 0.7
<i>Larvae survival rate</i> (%)	82.3 ± 1.5 <sup>a</sup>	70.2 ± 2.1 <sup>b</sup>	82.2 ± 0.9	79.3 ± 1.2	75.4 ± 1.4 <sup>B</sup>	80.8 ± 0.7 <sup>A</sup>
<i>Fertilized eggs</i> ( $10^3$ )	4.6 ± 0.4	7.4 ± 0.5	36.8 ± 1.0 <sup>a</sup>	29.2 ± 0.9 <sup>b</sup>	6.0 ± 0.3 <sup>B</sup>	33.0 ± 0.7 <sup>A</sup>
<i>Viable eggs</i> ( $10^3$ )	2.0 ± 0.2	3.6 ± 0.4	18.7 ± 0.7 <sup>a</sup>	13.6 ± 0.8 <sup>b</sup>	2.8 ± 0.2 <sup>B</sup>	16.1 ± 0.5 <sup>A</sup>
<i>Hatched eggs</i> ( $10^3$ )	1.9 ± 0.2	3.0 ± 0.3	16.7 ± 0.6 <sup>a</sup>	11.9 ± 0.7 <sup>b</sup>	2.5 ± 0.2 <sup>B</sup>	14.1 ± 0.4 <sup>A</sup>
<i>N° alive larvae</i> ( $10^3$ )	1.6 ± 0.2	2.1 ± 0.2	13.7 ± 0.5 <sup>a</sup>	9.5 ± 0.5 <sup>b</sup>	2.1 ± 0.2 <sup>B</sup>	12.3 ± 0.4 <sup>A</sup>

Results are expressed as means ± SEM. For each trait, values in the same row followed by different letters are significantly different ( $P < 0.05$ ), values between genetic groups are expressed with capital letters, and values within genetic groups between tanks with lower-case letters.

## RESULTS

In all experimental tanks, breeders spawned daily between December 2018 and June 2019. The numbers of spawning days registered were 86, 116, 176, and 177 days for tanks D1, D2, N1, and N2, respectively. In **Table 1**, the mean values of all spawning quality traits are reported per tank and type of cross. In **Table 2**, the comparisons for all spawning quality traits are presented: within fortnight per types of crosses, and within type of cross per fortnights.

### Oocyte Yield

*Oocyte yield* contribution among tanks, for both types of crosses, was generally higher and more reproducible in the middle of the spawning season and lower at either ends of it (**Figure 3**).

Across the whole spawning season the values between tanks were only significantly different within the *gN* group. Between genetic groups, the *gN* group reported a value 5.27 times higher than the *gD* group (**Table 1**). This significant difference was maintained across all fortnights (**Table 2** and **Figure 4A**). Over the fortnights, within each genetic group both registered their best values in the first phase of the spawning season (fortnights two–five for *gD*, and fortnights one–seven for *gN*). Within fortnights *oocyte yield* was explained by genetic factor, from 29.1% in fortnight twelve to 90.9% in fortnight six (**Figure 5A**) and a mean value across fortnights of 59.6%. The tank factor explained less in the middle (0% in fortnight six) and more at the beginning (44% in fortnight two) and at the end (65% in fortnight eleven) of the spawning season, with a mean value of 23.5%.

### Fertilization, Viability, and Hatching Rates

In the whole spawning season these three traits did not report statistical differences between genetic groups, while statistical differences were estimated within genetic groups between tanks (**Table 1**).

Within fortnights, statistical differences were detected between genetic groups in fortnight two for *fertilization rate*, in fortnights three, five, six, eight, and nine for *viability rate*, and

in fortnights one, three, and nine for *hatching rate* (**Table 2** and **Figures 4B–D**). The *fertilization rate* of *gD* was lower than for *gN* across the fortnights. The *viability rate* of *gD* was higher than *gN* across fortnights at the beginning of the spawning season (fortnights one, two, and three) and lower than *gN* in the rest of the fortnights. The trend for *hatching rate* between *gD* and *gN* groups did not follow any regular pattern (**Table 2**).

Between fortnights, within genetic groups, *fertilization rate* for both groups showed a mild increase toward the end of the spawning season. In the case of *gN* group, this rate decreased in the last two fortnights. For both genetic groups, the *viability rate* reported increases from the beginning of the spawning season, peaking in fortnight three and then decreasing until the end. The *viability rate* was more stable for the *gN* group. The *hatching rate* did not report any differences (**Figures 4B–D**).

### Larval Survival Rate

For the whole spawning season, the *gN* group reported a value 7.2% higher than the *gD* group (**Table 1**). Within the same genetic group between tanks, only significant differences were found for the *gD* group.

Within fortnights, at the beginning of the spawning season (fortnights one–three), the *gD* group showed higher values than the *gN* group, a trend which was reversed for the rest of the spawning season (**Table 2** and **Figure 4E**). Regarding the evolution of this trait across the fortnights, it was observed that the values of the *gN* crosses were generally constant throughout the spawning period, while in the *gD* crosses the values declined slightly at the end of the spawning period (**Figure 4E**).

### Fertilized Eggs, Viable Eggs, Hatched Eggs, and Number of Alive Larvae

In the whole spawning season these traits all differed significantly between genetic groups, the *gN* group being almost 6 times higher than *gD* group (**Table 1**). Within the same genetic group between tanks there were significant differences only in the *gN* group. Within fortnights the *gN* group was superior throughout the whole spawning season (**Table 2** and **Figure 4F**). Between

**TABLE 2** | The mean values of spawning quality traits throughout the spawning season, by fortnight (oocyte yield, fertilization rate, viability rate, hatching rate, larval survival rate, fertilized eggs, viable eggs, hatched eggs, number of alive larvae) per Genetic groups (gD, gN).

Trait	Genetic	14-31/12/2018	01-15/01/2019	16-31/01/2019	01-14/02/2019	15-28/02/2019	01-15/03/2019	16-31/03/2019	01-15/04/2019	16-30/04/2019	01-15/05/2019	16-31/05/2019	01-15/06/2019
Oocyte yield (10 <sup>5</sup> )	gD	2.8 ± 1.3 <sup>b</sup>	DE 11.5 ± 2.2 <sup>b</sup>	ABC 13.2 ± 0.8 <sup>b</sup>	A 11.8 ± 1.3 <sup>b</sup>	AB 12.5 ± 0.9 <sup>b</sup>	A 9.3 ± 0.7 <sup>b</sup>	AB 7.4 ± 0.6 <sup>b</sup>	ABC 5.0 ± 0.8 <sup>b</sup>	BCD 2.8 ± 0.7 <sup>b</sup>	CDE 0.1 ± 0.1 <sup>b</sup>	E 0.0 ± 0.0 <sup>b</sup>	E 0.0 ± 0.0 <sup>b</sup>
	gN	39.1 ± 2.8 <sup>a</sup>	AB 48.9 ± 2.3 <sup>a</sup>	A 43.2 ± 1.7 <sup>a</sup>	A 38.8 ± 1.5 <sup>a</sup>	AB 37.8 ± 1.6 <sup>a</sup>	AB 38.5 ± 1.0 <sup>a</sup>	AB 36.6 ± 1.7 <sup>a</sup>	AB 31.7 ± 1.1 <sup>a</sup>	BC 26.5 ± 1.4 <sup>a</sup>	CD 25.3 ± 1.4 <sup>a</sup>	CD 23.8 ± 2.0 <sup>a</sup>	CD 8.8 ± 2.0 <sup>a</sup>
Fertilization rate (%)	gD	84.3 ± 3.9	B 86.3 ± 4.6 <sup>b</sup>	AB 89.6 ± 3.3	AB 94.7 ± 1.4	AB 96.3 ± 1.0	A 95.3 ± 1.1	A 97.1 ± 0.6	A 96.8 ± 0.9	AB 97.9 ± 0.8	A 98.3 ± 0.2	A 98.9 ± 0.8	A 98.9 ± 0.8
	gN	88.7 ± 2.1	D 97.1 ± 0.4 <sup>a</sup>	AB 96.7 ± 0.5	ABC 94.6 ± 2.0	ABC 97.3 ± 0.5	AB 96.9 ± 0.5	ABC 98.2 ± 0.3	A 98.2 ± 0.3	A 98.2 ± 0.3	A 98.2 ± 0.3	A 98.2 ± 0.3	A 98.2 ± 0.3
Viability rate (%)	gD	65.8 ± 6.3	ABC 68.8 ± 4.0	AB 78.6 ± 2.2 <sup>a</sup>	A 50.9 ± 6.6	BC 22.7 ± 3.8 <sup>b</sup>	CDE 42.1 ± 5.3 <sup>b</sup>	BC 35.0 ± 4.7	BCD 11.7 ± 3.1 <sup>b</sup>	DE 2.3 ± 0.6 <sup>b</sup>	E 32.7 ± 2.0 <sup>a</sup>	ED 32.7 ± 2.0 <sup>a</sup>	E 32.9 ± 7.2
	gN	50.1 ± 4.1	ABC 58.6 ± 4.2	AB 58.8 ± 1.8 <sup>b</sup>	A 56.9 ± 2.0	AB 56.4 ± 1.6 <sup>a</sup>	AB 55.1 ± 1.6 <sup>a</sup>	AB 44.9 ± 2.4	BCD 33.7 ± 2.0 <sup>a</sup>	ED 32.7 ± 2.0 <sup>a</sup>	CDE 35.1 ± 2.8	CDE 24.0 ± 3.2	E 32.9 ± 7.2
Hatching rate (%)	gD	95.5 ± 2.0 <sup>a</sup>	88.0 ± 3.0	83.3 ± 1.1 <sup>a</sup>	87.6 ± 3.2	88.0 ± 1.8	88.1 ± 1.8	91.6 ± 1.6	92.0 ± 1.7	70.6 ± 10.2 <sup>b</sup>	92.9 ± 1.0	92.7 ± 0.8	94.9 ± 1.5
	gN	80.4 ± 4.0 <sup>b</sup>	80.1 ± 4.1	83.5 ± 3.0 <sup>b</sup>	89.1 ± 1.2	85.7 ± 2.0	92.1 ± 0.9	92.7 ± 1.4	92.1 ± 0.9	91.6 ± 1.0 <sup>a</sup>	92.9 ± 1.0	92.7 ± 0.8	94.9 ± 1.5
Larval survival rate (%)	gD	93.6 ± 1.3 <sup>a</sup>	A 83.7 ± 2.3	AB 89.8 ± 1.1 <sup>a</sup>	A 79.9 ± 2.9	AB 64.3 ± 4.6 <sup>b</sup>	B 70.3 ± 3.0 <sup>b</sup>	B 69.1 ± 3.3 <sup>b</sup>	B 59.0 ± 6.2 <sup>b</sup>	B 39.6 ± 5.9 <sup>b</sup>	B 75.4 ± 2.5	B 81.5 ± 1.8	AB 85.8 ± 2.4
	gN	83.7 ± 1.5 <sup>a</sup>	AB 83.9 ± 1.8	AB 85.9 ± 1.5 <sup>b</sup>	A 85.6 ± 1.4	AB 77.6 ± 3.8 <sup>a</sup>	AB 81.5 ± 2.4 <sup>a</sup>	AB 80.7 ± 2.0 <sup>a</sup>	AB 78.8 ± 2.6 <sup>a</sup>	AB 72.2 ± 3.6 <sup>a</sup>	B 75.4 ± 2.5	B 81.5 ± 1.8	AB 85.8 ± 2.4
Fertilized eggs (10 <sup>5</sup> )	gD	2.3 ± 1.0 <sup>b</sup>	DE 9.8 ± 1.9 <sup>b</sup>	ABC 11.9 ± 0.8 <sup>b</sup>	A 11.0 ± 1.1 <sup>b</sup>	AB 12.1 ± 0.9 <sup>b</sup>	A 8.8 ± 0.7 <sup>b</sup>	AB 7.1 ± 0.6 <sup>b</sup>	ABC 4.8 ± 0.8 <sup>b</sup>	BCD 2.8 ± 0.6 <sup>b</sup>	CDE 2.8 ± 0.6 <sup>b</sup>	CDE 2.8 ± 0.6 <sup>b</sup>	CDE 2.8 ± 0.6 <sup>b</sup>
	gN	35.5 ± 2.8 <sup>a</sup>	AB 47.5 ± 2.2 <sup>a</sup>	A 41.8 ± 1.7 <sup>a</sup>	A 36.8 ± 1.6 <sup>a</sup>	AB 36.9 ± 1.6 <sup>a</sup>	AB 37.3 ± 1.0 <sup>a</sup>	AB 36.0 ± 1.6 <sup>a</sup>	AB 31.1 ± 1.0 <sup>a</sup>	BC 26.1 ± 1.4 <sup>a</sup>	CD 24.6 ± 1.3	CD 22.5 ± 1.8	CD 7.6 ± 1.7
Viable eggs (10 <sup>5</sup> )	gD	1.3 ± 0.6 <sup>b</sup>	D 6.6 ± 1.3 <sup>b</sup>	BC 9.6 ± 0.7 <sup>b</sup>	A 24.6 ± 1.3 <sup>a</sup>	A 20.8 ± 1.1 <sup>a</sup>	A 20.5 ± 0.7 <sup>a</sup>	A 16.2 ± 1.3 <sup>a</sup>	ABC 10.3 ± 0.6 <sup>a</sup>	CD 9.7 ± 1.2 <sup>a</sup>	BCD 9.4 ± 1.1	CD 6.9 ± 1.2	D 3.2 ± 1.1
	gN	18.1 ± 2.0 <sup>a</sup>	AB 26.8 ± 2.1 <sup>a</sup>	A 26.8 ± 2.1 <sup>a</sup>	A 21.5 ± 1.3 <sup>a</sup>	A 20.8 ± 1.1 <sup>a</sup>	A 20.5 ± 0.7 <sup>a</sup>	A 16.2 ± 1.3 <sup>a</sup>	ABC 10.3 ± 0.6 <sup>a</sup>	CD 9.7 ± 1.2 <sup>a</sup>	BCD 9.4 ± 1.1	CD 6.9 ± 1.2	D 3.2 ± 1.1
Hatched larvae (10 <sup>3</sup> )	gD	1.3 ± 0.6 <sup>b</sup>	D 5.8 ± 1.1 <sup>b</sup>	BC 8.9 ± 0.6 <sup>b</sup>	A 4.8 ± 0.9 <sup>b</sup>	AB 2.2 ± 0.4 <sup>b</sup>	AB 3.2 ± 0.5 <sup>b</sup>	AB 2.0 ± 0.3 <sup>b</sup>	BC 0.5 ± 0.1 <sup>b</sup>	CD 0.0 ± 0.0 <sup>b</sup>	D 0.0 ± 0.0 <sup>b</sup>	D 0.0 ± 0.0 <sup>b</sup>	D 0.0 ± 0.0 <sup>b</sup>
	gN	14.5 ± 1.6 <sup>a</sup>	BCD 21.4 ± 1.6 <sup>a</sup>	AB 20.4 ± 1.0 <sup>a</sup>	A 19.3 ± 1.3 <sup>a</sup>	AB 17.9 ± 1.0 <sup>a</sup>	AB 18.9 ± 0.7 <sup>a</sup>	AB 15.0 ± 1.1 <sup>a</sup>	ABC 9.5 ± 0.6 <sup>a</sup>	CD 9.0 ± 1.2 <sup>a</sup>	D 8.9 ± 1.0	CD 6.4 ± 1.1	D 3.0 ± 1.0
N° alive larvae (10 <sup>3</sup> )	gD	1.2 ± 0.6 <sup>b</sup>	D 5.0 ± 0.9 <sup>b</sup>	BC 8.0 ± 0.6 <sup>b</sup>	A 4.1 ± 0.7 <sup>b</sup>	AB 1.8 ± 0.3 <sup>b</sup>	AB 2.6 ± 0.4 <sup>b</sup>	AB 1.6 ± 0.2 <sup>b</sup>	BC 0.3 ± 0.1 <sup>b</sup>	CD 0.0 ± 0.0 <sup>b</sup>	D 0.0 ± 0.0 <sup>b</sup>	D 0.0 ± 0.0 <sup>b</sup>	D 0.0 ± 0.0 <sup>b</sup>
	gN	11.7 ± 1.3 <sup>a</sup>	BCD 17.1 ± 1.3 <sup>a</sup>	AB 17.0 ± 0.8 <sup>a</sup>	A 17.4 ± 1.2 <sup>a</sup>	A 15.4 ± 0.9 <sup>a</sup>	AB 17.4 ± 0.6 <sup>a</sup>	A 13.8 ± 1.0 <sup>a</sup>	ABC 8.7 ± 0.5 <sup>a</sup>	CDE 8.4 ± 1.1 <sup>a</sup>	CDE 8.3 ± 1.0	CDE 6.0 ± 1.0	DE 2.8 ± 0.9

Results are expressed as means ± SEM. For each trait, values in the same column followed by different lower-case letters are significantly different ( $P < 0.05$ ) between genetic groups crosses during the same spawning period. Values in the same row followed by different capital letters are significantly different ( $P < 0.05$ ).

fortnights, the gD and gN groups showed the same pattern in the distribution of values across all traits: low values at the beginning, high values in the middle and low values at the end (Table 2).

For the number of alive larvae, the explanation of its variability within fortnights due to genetic and tank factors is shown in Figure 5B. Thus, genetic factors were highest in the middle of the spawning season (from fortnights five to eight), ranging from 63 to 87%. Conversely, the impact of the tank was high after the beginning of the spawning season, low in the middle and at its highest at the end (80–84%). However, environmental factors (not explained) were especially influential at the beginning (56–46% in fortnights one to two, respectively) and at the end (66% in fortnight twelve) of the spawning season.

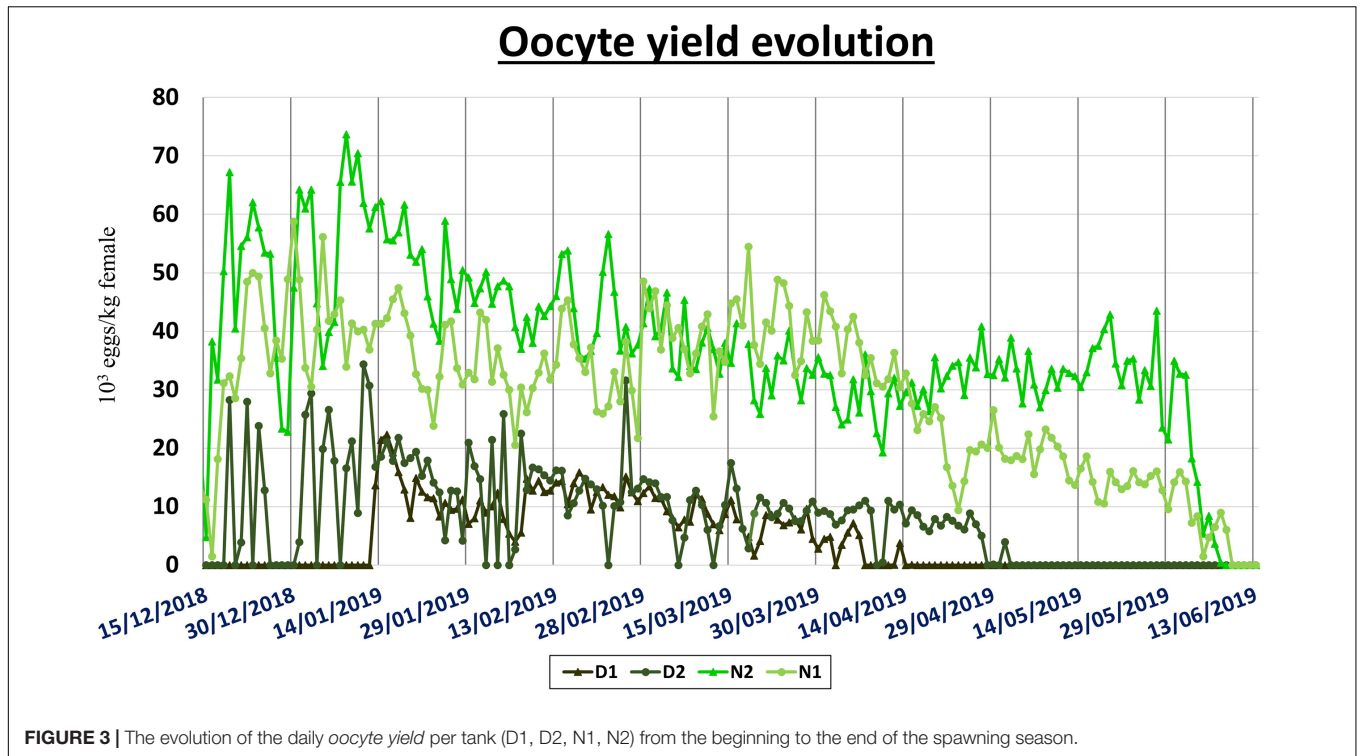
Figure 5C shows the importance of the oocyte yield, fertilization rate, viability rate, hatching rate, and larvae survival rate on the number of alive larvae. It can be seen that oocyte yield and viability rate explain the majority of variance. The influence of oocyte yield was at its maximum between fortnights three and seven (70.8–89.1%). Conversely, viability rate was at its maximum between fortnights nine and eleven (58–89.7%). Taking all traits into account, the best linear model explained between 84.5 and 98.7% of total variation of the number of alive larvae.

## DISCUSSION

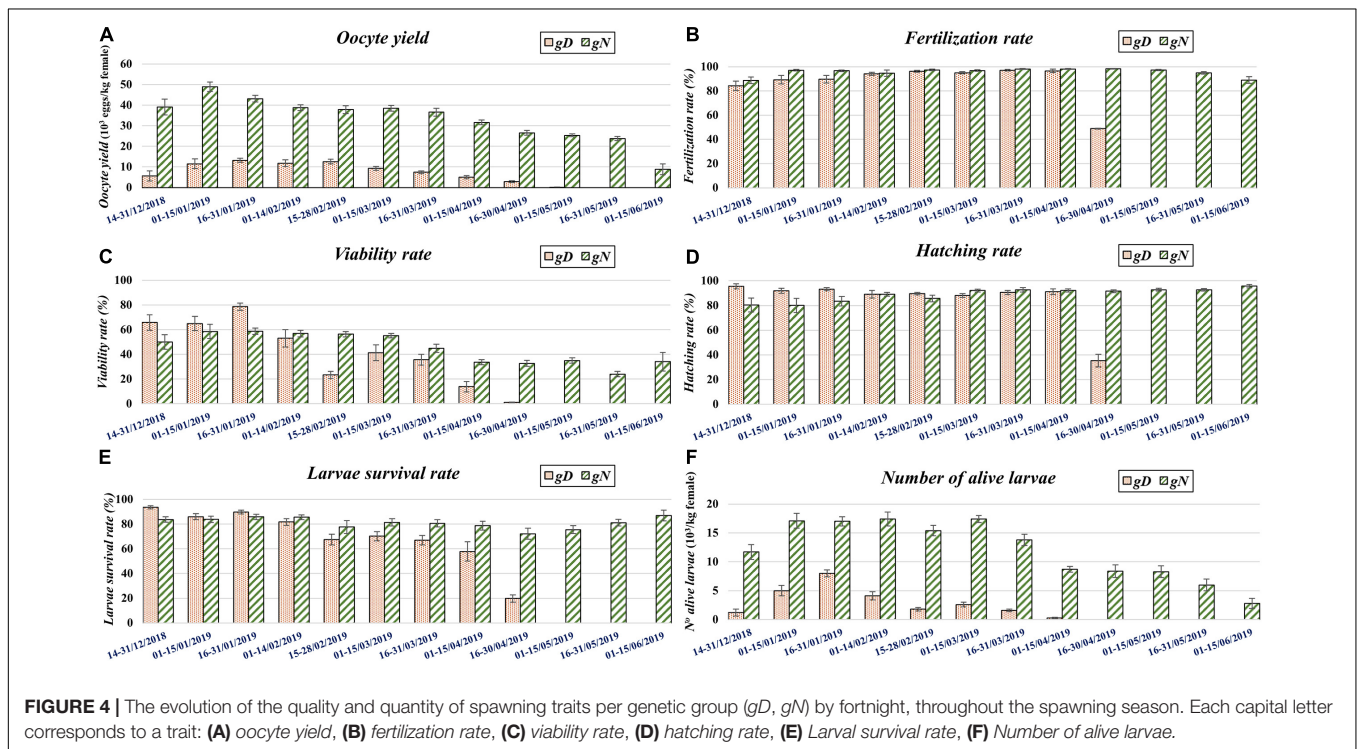
Body malformation is one of the most important traits defining the quality of batches. Deformities have a negative effect on the profit of aquaculture companies, especially hatcheries, because on-growing companies do not accept deformed fry (Afonso and Roo, 2007). Most skeletal anomalies in marine aquaculture species occur during their embryonic, larval and metamorphosis stages (Koumoundouros, 2010), and poor spawning quality is often associated with the appearance of such deformities. In gilthead seabream, variation in the inbreeding of breeders produces differences in spawning quality (Astorga, 2005).

In this study the effect of the EBV<sub>def</sub> on spawning quality traits (oocyte yield, fertilization rate, viability rate, hatching rate, larval survival rate, fertilized eggs, viable eggs, hatched eggs and number of alive larvae) is reported for first time in gilthead seabream. Breeders started spawning naturally at the beginning of the species' spawning season in December-January and continued until April-June (Arias, 1980; Fernández-Palacios et al., 1995; Kissil et al., 2001; Ibarra-Zatarain and Duncan, 2015). The number of spawning days from all tanks ranged between 86 and 116 days for the gD group, and 176–177 for the gN group. These results agree with values reported by Jerez et al. (2012) (121–130 days of spawning). The difference in spawning days observed between both genetic groups was robust because it was reproduced in the same way within the genetic groups between tanks, suggesting at least a genetic effect based on the EBV<sub>def</sub>.

The oocyte yield trait reported the greatest variability, showing differences between genetic groups (fed with commercial diets). In any event, their values ranged according to the locality and environmental conditions (Fernández-Palacios et al., 1995). While the oocyte yield of gN group was in line with



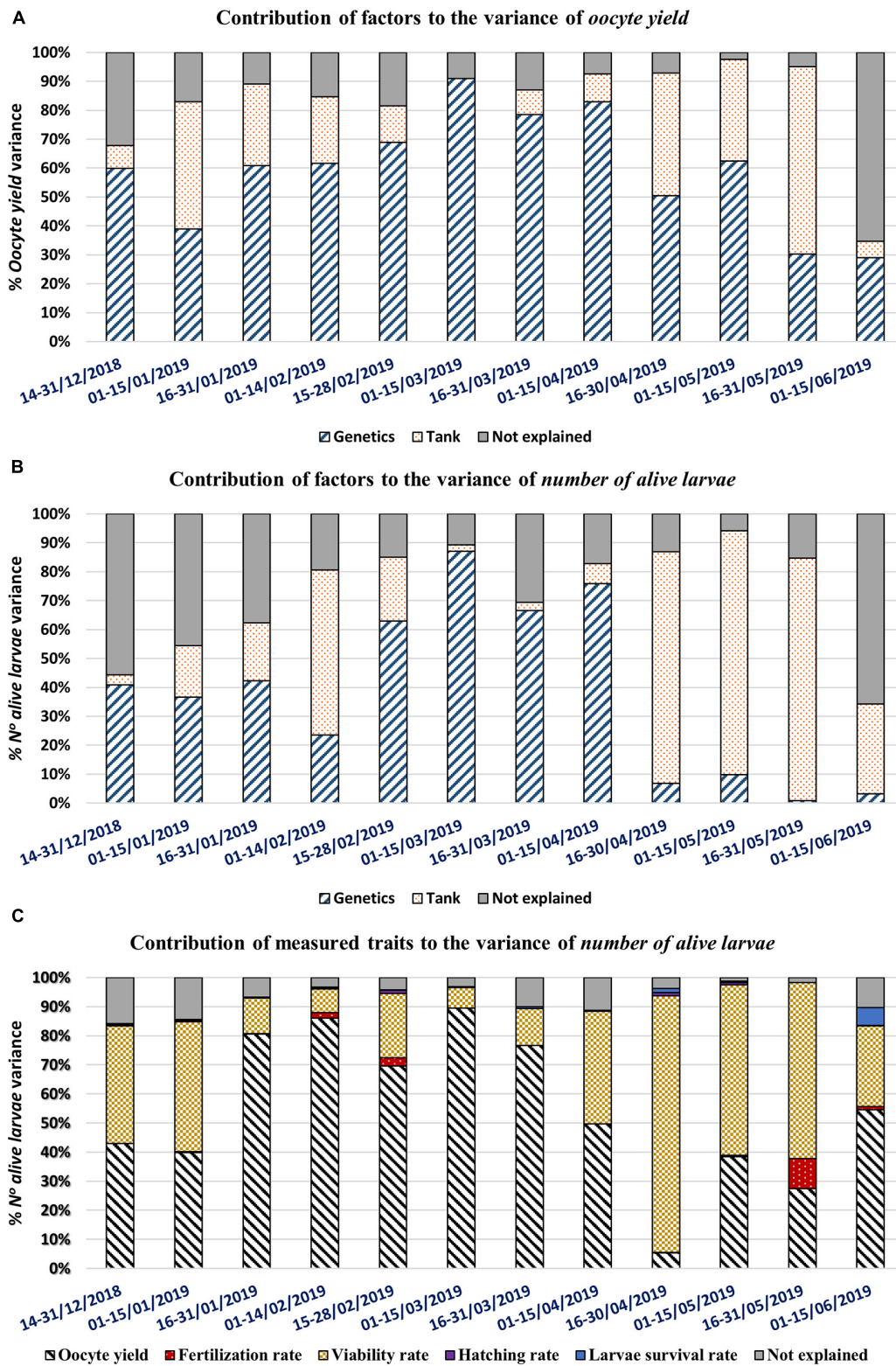
**FIGURE 3 |** The evolution of the daily oocyte yield per tank (D1, D2, N1, N2) from the beginning to the end of the spawning season.



**FIGURE 4 |** The evolution of the quality and quantity of spawning traits per genetic group (gD, gN) by fortnight, throughout the spawning season. Each capital letter corresponds to a trait: (A) oocyte yield, (B) fertilization rate, (C) viability rate, (D) hatching rate, (E) Larval survival rate, (F) Number of alive larvae.

Xu et al. (2019), oocyte yield of gD group was 5.3 times lower in comparison to the gN group for the same time period. These differences were kept within fortnights. On the other hand, the gD group reported values lower than those reported by Jerez et al. (2012), and much lower than those reported by Xu et al.

(2019), using diets with nutritional deficiencies. Oocyte yield trait was widely influenced by genetic factor throughout the whole spawning season, while environmental factors were only relevant at the beginning and end of the spawning season. In line with this, breeders with high genetic content in morphological deformity



**FIGURE 5 |** Histograms of the variance explained in the linear regression analysis by fortnight, throughout the spawning season. Each capital letter represents a different analysis: **(A)** the importance of environmental and genetic factors (Tank and Genetic, respectively) on the *oocyte yield*. **(B)** The importance of environmental and genetic factors (Tank and Genetic, respectively) on the *number of alive larvae*. **(C)** The importance of measured traits (*oocyte yield*, *fertilization rate*, *viability rate*, *hatching rate*, *larval survival rate*) on the *number of alive larvae*.



stimulate poor spawn quality through a lower level of *oocyte yield* and would be characterized for their  $EBV_{def}$  content.

Traits as *fertilization*, *viability*, and *hatching rates* are widely used for controlling spawning quality but in this study, they did not show significant differences between genetic groups, although *viability rate* and *hatching rate* estimates were, respectively, lower and similar to other studies (Fernández-Palacios et al., 1995, 1997; Scabini et al., 2011; Xu et al., 2019; Ferosekhan et al., 2020). The number of breeders and sex-ratio used in both types of crosses per tank (*gN* and *gD* groups) were in concordance with value used by Ibarra-Zatarain and Duncan (2015), in reproductive season of gilthead seabream. In fact, the *fertilization rate* registered was in line with values reported by other authors, also using mass spawning to study this species (Fernández-Palacios et al., 1995, 1997; Scabini et al., 2011; Jerez et al., 2012; Ibarra-Zatarain and Duncan, 2015), and higher than other research using mating with only one male or female (Gorshkov et al., 1997; Xu et al., 2019; Ferosekhan et al., 2020).

*Larval survival rate* reported in this study was within range for this species (Carrillo et al., 1989; Fernández-Palacios et al., 1995, 1997; Scabini et al., 2011; Xu et al., 2019; Ferosekhan et al., 2020) and the higher survival of the *gN* group compared to the *gD* group suggest a major susceptibility of the latter to environmental factors.

The large differences observed for *fertilized eggs*, *viable eggs*, *hatched eggs* and the *number of alive larvae* between genetic groups were similar along the whole spawning season, having significant differences between groups within fortnights (6 times higher in the *gN* group vs. the *gD* group). Moreover, between fortnights and independently of the genetic group, the same pattern in the distribution of values across these traits was reported: significantly low values at the beginning, high values in the middle, and low values at the end. *Oocyte yield* values described a distribution pattern practically equal but with differences of 5.3 times between groups, showing the high responsibility of it on all those traits, as expected.

The *number of alive larvae* is determinant for defining the effective production of hatcheries, in order to satisfy the on-growing companies' demand, which requires planning. It is therefore essential to know the spawning window within which the spawning quality is optimal. Genetic improvement is accumulative, permanent, and extendable to the whole production chain (Falconer and Mackay, 2001). In this study, the genetic factor showed a significant impact on the *number of alive larvae* (63–87%), mainly in the middle of the spawning season, in line with the period when spawning is considered optimal at an industrial level (Navarro et al., 2009).

A linear regression modeling of spawning for *number of alive larvae*, revealed that *oocyte yield* and *viability rate* traits explained the majority of its variance, being maximum in the middle of the spawning season by the *oocyte yield*. These results are in concordance with the empiric suggestions reported by Fernández-Palacios et al. (1995).

In conclusion, this study demonstrates how the deformity genetic background of phenotypically normal breeders affects and explains their spawning quality, for the first time in gilthead seabream. Shorter spawning periods, lower *oocyte yield* and,

consequently, a lower *number of alive larvae* were found among *gD* breeders. The *number of alive larvae*, the most determinant trait of spawning quality, peaked in the middle of the spawning season on the basis of *oocyte yield* and genetic factor, reflecting the importance of controlling the breeders' genetics and the utility of *oocyte yield* trait for tracking spawning quality. Conversely, the *viability rate* and tank factor supported the *number of alive larvae* at the beginning and end of the spawning season. Given the results obtained, for *oocyte yield* trait and *number of alive larvae*, the *gN* breeders showed values 5.3 and 6 times higher than *gD* breeders, respectively. For future studies, it would be useful to estimate the differential gene expression patterns between breeders with different  $EBV_{def}$  values, as well as between their offspring, in order to discover the genetic structure involved in skeletal deformity determination, in gilthead seabream.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

Ethical review and approval was not required for the animal study because all the animal experiments were performed according to the European Union Directive (2010/63/EU) on the protection of animals for scientific purposes, at Fundación Canaria Parque Científico Tecnológico (FCPCT) of the Universidad de Las Palmas de Gran Canaria (Canary Islands, Spain).

## AUTHOR CONTRIBUTIONS

ÁL-F, HS, MZ, and JA-L: conceptualization, methodology, and data curation. ÁL-F, HS, SL-B, JP-S, and CP-G: investigation. ÁL-F and JA-L: writing—original draft preparation. HS, MZ, SL-B, CP-G, and JP-S: writing—review and editing. HS, MZ, JP-S, and JA-L: funding acquisition. All authors have read and agreed to the published version of the manuscript.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2021.656901/full#supplementary-material>

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- Conflict of Interest:** SL-B was employed by company AQUANARIA S.L.
- The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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