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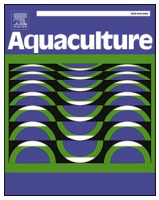
Aquaculture 556 (2022) 738292

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture



# Genetic parameters of fillet fatty acids and fat deposition in gilthead seabream (*Sparus aurata*) using the novel 30 k Medfish SNP array

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ARTICLE INFO

*Keywords:*
Omega-3
Gilthead seabream
Lipid metabolism
Lipid deposition
Genetic parameters

ABSTRACT

Lipid-related traits are important candidates for a breeding goal for gilthead seabream, because they affect both fish and human health, as well as production efficiency. However, to date there have been very few estimates of genetic parameters for these traits, and the genetic relationship between fatty acids and other important traits have never been reported for gilthead seabream. Therefore, the aim of this study was to estimate genomic heritability and genetic relationships of fat deposition traits and individual muscle fatty acids in a commercial population of gilthead seabream using the novel ~30 k MedFish SNP array.

In total 967 gilthead seabream fed with a commercial feed were genotyped with the MedFish SNP chip which included ~30 K informative markers for this species. On average, the fish weighed 372 g. The mean content of eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) was 822 mg per 100 g fillet. The heritability of muscle fat, viscera weight and percentage viscera were in the range of 0.34–0.46. The genetic correlation of body weight with muscle fat was 0.12, indicating that genetic variation in muscle fat is largely independent of the weight of the fish. The heritability of the product of endogenous fatty acid synthesis ($n = 240$), palmitoleic acid (16:1n-7), was high (0.43). The estimated heritability of EPA (%) and DHA (%) was 0.39 and 0.33, respectively. Both EPA and DHA had low, non-significant genetic correlations with body weight, and DHA had a negative genetic correlation with muscle fat (−0.53).

It is possible to increase EPA and DHA content in gilthead seabream fillets by selective breeding. The high heritability of 16:1n-7, a marker of *de novo* lipogenesis, suggests that there is a strong genetic component to this metabolic pathway in gilthead seabream. Muscle fat deposition and body weight seem to be independent traits, and selective breeding for faster growth is not likely to influence the proportional content of EPA and DHA.

## 1. Introduction

Gilthead seabream is one of the major aquaculture species in Europe, ranked as number four, with a total production of almost 200,000 tons in 2019 (FEAP, 2020), but is at an early stage regarding selective breeding. The first commercial breeding programs of seabream were initiated in the early 2000's and have primarily focussed on improving growth rate (Brown, 2004; Janssen et al., 2017; Thorland et al., 2007).

Lipids are important in production of all farmed animals because they are linked to production efficiency, health, and product quality. Excess dietary lipids that are not deposited in the edible muscle or used as energy source for growth are considered a loss and an indicator of low efficiency. Constant energy excess may lead to excessive fat deposition in and around internal organs and thereby increase the risk of metabolic disorders, oxidative stress and inflammation (Fontana et al., 2007; Todorčević et al., 2010).

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https://doi.org/10.1016/j.aquaculture.2022.738292
Received 22 December 2021; Received in revised form 22 April 2022; Accepted 23 April 2022
Available online 27 April 2022

The lipid content of muscle tissue affects organoleptic and processing qualities of fish fillets. In seabream, it has been shown that the taste, colour, and juiciness of fillets are affected by the fat content (Grigorakis et al., 2003; Grigorakis, 2007). Muscle fat also affects the nutritional quality of fillets. Fish is an important source of the essential omega-3 long-chain polyunsaturated fatty acids (LC-PUFAs), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) in human diets. EPA and DHA have several beneficial health effects, such as preventing and attenuating a range of inflammatory disorders, including cardiovascular disease, immune dysfunction, and obesity (Calder, 2015; Thota et al., 2018; Todorcevic and Hodson, 2015). Hence, improving the omega-3 content of fish fillet is a point of focus in aquaculture. As a significant part of the Mediterranean diet, seabream is an important source of these essential and health-promoting fatty acids.

In general, omega-3 fatty acid content of fish muscle is largely determined by the dietary fatty acid composition, which is well documented also for gilthead seabream (Ballester-Lozano et al., 2011; Grigorakis et al., 2002; Izquierdo et al., 2003; Mnari et al., 2007). In addition to feed, other factors including environmental factors (*e.g.* water temperature) have also been shown to influence the omega-3 fatty acid content of seabream muscle (Ibarz et al., 2005). Moreover, metabolic factors are likely to play a role, as higher DHA level in muscle compared to the feed have been observed in gilthead seabream (Izquierdo et al., 2003). Omega-3 content in fish fillets can also be regulated genetically: a recent study in gilthead seabream showed that there is a heritable component to variation in muscle content of EPA and DHA in gilthead seabream (Vallecillos et al., 2021). Dong et al. (2016) estimated a heritability of 0.44 for the omega-3 levels of fillets of the marine fish species large yellow croaker. Studies in Atlantic salmon have revealed medium to high heritability for the omega-3 levels of fillets and showed that genetic variation is possibly linked to omega-3 bioconversion capacity (Horn et al., 2018; Leaver et al., 2011). Although marine species have very limited capacities for omega-3 bioconversion (conversion of the shorter chain alpha-linolenic acid (ALA) to the longer chain EPA and DHA), some species like gilthead seabream and European seabass do express several of the required genes. Fatty acid elongase genes, as well as Delta-6 desaturase have been described, and are up-regulated by dietary factors in the same manner as in salmonids (Seiliez et al., 2003; Tocher and Ghioni, 1999). Still, in gilthead seabream, the omega-3 fatty acid bioconversion capacity is shown to be limited due to a deficiency in Delta5 desaturase activity (Tocher and Ghioni, 1999). There are, however, other metabolic factors that can influence the omega-3 fatty acid composition in fish, such as fatty acid uptake, deposition, and beta-oxidation (Henderson and Sargent, 1985; Horn et al., 2019; Vegusdal et al., 2004). Although knowledge is limited in gilthead seabream, studies have shown a preferential retention of arachidonic acid (20:4 n-6) and DHA over EPA (Izquierdo, 1996; Kalogeropoulos et al., 1992; Koven et al., 2001).

The first publicly available combined-species SNP array for European seabass and gilthead seabream, the new Axiom® MedFish SNP array, was recently developed (Peñaloza et al., 2021). This array enables the accurate high-throughput genotyping of ~ 30 K SNPs distributed throughout the seabream genome. The availability of genotype data on individuals allows the computation of genomic relationship matrix (GRM) where the coefficient of relationships between relatives are described more precisely with possibility of covering the deviations in relatedness caused by Mendelian sampling (Houston et al., 2020; Nielsen et al., 2009). Although heritability can be underestimated or overestimated in influential regions with high or low linkage disequilibrium (LD), GRM reflects more precise relatedness among individuals than the pedigree-based relationships (Da et al., 2014). The genetic variance components estimated using GRM are therefore possibly more precise than pedigree-based estimates, especially when the available pedigree is not very deep.

A key factor in the rate of genetic improvement possible for any given trait in a breeding programme is its heritability, and its genetic correlations with other key traits in the breeding goal. Only a few studies have reported genetic parameters of fat deposition traits in gilthead seabream (Elalfy et al., 2021; García-Celdrán et al., 2015b; Navarro et al., 2009b), but never using high-throughput genotyping. One study has reported heritability of muscle fatty acids (Vallecillos et al., 2021), but the genetic correlations between fatty acids and other important traits have never been reported for gilthead seabream.

The aim of the current study was to estimate genomic heritability and genetic relationships of fat deposition traits and individual muscle fatty acids in gilthead seabream using the novel ~30 k MedFish SNP array.

## 2. Material and methods

Gilthead seabream originating from the selective breeding program of Galaxidi Marine Farm SA (Galaxidi, Greece) were used in this experiment. All fish used in this study were the offspring of 33 males and 20 females mass spawned in a single broodstock tank over one day. The fish were stocked in a sea cage at an average weight of 2.6 g. During the sea phase, the fish were fed commercial feed and kept in commercial sea cages in Galaxidi, Greece. They were slaughtered in September 2019, after 15 months at sea and fasted prior to slaughter. From the average body weight of ≈165 g to 270 g, the fish were fed a diet with a protein/lipid content of 45/20, that only contained fish oil. From six to eight months, the fish were fed a second experimental diet of a 45/16 protein/lipid content, that included some sunflower oil. During the last two-week phase of the trial before slaughter, the fish returned to the first diet. Due to the logistics of sampling, slaughtering took place over several days, therefore the number of days fasted prior to slaughter varied from 4 to 12, which is considered in the statistical analysis of the data.

At the time of slaughter, body weight, viscera weight, and muscle fat measured by using Distell meter were recorded on 959 fish. Total viscera weight was measured directly after slaughter by removing and weighing the entire viscera, including liver and heart. The liver and heart were also weighed separately, so for the analyses in the current study, the viscera weight was calculated by subtracting heart weight and liver weight from total viscera weight. Visceral fat was not measured directly, but viscera percentage of body weight (viscera weight/body weight*100) was used as an indicator of amount of visceral fat in the current study (Viscera %).

### 2.1. Lipid measurements

Two methods of measuring muscle fat percentage were applied on the fish studied, the non-invasive Distell fat meter (Distell (Model-FM 692, www.distell.com), and the gold standard laboratory Folch method ($MFAT_F$). Directly after slaughter, a non-invasive fat measurement was made on each of the 959 fish using the Distell fat meter, a handheld microwave dielectric spectrometer. Two measurements were recorded above the lateral line and two below, repeated on both sides of the fish. The average of these eight measurements was used as a measure of the total muscle fat percentage ($MFAT_D$). A calibration equation for muscle fat in sea bream using the internal "custom calibration" setting was generated as per the manufacturer's specifications (Distell, 2011). The resulting calibration equation on 20 fish had a coefficient of determination of $R^2 = 0.79$. The average of the eight measurements per fish after use of the custom calibration was used as measure of the total muscle fat percentage ($MFAT_D$).

The whole skinless fillet of 240 of the 959 fish were individually homogenized, and total lipids were extracted using the Folch extraction method (Folch et al., 1957) to record total muscle fat percentage ($MFAT_F$). The fatty acid composition of the total lipids was determined using the Mason & Waller method by means of methyl ester gas chromatography separation and flame ionization detection (Mason and Waller, 1964). This gave fatty acid composition estimates for both

proportional (% of total fatty acids) and quantitative (mg per g fillet, based on internal standard) content of each fatty acid. For the data analysis, outliers and error datapoints were removed through the Interquartile Range (IQR) method. The results of the current study will focus on the five most prevalent fatty acids of the muscle (18:1n-9, 16:0, 18:2n-6, 22:6n-3 (DHA) and 16:1n-7), as well as the omega-3 fatty acids 20:5n-3 (EPA) and 18:3n-3 (ALA).

### 2.2. DNA extraction and genotyping

DNA was extracted from fin clips by IdentiGEN (Dublin, Ireland), using a crude DNA isolation method consisting of PK/Chelex extraction followed by a dilution step. The fish were genotyped using the MedFish array, which contains approximately 29,800 validated SNPs for gilthead seabream (Peñaloza et al., 2021).

Genotype data were filtered using Plink software (Purcell et al., 2007), excluding SNPs with minor allele frequency (MAF) lower than 2%, missing call rates exceeding 10%, and variants which had Hardy-Weinberg equilibrium exact test *p*-value below 1e-15. In total 25,919 SNPs passed filters and quality control.

### 2.3. Statistical analysis

The genomic relationship matrix was generated with the GCTA software, following the method by Yang et al. (2011), using the following equation to estimate the genetic relationship between individuals j and k:

$$G_{jk} = \frac{1}{N}\sum_{i=1}^{N}\frac{(x_{ij} - 2p_i)(x_{ik} - 2p_i)}{2p_i(1-p_i)},$$

where $x_{ij}$ is the number of copies of the reference allele for the $i^{th}$ SNP of the $j^{th}$ individual, $x_{ik}$ is the number of copies of the reference allele for the $i^{th}$ SNP of the $k^{th}$ individual, and $p_i$ is the frequency of the reference allele, estimated from the observed allele frequencies.

Variance and covariance components were estimated by residual maximum likelihood procedures using the CGTA program. Bivariate analyses were performed to estimate genetic correlations between traits, using the following bivariate animal model (Henderson, 1984):

$$\mathbf{Y} = \mathbf{XB} + \mathbf{U} + \mathbf{E}$$

where **Y** is a matrix of phenotypic records for individuals $i = 1, 2, \ldots, n$ and traits $j = 1, 2$, **X** is a matrix of the fixed effects of animal i on trait j, **B** is a matrix of fxed efect solutions. **U** is a matrix containing the random effects of animal i on trait j, with variance with variance $G_0 \otimes G$, where $G$ is the genomic relationship matrix between individuals and $G_0$ is a genetic variance–covariance matrix among traits. **E** is a matrix of residual effects, that is assumed to have a variance of

$$R = \begin{bmatrix} \sigma^2_{e1} & \sigma_{e1,e2} \\ \sigma_{e1,e2} & \sigma^2_{e2} \end{bmatrix}, \text{ where 1 and 2 indicate traits.}$$

Univariate analyses were performed to estimate heritability for all traits. For the univariate analyses, matrices **Y**, **B**, **U** and **E** were reduced to vectors and matrices G and R were reduced to scalars. Heritability (narrow sense) was estimated as the ratio of additive genetic variance to total phenotypic variance. Slaughter day (representing number of days fasted) was included as a fixed effect for all traits.

Genetic coefficient of variation was determined based on the method suggested by Burton and Devane (1953) as follows:

$$GCV = \frac{\sqrt{\sigma^2 g}}{\mu} * 100,$$

Where $\mu$ is the mean of the trait, and $\sigma^2 g$ is the genetic variance.

As parent fish were not genotyped, it was not possible to perform parentage assignment and develop pedigree, therefore, principal component analysis (PCA) was performed to evaluate population structure and get an idea on number of clusters possibly representing full and/or half-sib families. The PCA based cluster analysis showed that the population is quite homogenous (little to no cluster differentiation), with relatively low variation explained by first two PCAs, 4.9% and 3.8% variation explained by PCA1 and PCA2, respectively (Fig. 1). The cluster analysis showed ~42 clusters with number of individuals ranging from 2 to 53 individuals per cluster with a mean cluster size of 23 individuals.

## 3. Results and discussion

The body size and muscle fat level were within the normal range for commercially produced harvest sized gilthead seabream, with a mean body weight of 372 g, ranging from 124 to 546 g (Table 1). The coefficient of variation for all production traits surpassed 0.15, showing that there was substantial individual variation in these traits.

### 3.1. Genetic parameters of lipid related production traits

The heritability of muscle fat, viscera weight and viscera % were all above 0.3, showing that there is a substantial genetic component to these traits (see Table 2). Viscera % had an especially high heritability estimate of 0.46, which was almost equal to the estimated heritability of viscera weight with body weight included as covariate in the statistical model (0.45 ± 0.06, result not shown). This result is in line with the previously reported heritability of 0.5 for visceral fat in gilthead seabream by Navarro et al. (2009b), although García-Celdrán et al. (2015a) reported a heritability of 0.2 for visceral fat with body weight as a covariate in the model. In the current study, the observed variation in viscera weight was assumed to mainly be due to the size of the fish and the amount of lipids stored here; hence the viscera weight expressed as % of body weight was used as an indicator of amount of visceral fat. The genetic correlation between body weight and viscera weight was 0.72, supporting that there is some genetic variation in viscera weight that is independent of the weight of the fish, which could be variation in distribution of lipid deposition.

Previously reported heritability of muscle fat ranges from 0.05 to 0.31 in gilthead seabream (Elalfy et al., 2021; García-Celdrán et al., 2015a; Navarro et al., 2009b; Vallecillos et al., 2021). In the current study, both methods of recording muscle fat showed heritability slightly higher than previous estimates: $MFAT_D$ had a heritability of 0.46, and $MFAT_F$ of 0.34. The considerably smaller dataset is reflected in the larger standard error for $MFAT_F$ (Table 2). The differences in heritability estimates between studies could be partly related to the accuracy of the recording technique. Moreover, there are differences in the statistical models used. García-Celdrán et al. (2015a) and Vallecillos et al. (2021) included body weight as a covariate. In the current study, body weight was not included as a covariate in the statistical model, but when tested, body weight as covariate did not have a significant effect on heritability estimates of the muscle fat traits (results not shown). This is due to that the genetic correlation between body weight and muscle fat was very low (rg = 0.12 for $MFAT_D$ and rg = 0.13 for $MFAT_F$), which was in accordance with the low phenotypic correlations observed (Fig. 2 & Table 3). A low genetic correlation between body weight and muscle fat is in agreement with the estimate reported by Navarro et al. (2009a) (rg = 0.12). However, estimates reported by García-Celdrán et al. (2015b) and Vallecillos et al. (2021) are higher (0.29 and 0.59, respectively). This could explain the differences in statistical models and heritability estimates. Furthermore, these differences in estimates of heritability and genetic correlations may be due to genetic differences among the studied populations, as well as genotype-by-environment interactions (Gulzari et al., 2022; Kause et al., 2002).

The low genetic correlation between body weight and muscle fat observed in the current study of gilthead seabream is contrary to findings of salmonid species such as Atlantic salmon where muscle fat percentage is highly genetically correlated with body weight, *i.e.* rg =

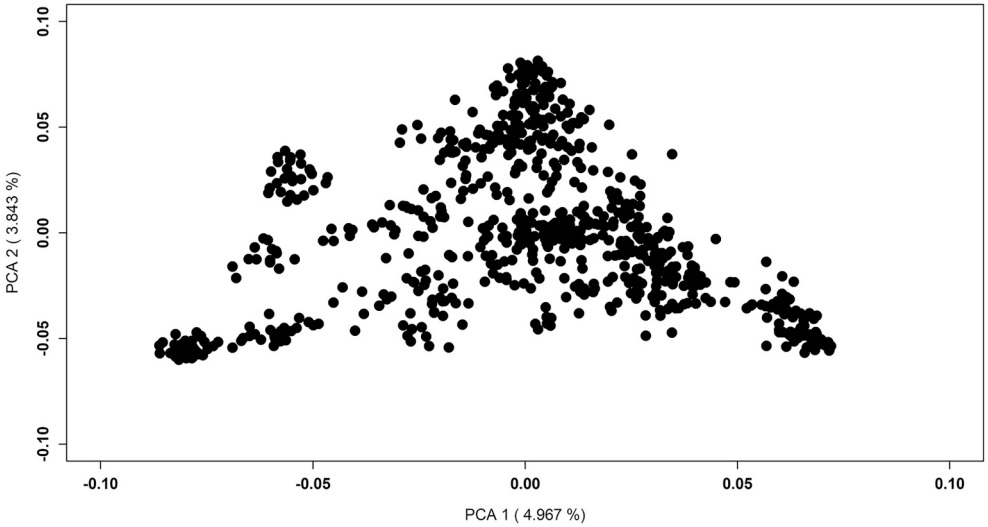


**Fig. 1.** PCA plot of the population structure.

**Table 1**
Descriptive statistics for production traits.

| | N | Mean (SD) | Min | Max | CV (%) |
|---|---|---|---|---|---|
| Body weight (g) | 967 | 372 (64) | 124 | 546 | 17 |
| Viscera weight (g) | 911 | 22.9 (6.0) | 5.4 | 39.6 | 26 |
| Viscera % | 911 | 6.1 (1.1) | 3.3 | 9.8 | 18 |
| $MFAT_D$ (%) | 960 | 7.6 (1.54) | 2.8 | 11.9 | 18 |
| $MFAT_F$ (%)* | 238 | 9.4 (0.1) | 3.9 | 14.8 | 20 |

SD = standard deviation. CV = coefficient of variation. Viscera % = (viscera weight/body weight), $MFAT_D$ = muscle fat % by Distell meter, $MFAT_F$ = muscle fat % by Folch chemical analysis.

**Table 2**
Heritability ($h^2$) of production traits.

| Trait | N | $V_a$ | $V_e$ | $h^2$ | GCV % |
|---|---|---|---|---|---|
| Body weight | 967 | 1435 (271) | 2842 (187) | 0.34 (0.05) | 10 |
| Viscera weight | 911 | 14.87 (2.73) | 23.31 (1.7) | 0.39 (0.06) | 17 |
| Viscera % | 911 | 0.58 (0.09) | 0.67 (0.05) | 0.46 (0.05) | 12 |
| $MFAT_D$ | 960 | 1.03 (0.16) | 1.21 (0.09) | 0.46 (0.05) | 13 |
| $MFAT_F$ | 238 | 1.13 (0.46) | 2.2 (0.38) | 0.34 (0.12) | 11 |

Standard errors in brackets. Va: Additive genetic variance. Ve: Residual variance. GCV = Genetic coefficient of variation. Viscera % = (viscera weight/body weight), $MFAT_D$ = muscle fat % by Distell meter, $MFAT_F$ = muscle fat % by Folch chemical analysis.

0.45–0.84 (Powell et al., 2008; Tsai et al., 2015; Vieira et al., 2007). There are similar findings in the salmonids Rainbow trout (rg = 0.30–0.52) (Blay et al., 2021) and European white fish (rg = 0.62–0.64) (Janhunen et al., 2017). This difference is likely explained by the inherent differences in the mechanisms of lipid deposition between the fish species. For example, in Atlantic salmon, the muscle is a main lipid storage site, while in gilthead seabream very little fat is stored in muscle, and the viscera is the main lipid storage site (McClelland et al., 1995). These differences in lipid storage are likely due to differences in behavioural adaptation to their natural habitats; seabream is a bottom-dwelling sedentary fish species, while Atlantic salmon is an active surface feeder species, and needs energy available in their locomotory muscles (McClelland et al., 1995).

There was a low genetic correlation between the two fat deposition traits $MFAT_D$ and Viscera % (rg = 0.23). This agrees with Navarro et al. (2009a), who found an even lower correlation of 0.05, and used a direct measure of visceral fat - manual removing and weighing of visceral fat

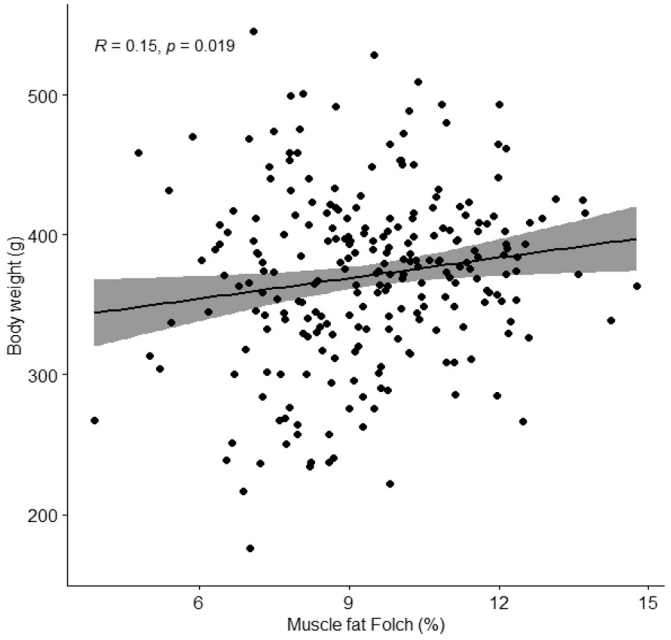


**Fig. 2.** Scatter plot of body weight against muscle fat, including the regression line and correlation coefficient, $n = 238$.

**Table 3**
Genetic (upper triangle) and phenotypic (bottom triangle) correlations.

| | Body weight | Viscera weight | Viscera % | $MFAT_D$ | $MFAT_F$ |
|---|---|---|---|---|---|
| Body weight | | 0.72 (0.06) | 0.25 (0.12) | 0.12 (0.12) | 0.13 (0.20)* |
| Viscera weight | 0.74 | | 0.86 (0.03) | 0.20 (0.12) | 0.19 (0.20)* |
| Viscera % | 0.22 | 0.81 | | 0.23 (0.11) | 0.11 (0.20)* |
| $MFAT_D$ | 0.28 | 0.24 | 0.18 | | 0.82 (0.09)* |
| $MFAT_F$ | 0.15** | 0.21 | 0.18 | 0.60 | |

Standard errors in brackets.
* *Only converged with no covariates in the statistical model.*
** $p > 0.01$.

deposits. This shows that deposition of fat in these two lipid deposits (muscle and viscera) are genetically different traits, similar to what has been described in Rainbow trout (Kause et al., 2007).

The genetic correlation between the two methods of measuring muscle fat was high; 0.82 (Table 3), but lower than reported by a recent study on European seabass, where the genetic correlation between the same two methods on the same trait was close to unity at 0.96 ± 0.03 (Difford et al., 2021). Pearson's correlation coefficient for the two methods was 0.6, showing that the Distell meter was not highly accurate for phenotypic recording of muscle fat percentage in this dataset.

### 3.2. Muscle fatty acid composition

The fatty acid composition of muscle was recorded as both proportional content (% of total fatty acids) and quantitative content (mg/g tissue) on 240 fish (Table 4). The phenotypic variation in proportional content was low, while the variation in quantitative content was higher, reflecting the variation in total muscle fat content. The major fatty acids in the muscle, constituting more than 60% of muscle fatty acids, were oleic acid (18:1n-9), palmitic acid (16:0), and linoleic acid (18:2n-6). Oleic and linoleic acid are the major fatty acids from plant oils in fish feed, while palmitic acid is the most common saturated fatty acid found in all animals. The mean proportional content of EPA and DHA in the gilthead seabream muscle was 2.43% and 6.95% of total lipids, respectively. The mean quantitative content of EPA and DHA was 2.14 and 6.08 mg/g, respectively, *i.e.* 822 mg per 100 g fillet in total. Considering the recommendations on daily intake of omega-3 FAs for the general human public of 250–500 mg EPA + DHA (Cunnane, 2004; GOED, 2014), farmed seabream is a good source of the essential nutrients EPA and DHA.

### 3.3. Heritability and genetic correlations of fatty acids in gilthead seabream fillets

The estimated heritability of the proportional content of both EPA and DHA was relatively high, at 0.39 and 0.33, respectively (Table 5). These estimates are substantially higher than those recently reported by Vallecillos et al. (2021), at 0.05 for EPA and 0.11 for DHA. This difference is likely due to the different statistical models used, as Vallecillos et al. (2021) used a Bayesian model, which is quite conservative, and included body weight as a covariate in the model, which could remove some of the genetic variation in the fatty acid traits. In the current study, we chose not to include body weight as a covariate for fatty acids, as both % EPA and % DHA had low and non-significant genetic correlations with body weight (Table 6). However, DHA (but not EPA) was significantly correlated with muscle fat, which is in agreement with Vallecillos et al. (2021). Thus, including muscle fat as a covariate in the model would likely reduce the heritability estimate for DHA in this data material.

**Table 4**
Descriptive statistics of proportional (% of total fatty acids) and quantitative (mg/g tissue) content of the top five most prevalent fatty acids in muscle, and the omega-3 fatty acids EPA (20:5n-3) and ALA (18:3n-3).

| Fatty acid | Proportional content (%) | | | | Quantitative content (mg/g) | | | |
|---|---|---|---|---|---|---|---|---|
| | Mean (SD) | Min | Max | CV (%) | Mean (SD) | Min | Max | CV (%) |
| 18:1 n-9 | 29.0 (0.05) | 27.4 | 30.6 | 2 | 25.6 (0.33) | 12.5 | 39.3 | 20 |
| 16:0 | 15.7 (0.03) | 14.4 | 16.9 | 3 | 13.8 (0.17) | 7.1 | 21.2 | 19 |
| 18:2 n-6 | 15.6 (0.04) | 13.9 | 17.3 | 4 | 13.8 (0.17) | 7.1 | 21 | 19 |
| 22:6 n-3 (DHA) | 7.0 (0.03) | 6.1 | 7.9 | 6 | 6.1 (0.06) | 3.5 | 8.5 | 16 |
| 16:1 n-7 | 5.1 (0.02) | 4.4 | 5.9 | 6 | 4.5 (0.06) | 2.1 | 7 | 22 |
| 20:5 n-3 (EPA) | 2.4 (0.01) | 2 | 2.9 | 7 | 2.1 (0.03) | 1.1 | 3.2 | 20 |
| 18:3 n-3 (ALA) | 2.4 (0.01) | 2.2 | 2.6 | 3 | 2.1 (0.03) | 1.1 | 3.2 | 20 |

$N = 240$. CV = coefficient of variation. SD = standard deviation.

One limitation of this study is the lack of pedigree, which may have biased the estimation of genetic parameters, as population stratification can inflate estimates of genetic relatedness (Dandine-Roulland et al., 2016). However, the evaluation of population structure by principal component analysis showed that the population is quite homogenous with little to no cluster differentiation (Fig. 1). Also, previous studies have shown that genomic information can more accurately reflect the relationships between individuals than pedigree information (Da et al., 2014; Wang and Da, 2014). The increased accuracy of genomic prediction compared with pedigree prediction is evident in a range of aquaculture species (reviewed in Houston et al. (2020)).

The high heritability for both EPA and DHA estimated in the current study demonstrate that selective breeding is a promising tool for increasing muscle content of these essential nutrients in the fillet of this species. This agrees with studies in Atlantic salmon that have shown significant heritability of EPA and DHA content of muscle (Leaver et al., 2011; Horn et al., 2018). Wang et al. (2019) also showed that there is a genetic component to omega-3 content of Asian seabass fillets. Heritability estimates of DHA in tilapia and common carp have been close to zero, which could be due to the very low quantities of DHA detected (Nguyen et al., 2010; Prchal et al., 2018). The estimates for both fish species indicated that EPA had a considerably higher heritability than DHA, while in Atlantic salmon, the heritability of EPA was lower than DHA (Horn et al., 2018).

Alpha-linolenic acid (18:3n-3; ALA) had the lowest heritability of all fatty acids (0.03). The ratio of DHA/ALA was included in the analysis as an indicator-trait for the conversion of ALA to DHA (omega-3 bioconversion), but is in this case likely reflecting variation in DHA due to the very low variation in ALA. This low variation, together with the low general content of ALA in the muscle of gilthead seabream, indicates that omega-3 bioconversion of ALA to DHA is not substantial, and not very important for the overall DHA content in the muscle of this species. This implies that the phenotypic and genetic variation observed in EPA and DHA levels are linked to other metabolic factors, such as deposition or beta-oxidation, rather than omega-3 bioconversion. This is in accordance with a study reporting that the omega-3 bioconversion capacity is limited in gilthead seabream due to a deficiency in Delta5 desaturase activity, catalyzing conversion of 20:4n-3 to EPA (Tocher and Ghioni, 1999).

The estimates of genetic correlations showed that the proportional content of each fatty acid was differently correlated with muscle fat (Table 6). The standard errors on the estimates were large due to the small dataset on fatty acids, but the genetic correlations agreed with the phenotypic correlations. The fatty acids with the strongest positive correlations with muscle fat were 18:1n-9 and 16:1n-7 (Table 6), both of which are products of *de novo* lipogenesis, synthesis of fatty acids, although 18:1n-9 is also found in the feed. This indicates that a genetic predisposition for higher muscle fat is due to a higher inherent *de novo* lipogenesis activity. The high heritability of % 16:1n-7, which is almost exclusively a product of *de novo* lipogenesis, also indicates that the rate of *de novo* lipogenesis in gilthead seabream muscle is a heritable trait (Table 5).

The genetic correlations indicated that the observed genetic variation in % EPA and % DHA is independent of body weight, as both % EPA and % DHA had low and non-significant genetic correlations with body weight (Table 6). The proportional content of EPA had a weak positive (non-significant) correlation with muscle fat, while DHA had a negative genetic correlation. This difference between EPA and DHA likely reflects the metabolic differences between the two fatty acids, and their roles in muscle tissue (Calder, 2006; Stillwell and Wassall, 2003). The observed relationship between DHA and muscle fat is seen in several species and

**Table 5**
Heritability ($h^2$) of fatty acid traits.

| Trait | % | | | | mg/g | | | |
|---|---|---|---|---|---|---|---|---|
| | Va | Ve | $h^2$ | GCV (%) | Va | Ve | $h^2$ | GCV (%) |
| 16:0 | 0.06 (0.03) | 0.16 (0.02) | 0.28 (0.11) | 1.57 | 2.17 (0.84) | 4.21 (0.70) | 0.33 (0.11) | 10.67 |
| 16:1n-7 | 0.04 (0.01) | 0.05 (0.01) | 0.43 (0.12) | 3.75 | 0.34 (0.12) | 0.56 (0.10) | 0.38 (0.12) | 12.88 |
| 18:1n-9 | 0.16 (0.07) | 0.32 (0.06) | 0.34 (0.12) | 1.40 | 9.25 (3.33) | 15.65 (2.68) | 0.36 (0.11) | 11.88 |
| 18:2n-6 | 0.13 (0.06) | 0.32 (0.05) | 0.28 (0.12) | 2.27 | 2.07 (0.78) | 4.27 (0.66) | 0.33 (0.11) | 10.44 |
| ALA | 0.00 (0.00) | 0.01 (0.00) | 0.03 (0.08) | 0. 59 | 0.06 (0.02) | 0.10 (0.02) | 0.35 (0.11) | 11.40 |
| EPA | 0.01 (0.00) | 0.02 (0.00) | 0.39 (0.11) | 4.28 | 0.07 (0.02) | 0.12 (0.02) | 0.37 (0.12) | 12.56 |
| DHA | 0.05 (0.02) | 0.10 (0.02) | 0.33 (0.12) | 3.14 | 0.32 (0.13) | 0.61 (0.10) | 0.35 (0.12) | 9.21 |
| DHA/ALA | 0.01 (0.00) | 0.03 (0.00) | 0.25 (0.11) | 3.40 | – | – | – | – |

% = proportional content of fatty acids, mg/g = quantitative content of fatty acids, $V_a$: Additive genetic variance. $V_e$: Residual variance. Standard errors in brackets. GCV = genetic coefficient of variation. $N = 240$.

**Table 6**
Phenotypic ($r_P$) and genetic ($r_G$) correlations between production traits and fatty acids.

| Trait | $r_P$ | $r_G$ (SE) |
|---|---|---|
| BW * % EPA | 0.24 | 0.17 (0.18) |
| BW * % DHA | 0.08** | −0.03 (0.20) |
| $MFAT_F$ * % EPA | −0.05** | 0.11 (0.25) |
| $MFAT_F$ * % DHA | −0.56 | −0.53 (0.20) |
| $MFAT_F$ * % 18:1n-9 | 0.5 | 0.81 (0.17) |
| $MFAT_F$ * % 16:1n-7 | 0.4 | 0.51 (0.20) |
| $MFAT_F$ * 18:2n-6 | −0.07** | −0.20 (0.29) |
| $MFAT_F$ * 16:0 | 0** | −0.15 (0.30) |
| DHA * EPA | 0.42 | 0.55 (0.19) |
| DHA * 18:2n-6 | −0.22 | −0.37 (0.29) |
| DHA * 16:0 | −0.23 | −0.48 (0.25) |
| DHA * 16:1n-7 | −0.04** | 0.05 (0.26) |
| DHA * 18:3n-3 | −0.22 | nc |
| DHA * 18:1n-9 | −0.63 | nc |

$MFAT_F$ = Muscle fat % by Folch chemical analysis, BW = body weight. SE = standard error. EPA = 20:5n-3. DHA = 22:6n-3. Nc = no convergence. ** = $p >$ 0.01.

tissues and can be explained by DHA being a PUFA with important roles in cell membranes that is therefore found in phospholipids (PL) to a larger degree than in triglycerides (TG). When muscle fat increases, the proportion of PL relative to TG decreases, and therefore the % DHA decreases (Tocher, 2003).

### 3.4. Selection strategies for increased omega-3 fatty acid content of fillets

The heritability of EPA and DHA indicated that the content of healthy omega-3 fatty acids in seabream fillets can be increased through selective breeding, and there are two possible strategies to achieve this: by increasing the quantitative content (mg per g muscle), or by increasing the proportional content (% of total muscle fatty acids).

As muscle fat percentage increases, so does the quantitative content of all fatty acids. Therefore, the heritability of the quantitative content of all fatty acids mirrored the heritability of muscle fat and were all close to 0.34 (Table 5). This was confirmed in the estimates of genetic correlations, where the quantitative content of all fatty acids had a genetic correlation to muscle fat close to unity (results not shown). This implies that an increase in the quantitative content of EPA and DHA can be achieved by selective breeding for increased muscle fat content, which could be achieved by *e.g.* the Distell fat meter for non-invasive phenotypic recording. It should then be considered that increased muscle fat would influence the organoleptic qualities of the fish (Grigorakis, 2007). It should also be considered that selective breeding for increased muscle fat content would result in an increase in all fatty acids, including the less desirable omega-6 fatty acids. This is critical from a nutritional health perspective, as it is not only the amount of EPA and DHA that is important, but also the ratio of omega-3 to omega-6 fatty acids (Saini and Keum, 2018; Simopoulos, 2002). In addition, the genetic correlations indicated that increasing muscle fat would actually reduce the proportional content of DHA in the muscle, leading to a less desirable fatty acid profile of the seabream fillet (Table 6). On the other hand, the genetic correlations between proportional contents of muscle fatty acids showed a potential to select for an overall healthier fatty acid profile in muscle (Table 6). DHA was positively correlated with EPA (rg = 0.55), and negatively correlated with the main saturated fatty acid (16:0) and the main omega-6 fatty acid (18:2n-6) in muscle (rg = −0.48 and − 0.37, respectively). Thus, according to these results, selection for increased proportional content of DHA will result in a fish fillet with a higher proportion of marine omega-3 fatty acids, and lower proportion of saturated fatty acids and pro-inflammatory omega-6 fatty acids. However, a lower selection response may be expected using this strategy as the genetic coefficients of variation was lower for proportional compared to quantitative content of EPA and DHA (Table 5).

Even though both quantitative and proportional content of EPA and DHA are highly heritable, getting genetic response in fatty acid traits is not straight forward. With low phenotypic and genetic coefficients of variation it is difficult to get a reasonable selection response, even when heritability is high. We recommend future research into economic weights and selection indices which are outside the scope of this paper. Another challenge with implementing selection for fatty acid traits is that phenotyping requires costly and time-consuming chemical analyses, which make large-scale data recording challenging. New rapid methods for predicting fatty acid composition in fish fillet are being developed, such as Raman and near infrared spectroscopy (*e.g.* EWOS SalmoNIR technology from Cargill) (Afseth et al., 2006; Bekhit et al., 2014; Blay et al., 2021), which can make practical implementation of selection for these traits more feasible.

## 4. Conclusions

In the gilthead seabream, muscle fat deposition and body weight seem to be independent traits, and selective breeding for faster growth is not likely to influence the proportional content of EPA and DHA. The fatty acid 16:1n-7, a marker of *de novo* lipogenesis, had a high heritability (0.43), indicating that there is a strong genetic component to this metabolic pathway in seabream.

It is possible to increase EPA and DHA content in gilthead seabream fillets by selective breeding, as the estimated heritability of EPA (%) and DHA (%) was 0.39 and 0.33, respectively, and there was a positive genetic correlation between the two fatty acids (0.55). There is a potential to select for a healthier overall fatty acid profile in muscle of seabream by selection for increased proportional content of DHA, as DHA was negatively correlated with the major omega-6 fatty acid (18:2n-6) and the major saturated fatty acid (16:0).

## CRediT authorship contribution statement

**S.S. Horn:** Methodology, Writing – original draft, Visualization, Writing – review & editing, Formal analysis. **M.L. Aslam:** Methodology,

Writing – review & editing, Formal analysis. **G.F. Difford:** Methodology, Writing – review & editing, Formal analysis. **K. Tsakoniti:** Resources, Conceptualization, Data curation. **S. Karapanagiotis:** Resources, Data curation, Writing – review & editing. **B. Gulzari:** Writing – review & editing. **J.W.M. Bastiaansen:** Writing – review & editing. **C. Peñaloza:** Resources, Writing – review & editing. **R. Houston:** Resources, Writing – review & editing. **B. Ruyter:** Data curation, Conceptualization, Writing – review & editing. **A.K. Sonesson:** Funding acquisition, Project administration, Supervision, Conceptualization, Methodology, Writing – review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

This study was made possible by the EU project MedAID (H2020 grant agreement No 727315). Authors would like to thank Galaxidi Marine Farm S.A. for their participation in the study and its staff for providing necessary help and facilities for raising the population and the experimental data collection. Authors are grateful to Målfrid Tofteberg Bjerke and Anne Marie Langseter for skilful technical assistance.

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