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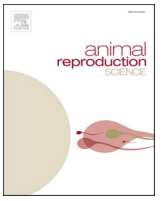

Selection for high growth improves reproductive performance of gilthead seabream *Sparus aurata* under mass spawning conditions, regardless of the dietary lipid source

Shajahan Ferosekhan [a,b,*], Samira Sarih [a], Juan Manuel Afonso [a], María Jesús Zamorano [a], Ramón Fontanillas [c], Marisol Izquierdo [a], Sadasivam Kaushik [a], Daniel Montero [a]

[a] IU-ECOAQUA, Universidad de Las Palmas de Gran Canaria, Taliarte, 35214, Telde, Las Palmas, Canary Islands, Spain
[b] ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar 751002, Odisha, India
[c] Skretting Aquaculture Research Centre, Sjohagen, 4016 Stavanger, Norway

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ABSTRACT

Genetic selection programmes in gilthead seabream mainly focus on traits related to growth, disease resistance, skeletal anomalies, or fillet quality. However, the effect of selection for growth on the reproductive performance of seabream broodstock has not received much attention. The present study aimed to determine the effect of selection for growth traits, high (HG) or low (LG) growth, and broodstock feeding with fish oil (FO diet) or rapeseed oil (RO diet) as main lipid sources, on reproductive performance of gilthead seabream. For the first part of the spawning season (Phase I) HG and LG broodstock were fed a commercial diet and the HG broodstock produced a higher number of larvae and higher viable eggs, hatching and larval survival rates than LG broodstock, affecting egg fatty acid profiles. For the second part of the study (Phase II) broodstock were fed one of the two diets containing FO or RO. Fecundity in terms of viable eggs, hatchlings, and larvae produced, as well as fertilization rates, were improved in HG broodstock. Some fatty acids such as 18:0, 20:2n-6, 20:3n-3 or EPA/ARA were also affected by the growth selection. According to the two-way ANOVA analysis, feeding the RO diet did not significantly affect fecundity parameters, but slightly reduced fertilization and hatching rates in HG broodstock. Nevertheless, HG broodstock showed better spawning quality parameters than LG broodstock, even when they were fed the RO diet. Egg fatty acid profiles reflected diet composition, although DHA contents were not affected. In conclusion, broodstock selected for high growth had a positive effect on broodstock performance, and FO replacement by RO did not markedly affect reproduction providing that fatty acid contents were sufficient to fulfill the essential fatty acid requirements of gilthead seabream broodstock.

1. Introduction

Gilthead seabream (*Sparus aurata*) is the major farmed marine fish in the European Union and Mediterranean region (FAO, 2020).

* Corresponding author at: ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar 751002, Odisha, India.
E-mail address: feroseaqua@gmail.com (S. Ferosekhan).

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Genetic improvement either through mass selection or family selection is practiced in different species of fish in order to improve growth (Fernandes et al., 2017; Regan et al., 2021; Gjedrem, 2012), disease resistance (Palaiokostas et al., 2016; Fjalestad et al., 1993), fillet quality and pigmentation (Horn et al., 2020; Garcia et al., 2017; Schlicht et al., 2019) or to reduce skeletal anomalies (Negrín-Báez et al., 2015; García-Celdrán et al., 2016; Lorenzo Felipe et al., 2021). In gilthead seabream, genetic selection programmes have tried to address several traits of importance such as improved growth (Borrell et al., 2011), disease resistance (Piazzon et al., 2020) or reduced skeletal anomalies (García-Celdrán et al., 2016; Lorenzo Felipe et al., 2021). Most of the selection programmes in fish are based on mass selection due to easy implication and management (Gorshkov et al., 1997). The genetic improvement programme in

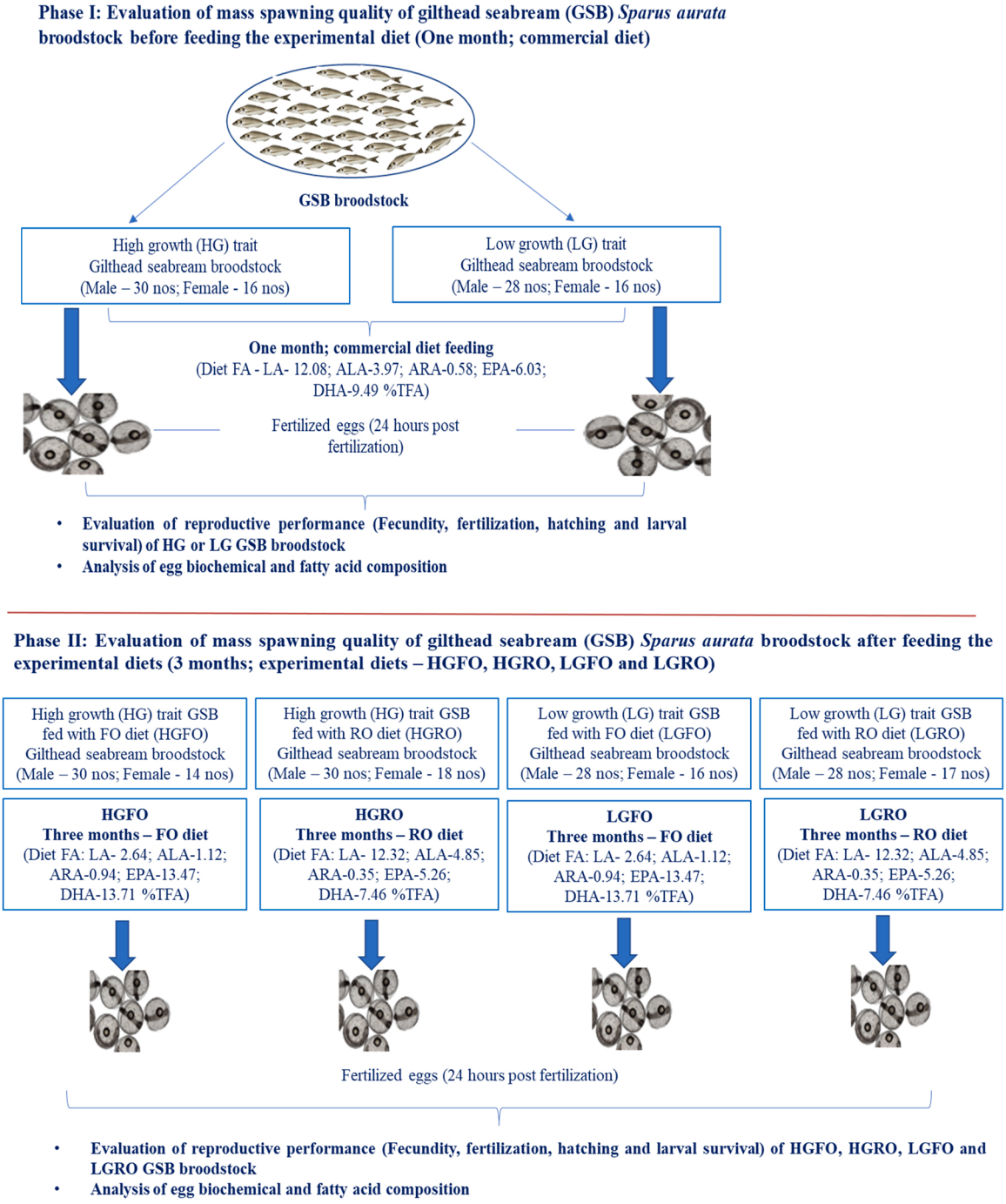

Fig. 1. Graphic representation of the mass spawning study of gilthead seabream (GSB) *Sparus aurata* broodstock selected for high growth (HG) or low growth (LG) trait fed with either commercial diet (Phase I) or experimental diets (Phase II).

gilthead seabream has led to a 5–29% increase in growth rate per generation, which eventually produced the genetically improved seeds for better performance in aquaculture conditions (Knibb et al., 1997; Brown et al., 2005). It was estimated that around 40% of seeds used in the European aquaculture are mostly coming from selective breeding programmes (Chavanne et al., 2016). PROGENSA, a Spanish National Breeding Program (Afonso et al., 2012; García-Celdrán et al., 2015; Lee-Montero et al., 2015; Lorenzo Felipe et al., 2021), is an ongoing selective breeding programme intended to improve the growth and skeletal anomalies of seabream through the Best Linear Unbiased Prediction (BLUP) methodology (Lorenzo Felipe et al., 2021).

Gilthead seabream is a batch spawner whose egg quality is affected by the broodstock diet composition (Fernández-Palacios et al., 2011). Besides, broodstock nutrition plays an important role in gamete quality and offspring performance (Izquierdo et al., 2001; Fernández-Palacios et al., 1995). Among other nutrients, lipids and fatty acid (FA) contents of broodstock diets have been identified as major dietary contributors for ensuring successful reproduction and the offspring survival (Izquierdo et al., 2001). The importance of broodstock diet composition in the development of the testis, ovary, oocyte quality and offspring performance is widely studied and reported for many fish species, particularly in improving the reproductive performance of gilthead seabream (Fernández-Palacios et al., 1995; Izquierdo et al., 2015; Xu et al., 2019; Ferosekhan et al., 2020, 2021). Marine fish have a limited ability to synthetize these essential fatty acids (EFA), from their fatty acid precursors linoleic acid (LA; 18:2n-6) and α-linolenic acid (ALA; 18:3n-3) (Monroig et al., 2011; Oboh et al., 2017; Tocher, 2015). Therefore, it is considered necessary to include sufficient amounts of these EFA for improving growth, health, immunity, reproduction, gamete quality and offspring performance of fish (Izquierdo and Koven, 2011; Tocher, 2015). Dietary supply of EPA and DHA are also essential to support neural and visual development, besides many physiological and behavioral activities (Benítez-Santana et al., 2014). Fish oils (FO) were traditionally being used as the main sources of dietary n-3 LC-PUFA, but their production has become stagnant, and their price and demand have increased, and therefore at present, different alternative oils are replacing FO in fish diets. However, most of these oils are plant oils devoid of the essential LC-PUFAs, although they contain the ALA and LA as precursors of EPA, DHA and ARA. Therefore, several strategies are being developed to improve the LC-PUFA synthesis from those precursors (Izquierdo et al., 2015). Nutritional programming of the offspring, through the modification of the broodstock diet, effectively stimulates LC-PUFA synthesis and improves the use of low fish meal (FM) and low FO diets along on-growing (Izquierdo et al., 2015; Turkmen et al., 2017; Turkmen et al., 2020; Xu et al., 2021; Xu et al., 2021). Recently, gilthead seabream broodfish were fed a low FO diet to nutritionally program the offspring to improve the use of low FO diets along on-growing (Turkmen et al., 2017; Ferosekhan et al., 2021; Xu et al., 2021). In such nutritional programming, FO was completely replaced by 1.5% linseed oil (LO) and 8.5% rapeseed oil (RO), oils rich in ALA (9.58% total fatty acids, TFA) and LA (13.49% TFA), both fatty acids precursors of LC-PUFA, to stimulate LC-PUFA biosynthesis. However, feeding this diet significantly reduced all fecundity and spawning quality parameters, regardless the selection for high (HG) or low (LG) growth (Ferosekhan et al., 2021). Since the dietary levels of EPA, DHA and ARA were sufficient to fulfill the EFA requirements of gilthead seabream broodstock, the poor reproductive performance was most probably linked to an excessive dietary content of linoleic and, particularly, linolenic acid.

Based on these findings, the present study was conducted to evaluate the reproductive performance of gilthead seabream selected for high growth and fed an improved diet under mass spawning conditions (Fig. 1). Therefore, the dietary content of LA and ALA was reduced by removing linseed oil, decreasing rapeseed oil to 7.54% and adding 1.76% fish oil, to reduce the possible negative effects of a diet totally devoid of FO on broodstock performance. This approach has direct implications for the gilthead seabream hatcheries for improved reproductive and offspring quality through appropriate broodstock selection programmes. In this context, the present study was carried out to delineate the effect of dietary fatty acid profiles on spawning quality, larval performance, biochemical and fatty acid composition of the eggs from broodstock of gilthead seabream selected for improved growth and reared under mass spawning conditions.

2. Materials and methods

2.1. Ethical statement

The study was conducted according to the European Union Directive (2010/63/EU) on the protection of animals for scientific purposes at Aquaculture Research Group (GIA) of ECOAQUA Institute, University of Las Palmas de Gran Canaria (ULPGC), Canary Islands, Spain. All experimentation performed at the (ULPGC) was approved by the Bioethical Committee of the University of Las Palmas de Gran Canaria (REF: OEBA-ULPGC-20/2018 R1).

2.2. Broodstock selection and management

The gilthead seabream broodstock used originated from the third generation of selection under the PROGENSA (Spanish National Breeding Program) project (Afonso et al., 2012; García-Celdrán et al., 2015; Lee-Montero et al., 2015; Lorenzo Felipe et al., 2021). Two broodstock groups expressing either high growth (H) or low growth (L), selected by Best Linear Unbiased Prediction (BLUP) methodology with VCE-v 6.0 software (Neumaier and Groeneveld, 1998), were used for the assessment of reproductive performance under mass spawning conditions. High and low growth trait broodstock fish were individually marked with PIT tags (EID Iberica SA-TROVAN, Madrid, Spain) and maintained separately for mass spawning in four tanks (10 m^3) at the facilities of ECOAQUA institute (ULPGC, Canary Islands, Spain). All the tanks were supplied with seawater (37 g L^{-1} salinity, 17.0–19.0 °C) with a daily water exchange of 600% and maintained under natural photoperiod. The four broodstock groups were maintained separately for the whole reproductive season as HG (tanks 1 and 2) and LG (tanks 3 and 4), with average biomass of 58 kg $tank^{-1}$ and an average ratio of female/male biomass range from 1.5 to 2.1 (Table 1). Male and female body weight and length were larger in H than L broodstock

(Table 1).

2.3. Phase I: evaluation of mass spawning quality before feeding the experimental diet

The selected male and female broodfish from HG and LG groups were assessed for spawning quality. At the beginning of the spawning season from 30 December 2019–22 January 2020 all the broodfish groups were fed with a commercial diet (crude protein 57.1%, crude lipid 20.8%, and ash 9.3%; Skretting, Burgos, Spain) to ensure that there were no significant differences in the spawning quality among broodfish from the same selection group (HG or LG) (Fig. 1). Broodfish were housed in natural photoperiod and the spawning were took place spontaneously without hormonal induction. The fatty acid composition of the commercial diet is provided in Table 3. For the evaluation of spawning quality, the spontaneously spawned eggs after 24 h of fertilization from each broodstock, HG or LG group were collected six times per week and estimated for all the spawning quality parameters as described in Ferosekhan et al. (2020, 2021). Fertilized egg samples (24 h post fertilization) were collected at the end of the feeding period and kept at − 80 °C for chemical and fatty acid composition analyses.

2.4. Phase II: Evaluation of mass spawning quality after feeding the experimental diets

The experimental broodstock feeds were formulated to be iso-proteic and iso-lipidic with either fish oil (FO diet) or a mixture of fish and rapeseed oils (RO diet) as the lipid source and were produced by Skretting ARC, Stavanger, Norway (Tables 2 and 3). Compared to the FO diet, the RO diet had higher levels of 18:2n-6 (LA) and 18:3n-3 (ALA) fatty acids and reduced levels of saturated, monoenoic, and n−3 LC-PUFA (20:5n−3; eicosapentaenoic acid, EPA and 22:6n−3; docosahexaenoic acid, DHA) (Table 3). The broodstock treatment groups were assigned as follows: HGFO, HGRO, LGFO, and LGRO. Both the HG and LG broodfish groups were fed with one of the two diets at the rate of 1% body weight, twice a day (9:00 and 14:00 h), over 3 months (24 February 2020–16 April 2020) (Fig. 1). Seawater temperature during the spawning period was in the range of 18.5–23 °C and fish were kept under natural photoperiod. Egg collection for spawning quality and biochemical composition followed the same protocol described in the commercial diet feeding phase (Ferosekhan et al., 2020, 2021). Finally, after 3 months of feeding the two diets, eggs were collected from all broodfish groups (HGFO, HGRO, LGFO, and LGRO) and analyzed for egg biochemical and fatty acid composition.

2.5. Evaluation of egg and larval quality

The naturally spawned eggs after 20 h of incubation were collected and placed in a 10 L container provided with aeration. Egg samples (n = 3; 10 mL) were randomly collected with thorough mixing and placed in a Bogorov chamber under the light microscope to calculate the total number of eggs and percentages of fertilized and viable eggs. Egg viability was determined by observing the percentage of morphologically normal eggs after 1-day post-fertilization (1 dpf) (Fernández-Palacios et al., 2011). Then, the viable eggs were individually placed in 96-well microtiter plates in two replicates filled with filtered and UV sterilized seawater. Eggs were incubated in a controlled temperature incubator at 19–21 °C to estimate the percentage of hatching (2 dpf) and larval survival rates at 3 days post-hatch (dph). From these values, other fecundity parameters were calculated, including total numbers of fertilized, viable, hatched, and larvae produced per kg female (Fernández-Palacios et al., 1995; Ferosekhan et al., 2020, 2021).

2.6. Egg biochemical analysis

After feeding either the commercial diet or the experimental diets, egg samples were collected from all the broodstock groups and stored at − 80 °C for analysis of proximate and fatty acid composition. Moisture contents were obtained after drying the samples in an oven at 110 °C for 24 h and then for 1 h until constant weight. Ash content was determined after incineration at 600 °C for 16 h. Crude protein content was determined by measuring the N content (N × 6·25) through automated Kjeldahl analysis (AOAC, 1995) and crude lipid extraction was carried out with chloroform: methanol (Folch et al., 1957). Fatty acid methyl esters (FAMEs) from egg total lipids

Table 1
Description of gilthead seabream Sparus aurata broodstock (Mean±SD) selected for mass spawning experiment.

Broodstock details	HGFO	HGRO	LGFO	LGRO
Broodstock density/tank				
Male (n)	30	30	28	28
Female (n)	14	18	16	17
Total (n)	44	48	44	45
Broodstock biometry				
Male length (cm)	43.05 ± 1.69	42.71 ± 1.88	42.20 ± 3.42	41.18 ± 2.95
Male weight (kg)	1.34 ± 0.21	1.35 ± 0.17	1.24 ± 0.28	1.15 ± 0.26
Female length (cm)	43.26 ± 2.14	43.43 ± 2.78	39.56 ± 2.12	41.19 ± 2.40
Female weight (kg)	1.38 ± 0.27	1.49 ± 0.28	1.03 ± 0.16	1.15 ± 0.23
Male biomass (kg)	40	41	35	32
Female biomass (kg)	20	27	16	20
Total biomass (kg)	60	67	51	52
Female/male biomass (kg)	2.0	1.5	2.1	1.6

Table 2
Ingredients and proximate composition of the gilthead seabream *Sparus aurata* broodstock experimental diets used for mass spawning study.

Feed ingredients (%)	Diet FO	Diet RO
Fish meal (North-Atlantic 12 C)	59.36	59.36
Krill meal	7.00	7.00
Squid meal	3.00	3.00
Wheat	20.57	20.57
Fish oil (South American)	9.30	1.76
Rapeseed oil	0.00	7.54
Vitamin-mineral premix	0.50	0.50
L-Histidine HCl	0.27	0.27
Proximate composition		
Crude protein (% dry matter, DM)	58.9	58.1
Crude lipid (% DM)	21.3	22.1
Ash (% DM)	9.8	9.8

were prepared by transmethylation method with 1% sulfuric acid in methanol, purified on NH_2 silica (Sep-pak; Waters, USA), and separated and quantified in a gas chromatograph (GC14A; Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and a Carbowax 20 M (30 m × 0.32 mm × 0.27 m) silica capillary column (length: 30 m; internal diameter: 0.32 mm; Supelco, Bellefonte, USA) using helium as a carrier gas. Column initial temperature was set to 170 °C for 10 min and then it was raised to 220 °C at 2.5 °C per min and finally maintained at 215 °C for a further 5 min. FAMEs were identified by comparison with previously characterized standards. Specific unclear peaks were identified by GLC-MS (TRACETM GC Ultra and PolarisQ mass spectrometer; Thermo Fisher Scientific, Spain) (Izquierdo et al., 1990).

2.7. Statistical analysis

Data are reported as mean ± standard deviation (SD). Data were compared statistically using the analysis of variance (ANOVA), at a significance level of < 0.05. All variables were checked for normality and homogeneity of variance (Sokal and Rohlf, 1979). Data which did not fit for normality was subjected to an arcsine transformation to attain normality. When arcsin-transformed data were not normally distributed, then Kruskal–Wallis, a non-parametric test was applied to the non-transformed data. An independent sample *t*-test was performed to compare, spawning quality, egg biochemical, and fatty acid composition for the commercial diet feeding phase to check the broodstock selection (HG or LG) effect. One-way and two-way ANOVA were applied to the results of spawning quality parameters (total eggs; fertilized eggs; viable eggs; hatched larvae; 3dph larvae per spawn per kg female and fertilization, egg viability, hatching, and larval survival rates), egg biochemical and fatty acid composition of experimental diet feeding phase to determine the combined effects of broodstock selection (HG or LG) and diet (FO or RO) and interaction of broodstock selection and diet. All data were analyzed using the program IBM SPSS version 20 for Windows (IBM SPSS Inc., Armonk, NY, USA).

3. Results

3.1. Phase-I: Evaluation of mass spawning quality before feeding the experimental diet

3.1.1. Egg and larval quality

After one month of feeding the commercial diet, at the beginning of the spawning season and prior to feeding the experimental diets the spawning quality was evaluated, it was observed that the total number of eggs and fertilized eggs per spawn per kg female were significantly higher for low growth trait broodstock (LG) than for high growth trait broodstock (HG) (Table 4). However, the number of viable eggs and hatched-out larvae were not significantly different between HG or LG broodstock (Table 4). Moreover, the HG broodstock group produced a 67% higher number of 3 dph larvae per spawn per kg female (7079 larvae) compared to LG (4736 larvae) broodstock group (Table 4). Regarding the spawning quality parameters (Table 5), despite the egg fertilization rate did not vary between broodstock groups, the percentage of viable eggs, hatching rate and larval survival (3 dph) were significantly higher in the HG broodstock group as compared to LG broodstock (Table 5).

3.1.2. Egg biochemical analysis

The chemical composition of eggs from either HG or LG broodstock before feeding the experimental diet did not show any significant difference (Table 6). Crude protein and crude lipid contents of eggs from HG or LG broodstock were 72.3% or 70.1% and 19.3% or 18.3% of dry matter, respectively (Table 6). Fatty acid profiles of eggs from high or low growth broodstock were very similar, only some derivatives from desaturases such as 18:2n-9, or 18:3n-6, were significantly reduced in HG broodstock, whereas the elongase products 20:1n-7 and 20:2n-6 were increased (Table 7). Besides, monoenes were also reduced in eggs from L broodstock, mainly due to the reduction in 18:1n-9 and 22:1n-7 (Table 7). Finally, the ratio EPA/ARA was mildly increased (Table 7).

Table 3
Fatty acid profiles (% total fatty acids) of commercial, FO and RO broodstock diet of gilthead seabream Sparus aurata.

%TFA	Commercial diet	FO diet	RO diet
14:0	2.74	6.01	2.22
14:1n-7	0.02	0.03	0.01
14:1n-5	0.11	0.20	0.07
15:0	0.30	0.45	0.18
15:1n-5	0.02	0.03	0.01
16:0 ISO	0.05	0.08	0.03
16:0	12.70	17.65	9.42
16:1n-7	3.74	6.93	2.20
16:1n-5	0.12	0.21	0.07
16:2n-6	0.01	0.02	0.01
16:2n-4	0.30	0.85	0.24
17:0	0.26	0.99	0.23
16:3n-4	0.21	0.14	0.10
16:3n-3	0.12	0.16	0.07
16:3n-1	0.03	0.07	0.03
16:4n-3	0.36	1.08	0.33
16:4n-1	0.01	0.01	0.01
18:0	3.13	3.45	2.41
18:1n-9	27.58	9.96	36.94
18:1n-7	2.67	2.70	2.74
18.1n-5	0.15	0.20	0.14
18:2n-9	0.06	0.10	0.02
18.2n-6 (LA)	12.08	2.64	12.32
18:2n-4	0.14	0.26	0.08
18:3n-6	0.15	0.24	0.08
18:3n-4	0.17	0.26	0.10
18:3n-3 (ALA)	3.97	1.12	4.85
18.3n-1	0.02	0.05	0.02
18:4n-3	1.15	2.70	1.30
18:4n-1	0.12	0.20	0.07
20:0	0.37	0.29	0.48
20:1n-9	0.30	0.30	0.23
20:1n-7	3.34	3.70	3.81
20:1n-5	0.19	0.27	0.12
20:2n-9	0.10	0.12	0.02
20:2n-6	0.68	0.20	0.14
20:3n-9	0.03	0.07	0.03
20:3n-6	0.16	0.13	0.04
20:4n-6 (ARA)	0.58	0.94	0.35
20:3n-3	0.34	0.13	0.08
20:4n-3	0.71	0.82	0.29
20:5n-3 (EPA)	6.03	13.47	5.26
22:1n-11	2.96	4.37	3.92
22:1n-9	0.55	0.43	0.78
22:4n-6	0.11	0.13	0.05
22:5n-6	0.24	0.32	0.13
22:5n-3	1.33	1.83	0.52
22:6n-3 (DHA)	9.49	13.71	7.46
Total saturates	19.49	28.84	14.94
Total monoenes	41.76	29.32	51.04
Total n-3	23.50	35.02	20.16
Total n-6	14.01	4.61	13.11
Total n-9	28.59	10.90	37.99
Sum n-3 LC-PUFA	17.90	29.96	13.61
EPA/ARA	10.43	14.39	15.08
DHA/EPA	1.57	1.02	1.42
DHA/ARA	16.41	14.65	21.39
n-3/n-6	1.68	7.60	1.54

3.2. Phase-II: evaluation of mass spawning quality after feeding the experimental diets

3.2.1. Egg and larval quality

The total number of eggs or fertilized eggs produced per spawn per kg female, was significantly (Table 8) lowest for LGRO broodstock and highest for LGFO. Thus, in LG broodstock, feeding the RO diet instead of the FO diet reduced both the total number of eggs and the number of fertilized eggs, whereas such effect was not found in HG broodstock, reflecting the interaction between selection and diet in these two parameters. However, regarding the number of viable eggs, hatchlings, and 3 dph larvae per spawn per kg female, all these parameters were significantly improved by broodstock selection, being increased in HGRO broodstock in comparison

Table 4
Reproductive performance of gilthead seabream *Sparus aurata* broodstock selected for high (HG) or low (LG) growth before experimental diet feeding period (n = 18).

Fecundity parameters	High growth (HG)	Low growth (LG)	*t*-test (*p* Value)
Total number of eggs/spawn/kg female	20,841^{b} ± 10,840	30,293^{a} ± 9599	0.009
Fertilized eggs/spawn/kg female	20,683^{b} ± 10,735	29,986^{a} ± 9515	0.009
Viable eggs/spawn/kg female	11,359 ± 6699	11,701 ± 4690	0.860
Hatched larvae/spawn/kg female	10,380 ± 6026	9214 ± 3810	0.493
Larval survival 3dph/spawn/kg female	7079 ± 5108	4736 ± 2381	0.091

Means bearing different superscript letters in each row differ significantly (Mean ± SD, $p < 0.05$, Independent sample *t*-test).

Table 5
Spawning quality (%) of gilthead seabream *Sparus aurata* broodstock selected for high (HG) or low (LG) growth before experimental diet feeding period (n = 18).

Spawning quality parameters (%)	High growth (HG)	Low growth (LG)	*t*-test (*p* Value)
Fertilization %	99.3 ± 0.5	99.0 ± 0.7	0.172
Egg viability %	53.8^{a}± 18.1	40.1^{b} ± 14.9	0.018
Hatching %	91.7^{a} ± 4.5	79.5^{b} ± 11.3	0.001
Larval survival (3 dph) %	67.4^{a} ± 18.5	51.6^{b} ± 12.3	0.005

Means bearing different superscript letters in each row differ significantly (Mean ± SD, $p < 0.05$, Independent sample *t*-test).

Table 6
Biochemical composition of eggs obtained from the gilthead seabream *Sparus aurata* broodstock selected for high (HG) or low (LG) growth before feeding the broodstock experimental diets (n = 3).

Egg biochemical composition	High growth (HG)	Low growth (LG)	*t*-test (*p* Value)
Crude protein (% DM)	72.3 ± 5.7	70.1 ± 3.9	0.545
Crude lipid (% DM)	19.3 ± 1.0	18.3 ± 0.4	0.131
Ash (% DM)	5.8 ± 1.9	6.2 ± 1.3	0.709

Means bearing different superscript letters in each row differ significantly (Mean ± SD, $p < 0.05$, Independent sample *t*-test).

to LGRO (Table 8). This effect was not observed in fish fed diet FO, reflecting the interaction between selection and diet in these three parameters. Thus, the hatchling and 3 dph larvae production were respectively 38% and 35% higher in the HGRO broodstock compared to LGRO broodstock.

Regarding the spawning quality parameters, fertilization rates were improved in eggs from high growth broodstock (Table 9) and fed the FO diet (Table 9). Therefore, the combination of selection for high growth and feeding the FO diet resulted in significantly highest fertilization rates. Similarly, the egg viability rate was significantly highest (86%) for HF broodstock followed by HGRO (74%) and LGRO broodstock, and there was a positive significant effect of selection for high growth, regardless the diet fed (Table 9). Besides, feeding the RO diet also had a significant effect on egg viability rates (Table 9), which were reduced in HG fish and increased in LG fish, denoting the interaction between both factors diet and selection (Table 9). Likewise, selection for H growth significantly improved hatching rates (Table 9) in broodstock fed FO diet, whereas this effect was not observed in fish fed RO diet, denoting the interaction between both factors (Table 9). Thus, the lowest hatching rate (86.8%) was observed in LF broodstock. The 3dph larval survival rate was not significantly affected by selection or diet (Table 9).

3.3. Egg biochemical analysis

Crude protein and ash content did not differ among eggs from the different broodstock groups (Table 10). However, crude lipid contents of eggs from HF and LF group were significantly higher than those from HGRO and LGRO groups, denoting the highly significant effect of RO diet.

Egg fatty acid composition markedly reflected the fatty acid profile of the broodstock diet (Table 11). Thus, feeding the rapeseed oil diet significantly reduced the egg contents in saturated fatty acids, such as 14:0, 15:0, 16:0, 17:0 or 18:0, and PUFA, such as 18:2n-4, 18:3n-4, 18:4n-3, 18:4n-1, 20:3n-9, 20:4n-6, 20:4n-3, 20:5n-3, 22:4n-6, 22:5n-6, 22:5n-3, as well as total n-3, n-3 LC-PUFA and n-3/n-6 (Table 11). On the contrary, feeding the RO diet significantly increased the egg contents in monounsaturated and n-9 fatty acids, such as 18:1n-9, 20:1n-7 or 22:1n-9, in n-6 fatty acids, such as 18:2n-6, 20:2n-6, as well as 18:3n-3, DHA/EPA and DHA/ARA. The egg DHA content was not significantly different among broodstock groups (Table 11). Two-way ANOVA analysis indicate that the egg fatty acid composition is altered by dietary fatty acid composition: fish oil based diet significantly increased egg ARA and EPA contents but had only a relatively smaller impact on DHA content although the total n-3 LC-PUFA contents and n-3/n-6 ratios were significantly improved (7–12% and 40–51%, respectively) in the groups fed the fish oil based diet (Table 11). Selection for high growth significantly increased 18:1n-5, 18:3n-4, 18:3n-3, and the EPA/ARA ratio, as well as the elongase products 20:2n-6 and 20:3n-3, and reduced 18:0,

Table 7
Fatty acid composition (% total fatty acids) of eggs obtained from the gilthead seabream *Sparus aurata* broodstock selected for high (HG) or low (LG) growth before feeding the broodstock experimental diets (n = 2).

Fatty acids (%TFA)	High growth (HG)	Low growth (LG)	*t*-test (*p* Value)
14:0	1.43 ± 0.12[a]	1.27 ± 0.11[b]	0.033
14:1n-5	0.06 ± 0.01	0.05 ± 0.01	0.156
15:0	0.2 ± 0.01	0.19 ± 0.02	0.097
15:1n-5	0.02 ± 0[a]	0.01 ± 0[b]	0.004
16:0 ISO	0.04 ± 0	0.04 ± 0	1.000
16:0	13.86 ± 0.32	13.75 ± 0.71	0.738
16:1n-7	3.2 ± 0.23	3.02 ± 0.22	0.199
16:1n-5	0.07 ± 0.01	0.06 ± 0.01	0.401
16:2n-4	0.12 ± 0.02	0.11 ± 0.02	0.210
17:0	0.12 ± 0.01	0.11 ± 0.01	0.292
16:3n-4	0.23 ± 0.01[a]	0.20 ± 0.02[b]	0.032
16:3n-3	0.08 ± 0.01	0.07 ± 0.01	0.687
16:4n-3	0.06 ± 0.01	0.06 ± 0.01	0.401
18:0	4.39 ± 0.06	4.46 ± 0.1	0.152
18:1n-9	24.18 ± 0.64	23.74 ± 0.57	0.234
18:1n-7	2.43 ± 0.08	2.38 ± 0.07	0.240
18:1n-5	0.13 ± 0	0.12 ± 0.01	0.092
18:2n-9	0.22 ± 0.03[b]	0.29 ± 0.02[a]	0.001
18:2n-6	9.95 ± 0.22	9.68 ± 0.26	0.091
18:2n-4	0.12 ± 0.01	0.11 ± 0.02	0.341
18:3n-6	0.26 ± 0.02[b]	0.33 ± 0.01[a]	0.001
18:3n-4	0.16 ± 0.02	0.15 ± 0.02	0.348
18:3n-3	2.15 ± 0.07	2.12 ± 0.14	0.642
18:3n-1	0.01 ± 0.01	0.01 ± 0	0.549
18:4n-3	0.41 ± 0.04	0.43 ± 0.03	0.344
18:4n-1	0.09 ± 0.01	0.08 ± 0.01	0.263
20:0	0.06 ± 0	0.06 ± 0	1.000
20:1n-9	0.12 ± 0	0.12 ± 0.01	0.203
20:1n-7	0.73 ± 0.01[a]	0.69 ± 0.03[b]	0.004
20:1n-5	0.1 ± 0	0.1 ± 0.01	0.175
20:2n-9	0.11 ± 0.02	0.1 ± 0.01	0.532
20:2n-6	0.38 ± 0.01[a]	0.35 ± 0.01[b]	0.001
20:3n-9	0.03 ± 0.01	0.03 ± 0.01	0.599
20:3n-6	0.26 ± 0.03	0.26 ± 0.01	0.888
20:4n-6	0.88 ± 0.05	0.87 ± 0.07	0.781
20:3n-3	0.31 ± 0.03	0.29 ± 0.01	0.086
20:4n-3	0.79 ± 0.01	0.76 ± 0.04	0.074
20:5n-3	5.2 ± 0.34	5.26 ± 0.4	0.789
22:1n-11	0.15 ± 0.01	0.14 ± 0.01	0.183
22:1n-9	0.08 ± 0.01	0.08 ± 0.01	0.599
22:4n-6	0.08 ± 0	0.08 ± 0.01	0.363
22:5n-6	0.27 ± 0	0.26 ± 0.02	0.127
22:5n-3	2.75 ± 0.09	2.66 ± 0.12	0.207
22:6n-3	23.65 ± 1.16	24.97 ± 1.14	0.076
Total Saturates	20.06 ± 0.47	19.84 ± 0.79	0.575
Total Monoenes	31.28 ± 0.63[a]	30.52 ± 0.53[b]	0.048
Total n-3	35.41 ± 1.13	36.63 ± 1.28	0.110
Total n-6	12.08 ± 0.22	11.83 ± 0.23	0.076
Total n-9	24.71 ± 0.66	24.33 ± 0.58	0.311
Sum n-3 LC-PUFA	32.71 ± 1.15	33.94 ± 1.3	0.112
EPA/ARA	5.93 ± 0.13	6.08 ± 0.12	0.070
ARA/EPA	0.17 ± 0	0.17 ± 0.01	0.260
DHA/EPA	4.57 ± 0.44	4.77 ± 0.42	0.426
DHA/ARA	27.08 ± 2.5	29.04 ± 2.95	0.244
n-3/n-6	2.93 ± 0.15	3.10 ± 0.15	0.080

Means bearing different superscript letters in each row differ significantly (Mean ± SD, $p < 0.05$, Independent sample *t*-test).

18:2n-4 and 22:1n-9.

4. Discussion

The present study aimed to determine the impacts of broodstock selected for growth trait (HG or LG) fed with either a high or low marine ingredients (FO or RO) diet on the reproductive performance, egg quality, larval performance, and egg biochemical and fatty acid compositions. Overall, broodstock selected for high growth (HG) and fed with a fish oil based diet (FO) showed the best spawning quality and egg fatty acid profiles.

Table 8
Reproductive performance of gilthead seabream *Sparus aurata* broodstock selected for high (HG) or low (LG) growth fed with either FO or RO diet over three months of reproductive months (n = 25).

Fecundity parameters	HGFO	HGRO	LGFO	LGRO	One-way ANOVA (*p* Value)	Two-way ANOVA (*p* Values)		
						Selection	Diet	S × D
Total number of eggs/spawn/kg female	$22,897^{b} \pm 10,989$	$43,176^{a} \pm 16,830$	$46,047^{a} \pm 18,533$	$16,459^{b} \pm 8789$	< 0.001	0.536	0.108	< 0.001
Fertilized eggs/spawn/kg female	$22,397^{b} \pm 10,756$	$41,533^{a} \pm 16,305$	$44,183^{a} \pm 18,118$	$15,749^{b} \pm 8661$	< 0.001	0.477	0.100	< 0.001
Viable eggs/spawn/kg female	$19,560^{bc} \pm 9141$	$31,669^{a} \pm 12,425$	$22,799^{ab} \pm 18,735$	$11,528^{c} \pm 7061$	< 0.001	< 0.001	0.869	< 0.001
Hatched larvae/spawn/kg female	$19,141^{bc} \pm 8950$	$29,629^{a} \pm 11,882$	$19,945^{b} \pm 16,647$	$11,112^{c} \pm 6884$	< 0.001	< 0.001	0.724	< 0.001
3dph larvae/spawn/kg female	$11,649^{ab} \pm 6627$	$16,977^{a} \pm 10,024$	$10,328^{bc} \pm 7048$	$5924^{c} \pm 4939$	< 0.001	< 0.001	0.755	0.001

Means bearing different superscript letters in each row differ significantly (Mean ± SD, $p < 0.05$, One-way ANOVA, Tukey Post-Hoc).

Table 9
Spawning quality (%) of gilthead seabream *Sparus aurata* broodstock selected for high (HG) or low (LG) growth fed with either FO or RO diet over three months of reproductive months (n = 25).

Spawning quality parameters (%)	HGFO	HGRO	LGFO	LGRO	One-way ANOVA (*p* Value)	Two-way ANOVA (*p* Values)		
						Selection	Diet	S × D
Fertilization %	$97.7^{a} \pm 1.0$	$96.0^{b} \pm 1.8$	$95.8^{b} \pm 1.8$	$95.5^{b} \pm 3.6$	0.003	0.007	0.030	0.132
Egg viability %	$86.3^{a} \pm 5.6$	$74.4^{b} \pm 9.9$	$44.9^{c} \pm 18.9$	$70.5^{b} \pm 11.6$	< 0.001	< 0.001	0.007	< 0.001
Hatching %	$97.6^{a} \pm 1.7$	$93.4^{a} \pm 5.6$	$86.8^{b} \pm 15.9$	$96.1^{a} \pm 2.6$	< 0.001	0.020	0.150	< 0.001
Larval survival (3 dph) %	61.4 ± 21.1	58.3 ± 21.4	55.6 ± 15.0	51.4 ± 20.4	0.326	0.108	0.357	0.880

Means bearing different superscript letters in each row differ significantly (Mean ± SD, $p < 0.05$, One-way ANOVA, Tukey Post-Hoc).

Table 10
Biochemical composition of eggs obtained from the gilthead seabream *Sparus aurata* broodstock selected for high (HG) or low (LG) growth fed with either FO or RO diet over three months of reproductive months (n = 3).

Egg biochemical composition	HGFO	HGRO	LGFO	LGRO	One-way ANOVA (*p* Value)	Two-way ANOVA (*p* Values)		
						Selection	Diet	S × D
Crude protein (% DM)	65.3 ± 1.7	64.0 ± 2.1	65.2 ± 0.9	64.3 ± 0.8	0.675	0.889	0.256	0.801
Crude lipid (% DM)	$24.4^{a} \pm 0.4$	$17.6^{b} \pm 0.2$	$24.6^{a} \pm 0.3$	$17.5^{b} \pm 0.4$	0.001	0.743	0.000	0.580
Ash (% DM)	6.9 ± 0.4	6.8 ± 0.3	7.0 ± 0.6	5.8 ± 0.8	0.085	0.188	0.072	0.114

Means bearing different superscript letters in each row differ significantly (Mean ± SD, $p < 0.05$, One-way ANOVA, Tukey Post-Hoc).

Selective breeding programmes in gilthead seabream have addressed improvement of somatic or skeletal growth (Borrell et al., 2011; García-Celdrán et al., 2015; Lee-Montero et al., 2015; Lorenzo Felipe et al., 2021), besides suggested impacts on intestinal functions (Perera et al., 2019). Gilthead seabream broodstock selected for either high (HG) growth or low (LG) growth and fed with diets containing two totally different fatty acid sources (FO or RO) were bred under mass spawning approach. The body weights of male and female from the high growth broodstock (HG) were significantly higher than those from the low growth broodstock (LG), denoting that the fish had been selected for growth. However, there was not any significant relation between fish growth and fecundity or spawning quality. The spawning period of gilthead seabream in the present study was observed from December to April in agreement to previous studies on this species (Fernández-Palacios et al., 1995; Izquierdo et al., 2015; Turkmen et al., 2019; Xu et al., 2019; Ferosekhan et al., 2020, 2021). The spawning quality parameters were in the range of previously reported for gilthead seabream broodstock (Scabini et al., 2011, 2015).

In the present study, prior to feeding the experimental diets, despite the high number of total eggs produced/kg female/spawn in low growth broodstock, selection for high growth significantly improved egg viability, hatching and larval survival and lead to a higher number of larvae. These results agree well with the increased viability and larval survival found in eggs from broodstock of fish selected for high growth in a previous study (Ferosekhan et al., 2021). In agreement, even after feeding the different experimental diets, selection for high growth increased the number of viable and hatched eggs and larval survival, particularly when fish were fed a diet with plant protein and oil sources. Even though fish selected for high growth had a larger body weight, no relation was found between the number of eggs and larvae produced and fish weight (R^2 =0.1902), in agreement with previous studies (Ferosekhan et al., 2021). Thus, the results on fecundity and spawning parameters during both phases confirm the superior spawning quality of gilthead seabream previously selected for high growth.

Besides, selection for high growth affected the egg fatty acid profiles, for instance reducing 18:0 and increasing 18:1n-5, 20:3n-3,

Table 11
Fatty acid composition (% total fatty acids) of eggs obtained from the gilthead seabream *Sparus aurata* broodstock selected for high (HG) or low (LG) growth fed with either FO or RO diet over three months of reproductive months (n = 2).

Fatty acids (%TFA)	HGFO	HGRO	LGFO	LGRO	One-way ANOVA *p* value	Two-way ANOVA (*p* Values)		
						Selection	Diet	S × D
14:0	2.45 ± 0.37^{ab}	1.38 ± 0.08^{c}	2.83 ± 0.59^{a}	1.53 ± 0.36^{bc}	0.005	0.272	0.001	0.620
14:1n-5	0.09 ± 0.01^{a}	0.05^{b}	0.1 ± 0.02^{a}	0.05 ± 0.02^{b}	0.003	0.328	0.001	0.549
14:1n-7	0.01 ± 0.01	0.01	0.02 ± 0.01	0.01	0.219	0.500	0.067	0.500
15:0	0.27 ± 0.02^{ab}	0.17 ± 0.01^{c}	0.3 ± 0.05^{a}	0.19 ± 0.03^{bc}	0.003	0.206	0.001	0.659
15:1n-5	0.02	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.487	1.000	0.282	0.282
16:0 ISO	0.05 ± 0.01^{a}	0.03 ± 0.01^{b}	0.05 ± 0.01^{a}	0.03 ± 0.01^{b}	0.005	1.000	0.001	0.397
16:0	15.02 ± 0.57^{ab}	11.21 ± 0.17^{c}	16.36 ± 2.08^{a}	12.51 ± 1.42^{bc}	0.005	0.115	0.001	0.979
16:1n-7	4.96 ± 0.33^{a}	2.62 ± 0.13^{b}	5.63 ± 0.77^{a}	2.74 ± 0.31^{b}	0.001	0.165	0.001	0.323
16:1n-5	0.09 ± 0.01	0.06 ± 0.01	0.1 ± 0.03	0.07 ± 0.01	0.060	0.372	0.012	0.855
16:2n-4	0.36 ± 0.02^{a}	0.16 ± 0.02^{b}	0.42 ± 0.05^{a}	0.16 ± 0.02^{b}	0.001	0.116	0.001	0.158
17:0	0.31 ± 0.02^{a}	0.13 ± 0.01^{b}	0.33 ± 0.03^{a}	0.14 ± 0.01^{b}	0.001	0.174	0.001	0.397
16:3n-4	0.21 ± 0.01^{ab}	0.17 ± 0.01^{b}	0.23 ± 0.04^{a}	0.17 ± 0.02^{b}	0.018	0.617	0.003	0.457
16:3n-3	0.15 ± 0.08	0.07	0.12 ± 0.02	0.07 ± 0.01	0.103	0.626	0.022	0.533
16:3n-1	0.06	0.06	0.08 ± 0.02	0.05 ± 0.01	0.129	0.446	0.098	0.098
16:4n-3	0.12 ± 0.02^{a}	0.05 ± 0^{b}	0.14 ± 0.02^{a}	0.06 ± 0.02^{b}	0.001	0.183	0.001	0.840
16:4n-1	0.01 ± 0.01	0.01 ± 0.01	0.01	0.01	0.596	0.195	1.000	1.000
18:0	3.9 ± 0.07^{ab}	3.36 ± 0.13^{b}	4.36 ± 0.33^{a}	3.66 ± 0.22^{b}	0.002	0.015	0.001	0.546
18:1n-9	18.13 ± 0.25^{b}	29.01 ± 1.23^{a}	16.67 ± 1.51^{b}	30.78 ± 2.16^{a}	0.001	0.860	0.001	0.091
18:1n-7	2.68 ± 0.01	2.59 ± 0.05	2.91 ± 0.28	2.68 ± 0.1	0.144	0.103	0.109	0.480
18:1n-5	0.16 ± 0.01^{ab}	0.14^{b}	0.18 ± 0.02^{a}	0.15 ± 0.01^{b}	0.009	0.043	0.004	0.218
18:2n-9	0.16 ± 0.01	0.17 ± 0.01	0.15 ± 0.02	0.18 ± 0.01	0.039	0.631	0.008	0.347
18:2n-6	5.8 ± 0.08^{b}	11.02 ± 0.44^{a}	4.84 ± 0.33^{b}	11.47 ± 0.54^{a}	0.001	0.290	0.001	0.014
18:2n-4	0.19^{b}	0.1^{c}	0.23 ± 0.02^{a}	0.09 ± 0.01^{c}	0.001	0.043	0.001	0.008
18:3n-6	0.23 ± 0.03	0.23 ± 0.02	0.22 ± 0.02	0.23 ± 0.01	0.878	0.792	0.601	0.601
18:3n-4	0.27 ± 0.02^{a}	0.16 ± 0.01^{b}	0.25 ± 0.02^{a}	0.12 ± 0.03^{b}	0.001	0.023	0.001	0.247
18:3n-3	1.59 ± 0.02^{b}	3.15 ± 0.18^{a}	1.17 ± 0.04^{c}	3.25 ± 0.06^{a}	0.001	0.024	0.001	0.002
18:3n-1	0.02 ± 0.01	0.01	0.02	0.01	0.045	0.631	0.008	0.631
18:4n-3	1.09 ± 0.04^{a}	0.66 ± 0.04^{b}	1.14 ± 0.05^{a}	0.62 ± 0.03^{b}	0.001	0.943	0.001	0.064
18:4n-1	0.15 ± 0.04^{a}	0.08 ± 0.02^{b}	0.18 ± 0.01^{a}	0.08 ± 0.01^{b}	0.001	0.211	0.001	0.308
20:0	0.07 ± 0.01^{b}	0.09^{a}	0.08 ± 0.01^{ab}	0.09 ± 0.02^{ab}	0.041	0.524	0.010	0.219
20:1n-9	0.16^{ab}	0.14 ± 0.01^{b}	0.18 ± 0.01^{a}	0.14 ± 0.01^{b}	0.001	0.076	0.001	0.076
20:1n-7	0.96 ± 0.03^{c}	1.19 ± 0.02^{a}	1.03 ± 0.08^{bc}	1.14 ± 0.06^{ab}	0.003	0.715	0.001	0.112
20:1n-5	0.14 ± 0.01^{a}	0.12 ± 0.01^{b}	0.15 ± 0.01^{a}	0.11 ± 0.01^{b}	0.001	0.500	0.001	0.195
20:2n-9	0.08^{a}	0.05^{b}	0.07 ± 0.01^{a}	0.05^{b}	0.001	0.081	0.001	0.081
20:2n-6	0.23^{b}	0.3^{a}	0.21 ± 0.02^{b}	0.29^{a}	0.001	0.016	0.001	0.471
20:3n-9	0.06 ± 0.01^{a}	0.03^{b}	0.05 ± 0.01^{a}	0.03^{b}	0.001	0.667	0.001	0.667
20:3n-6	0.16 ± 0.01^{a}	0.13 ± 0.01^{b}	0.16^{a}	0.13^{b}	0.001	0.195	0.001	1.000
20:4n-6	1.05 ± 0.05^{a}	0.65 ± 0.02^{b}	1.11 ± 0.04^{a}	0.63 ± 0.05^{b}	0.001	0.379	0.001	0.111
20:3n-3	0.21 ± 0.01^{a}	0.22 ± 0.01^{a}	0.18 ± 0.01^{b}	0.22 ± 0.01^{a}	0.002	0.010	0.002	0.029
20:4n-3	0.96 ± 0.02^{a}	0.68 ± 0.02^{b}	0.99 ± 0.04^{a}	0.62 ± 0.05^{b}	0.001	0.455	0.001	0.059
20:5n-3	9.33 ± 0.22^{a}	5.65 ± 0.19^{b}	9.57 ± 0.92^{a}	5.24 ± 0.74^{b}	0.001	0.825	0.001	0.378
22:1n-11	0.28 ± 0.01^{b}	0.3 ± 0.02^{ab}	0.32 ± 0.02^{a}	0.27 ± 0.01^{b}	0.017	0.565	0.347	0.003
22:1n-9	0.10 ± 0.01^{b}	0.15 ± 0.01^{a}	0.10 ± 0.02^{b}	0.12 ± 0.01^{ab}	0.001	0.043	0.001	0.098
22:4n-6	0.09 ± 0.01^{a}	0.07 ± 0.01^{b}	0.10 ± 0.01^{a}	0.06 ± 0.01^{b}	0.001	0.347	0.001	0.081
22:5n-6	0.27 ± 0.01^{a}	0.21 ± 0.01^{b}	0.29 ± 0.04^{a}	0.18 ± 0.02^{b}	0.001	0.696	0.001	0.078
22:5n-3	2.85 ± 0.12^{a}	2.16 ± 0.14^{ab}	2.95 ± 0.5^{a}	1.77 ± 0.33^{b}	0.005	0.439	0.001	0.213
22:6n-3	24.45 ± 1.01	20.99 ± 1.93	23.41 ± 4.75	17.78 ± 4.1	0.144	0.299	0.045	0.589
Total Saturates	22 ± 0.92^{ab}	16.34 ± 0.13^{c}	24.25 ± 3.05^{a}	18.1 ± 2.03^{bc}	0.003	0.104	0.001	0.829
Total Monoenes	27.77 ± 0.4^{b}	36.38 ± 1.43^{a}	27.39 ± 2.71^{b}	38.29 ± 2.66^{a}	0.001	0.535	0.001	0.360
Total n-3	40.76 ± 1.31^{a}	33.64 ± 2^{ab}	39.67 ± 6.13^{ab}	29.63 ± 5.18^{b}	0.036	0.323	0.008	0.563
Total n-6	7.84 ± 0.07^{b}	12.61 ± 0.41^{a}	6.95 ± 0.29^{b}	13 ± 0.47^{a}	0.001	0.239	0.001	0.012
Total n-9	18.62 ± 0.25^{b}	29.53 ± 1.24^{a}	17.16 ± 1.55^{b}	31.27 ± 2.16^{a}	0.001	0.872	0.001	0.096
Sum n-3 LC-PUFA	37.81 ± 1.38^{a}	29.7 ± 2.22^{ab}	37.11 ± 6.16^{a}	25.63 ± 5.23^{b}	0.021	0.358	0.004	0.512
EPA/ARA	8.93 ± 0.15	8.77 ± 0.07	8.61 ± 0.54	8.33 ± 0.51	0.321	0.120	0.347	0.803
ARA/EPA	0.11	0.11	0.12 ± 0.01	0.12 ± 0.01	0.099	0.028	0.397	0.397
DHA/EPA	2.62 ± 0.05^{b}	3.71 ± 0.22^{a}	2.43 ± 0.29^{b}	3.36 ± 0.33^{a}	0.001	0.092	0.001	0.596
DHA/ARA	23.4 ± 0.09^{b}	32.54 ± 1.8^{a}	20.99 ± 3.56^{b}	28.13 ± 4.37^{ab}	0.006	0.081	0.001	0.575
n-3/n-6	5.2 ± 0.17^{a}	2.67 ± 0.24^{b}	5.74 ± 1.1^{a}	2.29 ± 0.47^{b}	0.001	0.827	0.001	0.228

Means bearing different superscript letters in each row differ significantly (Mean ± SD, $p < 0.05$, One-way ANOVA, Tukey Post-Hoc).

20:2n-6 and the EPA/ARA ratio, suggesting the modulation of lipid metabolism, which could be partially responsible for the improved spawning quality through a better utilization of dietary lipids. The essential fatty acids ARA and EPA are precursors of eicosanoids, locally acting hormones that regulate a long list of physiological processes, including several related to reproduction (Izquierdo and Koven, 2011). EPA/ARA ratio is known to alter the production of eicosanoids (Wada et al., 2007; Fernández-Palacios et al., 2011), such

as prostaglandins of the II and III series in gonads, what may affect a series of factors related to fertilization such as sperm production and quality, male sexual behavior and synchronizing spawning of the females and males (Sorensen et al., 1988; Fernández-Palacios et al., 2011). Thus, both ARA and EPA affect fertilization rates in gilthead seabream (Fernández-Palacios et al., 2011). Interestingly the egg DHA contents were not influenced by selection or diet, denoting the importance of this fatty acid and its selective retention in marine fish tissues (Koven et al., 1998; Izquierdo and Koven, 2011).

Gilthead seabream has a high requirement for EPA and DHA during gametogenesis for the continuous supply of these EFAs to support the gamete development (Izquierdo et al., 2015; Ferosekhan et al., 2020, 2021). Indeed, an inadequate supply of these EFAs has a deleterious effect on the spawning quality in fish (Izquierdo et al., 2001; Henrotte et al., 2010; Luo et al., 2015). In the present study, feeding the RO diet did not significantly affect the fecundity parameters, which were in the range of those previously reported (Xu et al., 2019; Izquierdo et al., 2015; Ferosekhan et al., 2020), suggesting that the levels of dietary essential fatty acids fulfill the dietary requirements of gilthead seabream broodstock (Izquierdo et al., 2001; Fernández-Palacios et al., 2011). In contrast, in a previous study with the same broodstock, feeding a diet with RO and LO caused a 30–40% reduction in viable eggs, hatched eggs and 3-day-old larvae in comparison to fish fed the FO diet (Ferosekhan et al., 2021). Besides, egg viability, hatching and larval survival rates were reduced by 5–25% in broodstock fed the LO and RO diet comparison to the broodstock fed the FO diet (Ferosekhan et al., 2021). The content in LO, and subsequently in ALA, was the main difference between the diets used in the previous study (9.6% ALA in total fatty acids) and in the present one (4.9% ALA in total fatty acids). Thus, although in the present study egg fatty acid profiles reflected dietary fatty acids contents, only ALA, LA, and oleic acid (18:1n-9) markedly increased by 130%, 111% and 70%, respectively, in eggs from broodstock fed the RO diet in comparison broodstock fed the FO diet. In contrast, in the previous study, the egg contents in ALA increased by 257% in comparison to broodstock fed FO diet (Ferosekhan et al., 2021). Therefore, in comparison with the previous trial, the good reproductive performance of broodstock fed the RO diet together with the lower increase in the ALA in the present trial, suggest the deleterious effect of a diet high in ALA on reproductive performance of gilthead seabream broodstock. This hypothesis agrees well with the alterations in steroid hormones production and release found in seabream fed diets high in LO (Montero et al., 2003) or the increase in cortisol release by seabream head kidney cells superfused with ALA (Ganga et al., 2011). In agreement, in our previous trial, feeding broodstock with a diet high in ALA, significantly altered steroid hormones production in females (Ferosekhan et al., 2021). Besides, the high ALA contents in diet and, subsequently, in tissues, also the ratio LA/ALA could be determinant of the negative effect of this fatty acid, since it may markedly affect lipid metabolism and steroid hormones (Yehuda et al., 2000). Thus, whereas in the present study the LA/ALA ratios were similar in eggs from broodstock fed FO or RO diets, in the previous study feeding the vegetable oil diet reduced LA/ALA ratios to half of those from broodstock fed the FO diet (Ferosekhan et al., 2021), what could be related to the reduction in egg viability, hatching and larval survival rates (Ferosekhan et al., 2021). In human platelets, incubation with ALA inhibits phospholipase A2, the key enzyme responsible for liberating the fatty acids that are eicosanoid precursors from the membrane phospholipids (Ballou and Cheung, 1985). The modulation of lipid metabolism and stress physiology by ALA is mediated by protein kinases, which in turn affect AMP activation (Matthys and Widmaier, 1998).

5. Conclusion

Data form the present work shows that gilthead seabream broodfish selected for high growth exhibited very similar reproductive performance, egg quality and larval survival, when fed diets containing different oil sources. Selection for high growth affected egg fatty acid profiles, with reduced EPA/ARA ratios which were inversely related to broodstock performance. Feeding the RO diet did not negatively affect fecundity, nor egg DHA contents, denoting a sufficient content in essential fatty acids for gilthead seabream. Egg ALA contents were only moderately affected by the RO diet. The present findings strongly suggest that gilthead seabream broodstock selected for high growth trait also has a positive impact on egg quality and larval performance to produce quality offsprings and that feeding a RO diet does not negatively affect reproduction provided that there is sufficient LC-PUFA in diet and no excess of ALA.

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Authors' contribution

Conceived and designed the experiment: MI DM SF SS SK. Broodstock selection: MI DM SS SF JMA MJZ. Diet formulation and preparation: MI RF SF DM SK. Egg and larval quality evaluation: SS SF. Biochemical analysis SS SF MJZ. Data analysis: SF MI SK. Wrote the paper: SF SS MI SK DM.

Conflict of interest

The authors declare that there is no any conflict of interest.

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