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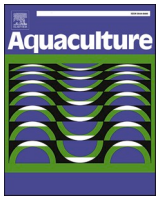

Genomic evaluation for body weight, length and growth estimates in meagre *Argyrosomus regius*

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ABSTRACT

Meagre (*Argyrosomus regius*) is a fast-growing sciaenid species which recently received a rising interest in aquaculture diversification and industry sustainability. The present study illustrates the first complete genomic evaluation of meagre for body weight, length and growth parameters using 810 offspring genotyped with a double-digest RAD sequencing approach. Offspring were assigned to both parents using SNP markers and a QTL associated with growth explaining approximately 3.25 % of the phenotypic variance was found. Using pedigree and genomic relationship matrix, moderate to high heritability estimates along with high genetic/genomic correlations between all studied traits were detected with the exception of the initial body weight. The predicted ability of the breeding values was higher using the genomic than the pedigree relationship matrix for body weight and growth. Present findings provide a better genetic insight of the production traits, as well as evidence that the implementation of the genomic information in breeding programs could be beneficial for the parental assignment as well as for the aquaculture production increase through genetic improvement using marker assisted or genomic selection.

1. Introduction

Meagre (*Argyrosomus regius*) is a teleost fish, belonging to the Sciaenidae family, and a relatively new species in the aquaculture industry in the Mediterranean area (Cárdenas, 2010; Carvalho et al., 2018). Wild populations can be located in the Atlantic coast of Europe, in the Mediterranean and Black Sea, as well as in the east coast of Africa and they can easily adapt in captivity (Cárdenas, 2010). Cultured populations are located in Egypt, Turkey, Spain, and Greece and their total aquaculture production is 64.9 %, 14.2 %, 10.6 %, and 6.5 %, respectively (Vallecillos et al., 2023). Some of the advantages in farming this species are the fast growth rate, since 800–1000 g can be achieved within 18 months, the good feed conversion rate (Fountoulaki et al., 2017) and the low-fat fillet content (Luna et al., 2006). Apart from the production benefits, the final product is a great source of unsaturated fatty acids (Poli et al., 2003).

Since meagre was introduced into aquaculture in the late 1990s (Duncan et al., 2013), several studies have been conducted to investigate the diseases or infections affecting the farmed populations (Soares et al., 2018; Ternengo et al., 2010), the diet composition (Piccolo et al., 2008; Chatzifotis et al., 2010; Fountoulaki et al., 2017), and reproduction traits (Mylonas et al., 2013, 2015). Regarding selective breeding, the utilization of the pedigree relationship matrix for the estimation of heritability for growth traits was first studied by Nousias et al. (2020). Moreover, Vallecillos et al. (2021) investigated growth, morphology and flesh-quality traits, and later the use of image analysis for growth and yield traits (Vallecillos et al., 2023). Based on a mass spawning protocol, which is applied for the reproduction in the aquaculture industry for meagre, Nousias et al. (2020) and Vallecillos et al. (2022) developed microsatellite multiplex PCR panels to infer parentage assignment for

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produced offspring. New methodologies such as the double-digest random amplified DNA sequencing (ddRAD-seq, Peterson et al., 2012) enabled to genotype larger DNA regions using SNP markers, construct a high-quality linkage map, and perform a QTL analysis which detected a SNP marker associated with body weight (Nousias et al., 2022). Recently, the first complete genome assembly for *Argyrosomus regius* was produced using long and short-read technologies (Papadogiannis et al., 2023), and is expected to provide beneficial results in the genetic improvement of the species.

Selective breeding programs are already applied in other Mediterranean species such as European sea bass and gilthead seabream for multiple generations and for multiple phenotypes such as growth, morphology and disease resistance (Chavanne et al., 2016). However, the selective breeding programs in meagre are scarce and in the initial stages and are focused mainly on growth performance (Vallecillos et al., 2021, 2023). In the aquaculture industry, the traditional pedigree selection method is used on a large scale while the Markers Assisted Selection (MAS) is gaining ground in Mediterranean species (European sea bass and gilthead seabream) (Chavanne et al., 2016). A disadvantage of the Markers Assisted Selection (MAS) is the necessity of the identification of a major QTL, while the Genomic Selection (GS) can be applied without it and it is more efficient in polygenic traits (Palaiokostas and Houston, 2017; Robledo et al., 2018). MAS and GS both gain ground, because of the Next Generation Sequence (NGS) techniques which are becoming more and more popular and cost efficient, such as RAD-seq (2b-RAD, ddRAD) (Robledo et al., 2018), or low/high density SNP panels (30 K MedFISH, Peñaloza et al., 2021, Thermo Fisher Axiom TM Seabass 57 K SNP Dlab-Chip, Griot et al., 2021) which are available in studied species such as the European sea bass, gilthead seabream. However, in cases of "new", in aquaculture, or less studied species, such as common dentex (*Dentex dentex*), sharpsnout seabream (*Diplodus puntazzo*) (Oikonomou et al., 2021), meagre (Nousias et al., 2022) and dusky kob (*Argyrosomus japonicus*) (Jackson and Rhode, 2024) the use of ddRAD sequencing in order to detect QTL, have provided beneficial results to identify genetic areas linked to traits. Using the RAD sequencing approach, the detected SNP markers are used to construct a genetic map, which could be used in the QTL analysis or to perform a comparative analysis (Robledo et al., 2018). As a result, the first linkage map for the new species such as meagre (Nousias et al., 2022), common dentex (*Dentex dentex*), sharpsnout seabream (*Diplodus puntazzo*) (Oikonomou et al., 2021), common pandora (*Pagellus erythrinus*) (Manousaki et al., 2016) and dusky kob (*Argyrosomus japonicus*) (Jackson and Rhode, 2024) were constructed. However, in cases where the reference genome was available, such as for the gilthead seabream, Kyriakis et al. (2019) used ddRAD sequencing approach and instead of constructing a linkage map, they performed an alignment of the detected SNPs against the reference genome.

The aim of this study was to provide the first complete genomic evaluation of the meagre (*Argyrosomus regius*) for the main breeding objectives, body weight and growth, in the aquaculture industry. We used offspring coming from a mass-spawning event, monitored and recorded their body weight, growth and length at three different timepoints, and finally genotyped them following ddRAD methodology. We performed the estimation of the genetic/genomic parameters of the main production traits along with the search for QTL related to those traits. Additionally, the calculation of the predicted ability of the breeding values using the pedigree and genomic relationship matrices was carried out for comparison purposes, as well as the identification of haplotype blocks.

2. Materials and methods

2.1. Ethical statement

All experiments were performed in accordance with the relevant guidelines and regulations. AVRAMAR S.A. research facilities are certified and have obtained the codes for the rearing and use of fish for scientific purposes (EL04-BIOexp-01).

2.2. Population and studied phenotypes

Meagre juveniles originating from a hormone-induced spawning event of thirteen breeders, seven females and six males, were reared together till 297 Days Post Hatching (DPH); at this time point, a grading into small- and big-sized was performed and fish were transferred into two different sea cages in Astakos, Aitoloakarnania, Greece (38oN, 21oE). At 394 DPH, 600 fish from each cage (2 cages) were randomly selected, individually tagged and fin-clipped, and put together into a single sea cage in the same location. Weight was measured on all offspring at 394 DPH (BW1), 770 DPH (BW2) and 978 DPH (BW3). Additionally, length was measured on all surviving offspring at 770 and 978 DPH (Len2 and Len3, respectively). Using the available information of the body weight at different time points, three growth periods were estimated as the difference between the aforementioned body weight measurements: "G1" between 770 DPH (BW2) and 394 DPH (BW1), "G2" between 978 DPH (BW3) and 770 DPH (BW2), "GT" between 978 DPH (BW3) and 394 DPH (BW1).

2.3. Preparation of ddRAD libraries

Fin clips were sampled from all broodstock and offspring fish at the beginning of the experiment and used for DNA extraction, as described by Miller et al. (1988). DNA concentration was quantified by spectrophotometry while DNA quality was evaluated with 1 % *w/v* agarose gel electrophoresis. Following DNA extraction, an RNase treatment (38 oC for 3 h) was performed to eliminate RNA residues and a final DNA quantification took place. Four ddRAD libraries (consisting of 266, 266, 156 and 184 samples, respectively) were constructed as described by Nousias et al. (2022). In short, 15 ng DNA from each of 872 samples (13 parents replicated four times, six parents replicated five times and 814 offspring) was separately but simultaneously digested by two restriction enzymes (RE): *SbfI* (CCTGCA|GG recognition site) and *NlaIII* (CATG|C recognition site) (New England Biolabs,NEB, UK) after incubation at 37oC for 90 min. Reaction inactivation was carried out at 65oC for 25 min and then an adapter mix was added to each sample for a 10-min incubation at 22oC. This adapter mix contained individual-specific combinations of P1 (SbfI-compatible) and P2 (NlaIII-compatible) adapters. The ratio of P1 to P2 adapter (1:16) was selected based on the relative abundance of *SbfI* and *NlaIII* cut sites present. P1 and P2 adapters included an inline five- to seven-base barcode for sample identification. For the ligation procedure, a ligation mixture, containing rATP, T4 ligase units (NEB) CutSmart buffer, was added and after incubation at 22oC for 2 h and 30 min the samples were left to cool down at room temperature. All samples of each library were pooled in a petri dish and each pool went through column purification (MinElute PCR Purification kit, Qiagen, UK) and was finally eluted in a 70 μl EB buffer (Qiagen, UK). All four library pools were size selected between 320 and 680 bp using a Blue Pippin machine (Sage Science) and 1.5 % Agarose Gel Cassette. The size-selected template DNA was amplified using a high-fidelity Taq polymerase. The PCR product was sequentially purified with a column (MinElute PCR Purification Kit) and with AMPure magnetic beads (Perkin-Elmer, UK). Finally, the libraries were eluted in 25 μl EB buffer and sequenced on Illumina NovaSeq PE150 aiming at 400–500 G raw data per library.

2.4. SNP discovery and genotyping

Raw reads were first demultiplexed and quality controlled using the STACKS v.54 (Catchen et al., 2013) component *process_radtags* which cleaned the data, corrected errors in barcodes and cutting sites (parameters -c -q -r). Then, the high-quality reads assigned to each individual were mapped against the meagre chromosome level genome

assembly (Papadogiannis et al., 2023) using bwa (Li and Durbin, 2009). The aligned reads were used for SNP calling and genotyping in STACKS with the component *gstacks* and were exported after excluding loci with minor allele frequency less than 0.05, with maximum observed heterozygosity more than 0.8 while selecting only one random SNP per *locus*. *populations* (parameters –write-random-snp –min-maf 0.05 –max-obs-het 0.8).

2.5. Quality control and parental assignment

The retrieved genotypes were further filtered using plink software (Purcell et al., 2007) and the following criteria were used, SNP call rate > 80 %, MAF > 1 % and HWE $< 10^{-6}$. The parentage assignment was performed using the Apparent (Dixon, 1951; Gower, 1971; Melo and Hale, 2019) in R (R Core Team, 2021). To estimate the expected progeny, only the 300 homozygous SNPs from all the available genomic information were used. The Gower Dissimilarity metric (GD) (Gower, 1971) for all the possible trinities (i.e. the parental pair and the offspring) was estimated to evaluate the genetic identity between the expected progeny and the potential offspring using the 0.1 as a threshold (MaxIdent). Finally, the Dixon test (Dixon, 1950, 1951) was used to separate the true from all the potential trinities using the 0.01 as a threshold (alpha).

2.6. Estimation of the genetic/genomic parameters

Heritability estimates for each studied trait using a restricted estimation of maximum likelihood method (REML) were produced and calculated using the additive genetic variance (σ_a^2) divided by the total phenotypic variance (σ_p^2) (which is the sum of additive genetic variance (σ_a^2) and residual variance (σ_e^2)) [$h^2 = \sigma_a^2/(\sigma_a^2 + \sigma_e^2)$] for the body weight, length and growth. In order to produce these estimates the above variance components (i.e. σ_a^2, σ_e^2) were produced using, the following univariate animal model was used in AIREMLF90 (Aguilar et al., 2014),

$$Y = \mu + X\,b + Z\,u + e \quad \text{(Model 1)}$$

where *Y* corresponds to the vector of the trait, μ is the mean of the trait, *b* is the vector of the fixed effect which is the cage (two levels) in the case of BW1 whereas *b* is the vector of the covariate (the use of the BW1) in the case of the BW2, BW3, L2 and L3. However, for growth (G1, G2 and GT) no fixed effect or covariate was used (no *b*). X, *Z* are the incidence matrices for the fixed and additive effects, respectively, *u* is the additive genetic effect either using the Genomic Relationship Matrix (GRM) and it is described as $\sim N(0, G\sigma_a^2)$(*G* is the GRM, and σ_a^2 is the additive variance) or using the Pedigree Relationship Matrix (PRM) and it is described as $\sim N(0, A\sigma_a^2)$ (*A* is the PRM, and σ_a^2 is the additive variance), and *e* is the residual.

Moreover, a bivariate animal model fitting all the possible pairs of traits was used in AIREMLF90 (Aguilar et al., 2014) in order to estimate genetic/genomic and phenotypic correlations among the traits. To estimate the genetic correlation (r_A) between trait X and trait Y, the genetic covariance between trait X and trait Y (cov_{XY}) was divided by the square root of proliferation of the additive genetic variance of trait X ($\sigma_{\hat{x}}^2$), and additive genetic variance of trait Y ($\sigma_{\hat{y}}^2$) [$r_A = cov_{XY}/\sqrt{\sigma_{\hat{x}}^2 * \sigma_{\hat{y}}^2}$]. The analysis was performed according to the following model,

$$Y = \mu + X\,b + Z\,u + e \quad \text{(Model 2)}$$

where *Y* corresponds to the matrix of each pair of traits, μ is the mean of the traits, *b* is the vector of the fixed effect which is the cage (2 levels) in the case of BW1 whereas *b* is the vector of the covariate (the use of the BW1) in the case of the BW2, BW3, L2 and L3. However, for the growth trait (G1, G2 and GT) no fixed effect or covariate was used (no *b*). X, *Z* are the incidence matrices for the fixed and additive effects, *u* is the additive genetic effect using the Genomic Relationship Matrix (GRM) and it is described as $\sim N(0, G\sigma_a^2)$(*G* is the GRM, and σ_a^2 is the additive variance) or using the Pedigree Relationship Matrix (PRM) and it is described as $\sim N(0, A\sigma_a^2)$(A is the PRM, and σ_a^2 is the additive variance) and *e* is the residual.

2.7. Haplotype blocks

The filtered genomic information was used in Plink software (Purcell et al., 2007) to identify the haplotype blocks in the population. Based on Gabriel et al. (2002), SNPs with a D′ value higher than 0.98 were considered as a haplotype block. Both the total number of SNPs and the total number of haplotype blocks were calculated. Additionally, the average number of SNPs and the average size (KB) were calculated per chromosome.

2.8. Univariate GWAS

In order to identify SNPs associated with body weight, growth and length for the meagre, a Genome Wide Association analysis was performed for those traits using Model 1 in which the same terms were fitted in GEMMA analysis software (Zhou and Stephens, 2012). Additionally, a multitrait Genome Wide Association analysis was performed including the last two body weight measurements (BW2, BW3), the last two length measurements (L2,L3) and all the growth measurements (GT, G1 and G2) using GEMMA (Zhou and Stephens, 2014).

An alternative Bonferroni correction was performed in the present study in which the thresholds of 0.05 and 0.01 were divided with the independent SNPs along with the haplotype blocks instead of the total number of SNPs (Nyholt, 2004). Manhattan plots were illustrated using *qqman* (Turner, 2018) in R (R Core Team, 2021). Finally, the proportion of phenotypic variance (PVE) explained by the statistically significant SNP was calculated as described in Oikonomou et al. (2022a).

The genomic regions of the significant SNPs were explored with the use of JBrowse 2 (Diesh et al., 2023) and the genes where the SNPs were in or nearby were identified. The genes which were linked to the significant SNPs were used in ShinyGO 0.80 (http://bioinformatics.sdstate.edu/go/, 14/04/2024) which is a graphical gene-set enrichment tool to get the pathways that are within the specified size limits and used for enrichment analysis.

2.9. The predicted ability of the breeding values

The Estimated Breeding Values (EBVs) were estimated using Model 1 and Best Linear Unbiased Prediction (BLUP) approach (Henderson, 1977) for the body weight (BW2, BW3) and the growth (GT, G2), while for the Genomic Best Linear Unbiased Prediction (GBLUP) approach (Meuwissen et al., 2001, 2013) was used to estimate the Genomic Estimated Breeding Values (GEBVs). Breeding values (EBVs / GEBVs) were estimated using BLUPF90 (Misztal et al., 2002, 2020).

To estimate the predicted ability, 20 % of the total population was randomly selected and its phenotypes were masked (162 fish, validation group). Thus, the breeding values of the validation group (EBVs/GEBVs) were calculated using only the available information from the remaining 80 % of fish from the population (training population). This procedure was repeated 20 times and each time the validation group was altered. Additionally, the corrected phenotype was estimated for the following phenotypes BW2 and BW3 (fitting the BW1 as covariate) while for G2 and GT no correction was performed. The correlation between breeding values and corrected phenotypes for the BW2 and BW3 was calculated for the selected 20 % of the population (validation group). In the case of G2 and GT, the correlation between breeding values and phenotypes was calculated for the selected 20 % of the population (validation group). A pairwise *t*-test comparison was performed between the two groups (PRM and GRM) for BW2, BW3, G2 and GT in R (R Core Team, 2021).

3. Results

3.1. Studied phenotypes and parental assignment

At the age of 978 DPH, the average body weight was 2604.1 g and the average length was 60.0 cm. At the age of 770 DPH, the average body weight was 1802.5 g and the average length was 51.6 cm (Table 1). Studying the growth trait, the average total growth (GT) was 1990.1 g from 394 to 978 DPH, the average G2 was 746.2 g from 770 to 978 DPH. For the traits, G2 and GT, negative estimations were detected in the population since there were cases in which fish lost weight instead of gaining at the age of 770 and 978 DPH. Fig. 1 illustrates the scatter plots between the body weight or total growth and the length.

After the quality control, 4573 SNPs remained in the 24 chromosomes and the average number of SNPs per chromosome was 190.54 (Table 2). For the parental assignment, 92.8 % of the offspring were successfully assigned to both parents. Out of the 810 fish, both parents were detected for 752 revealing 23 families in the population (Table 3). In the present study, 13 broodstock were used, but only 12 of them participated in the mattings. Focusing on the males, some of them participated with higher percentages in the offspring population i.e., M 896 and M 902 with 46.42 % and 27.41 %, respectively, while others showed much lower contribution (M 901 and M 907 with 0.99 % and 0.25 %, respectively). The same pattern also appears in females, i.e., F 903, F 895 and F 905 with 36.3 %, 21.36 % and 17.16 %, respectively. The aforementioned patterns also influenced the family structure, since a high variance of the offspring per family between 30.99 % and 0.12 % was also noted. (Table 3)

3.2. Haplotype blocks

In total, 327 haplotype blocks based on the Gabriel et al. (2002) were found including 1075 SNPs (Table 2). Approximately, 23.5 % of the SNPs participated in the construction of the haplotype blocks. Chromosome (chr) 23 included the lowest number of haplotype blocks while chr 5 and chr 6 the highest. In chr 24, no haplotype block was found. The average number of SNPs per block was 3.12 (ranging from 2.33 to 4.08) and the average size was 168.61 KB (ranging from 62.63 to 345.62 KB).

3.3. GWAS

A QTL associated with the G2 was detected in chr 17 explaining approximately 3.25 % of the phenotypic variance (Table 4). Analyzing the GT, a high trailing of the test statistic for multiple SNPs also appeared in the same chromosome but none of those SNPs were statistically significant. Also, for the same trait (GT), two SNPs in chr 13 were close to the threshold line (Fig. 2). Additionally, for BW1, trailing of the test statistic appears in chr 3 and 8, while in BW2, trailing appears in chr 1. For the length trait, no significant QTL or trailing of the test statistic was noticed in either the multivariate or univariate GWAS. The Manhattan plots for the univariate GWAS for the G1, BW1, BW2, BW3, L2, and L3 count with the multitrait GWAS for BW and Len are illustrated in the Supplementary material 1.

Using the ShinyGO 0.80, for the *TCF4* gene which contributed to the Myogenesis, Developmental Biology and Regulation of cell development, the *RABGAP1* gene which contributed to the Enzyme activator activity and the *ATP8B1* gene which increases the susceptibility to weight loss among other pathways. More details illustrated in Supplementary material 2.

3.4. Genetic and genomic parameters

Starting with the model in which the pedigree relationship matrix was used, high heritability was estimated for the last two body weight measurements (BW2 & BW3) along with the growth at all stages (G1, G2 and GT). An exception was the first measurement of the body weight (BW1) where a low heritability was estimated. Additionally, high heritability was also estimated for the body length. In general, the estimates had high standard errors; however, this did not seem to affect the significance of the heritability estimates. When the genomic relationship matrix was utilized, all the heritability estimates were lower compared to the estimations using the PRM apart from the first measurement of the body weight (BW1). Additionally, using the GRM all the standard errors were lower compared to the standard errors using the PRM.

High genetic and genomic correlations were estimated between the body weight at 770 and 978 DPH, growth and body length. Nevertheless, moderate genetic correlations were estimated between BW1 and the above phenotypes, and none of them were significant (Table 6), while in Table 7 higher genomic correlation were estimated between BW1 and BW2/BW3 and they were significant. Generally, there were cases in which a bivariate model did not successfully converge i.e. growth and body weight (G1 – BW2). Using the pedigree relationship matrix, high standard errors were produced while fitting the Genomic relationship matrix, smaller standard errors were produced for the genetic and genomic correlations. Focusing on the length and growth, high genetic and genomic correlations were estimated in both models and of similar magnitude between the length and body weight. It is worth mentioning that there are small differences between the phenotypic correlations in Tables 5 and 6 due to the number of offspring used in each analysis. Using the PRM, 58 fish were not used in the analyses because of their unknown pedigree, while using the GRM, all the fish with phenotypes were analyzed.

3.5. Predicted ability of the breeding values

In all cases, a higher predicted ability of the breeding values was detected using the genomic relationship matrix than pedigree and the difference was statistically significant using the *t*-test (Table 8, Fig. 3). Highest difference of the average predicted ability between the traditional pedigree and genomic selection was detected using the total growth (GT). In both approaches, low standard deviations and high average estimations were produced.

4. Discussion

In the present study, 810 offspring originating from mass spawning of meagre were recorded for the most important production traits, body weight and growth. Due to the lack of studies involving genomic

Table 1
Descriptive statistics of the studied phenotypes in meagre.

	BW1 (g)	BW2 (g)	L2 (cm)	BW3 (g)	L3 (cm)	G1 (g)	G2 (g)	GT (g)
Days Post Hatching (DPH)	394	770		978		770-394	978-770	978-394
Number of observations	804	810	810	633	634	804	633	628
Average	606.7	1802.5	51.6	2604.1	60	1197.1	746.2	1990.1
SD	161.8	558.6	5.18	767.4	6.06	456.05	330.7	681.2
Min	238.0	450.0	33.5	620.0	38.50	14	−510	−10
Max	1.14	3.71	64	5.020	75	3975	2330	2562

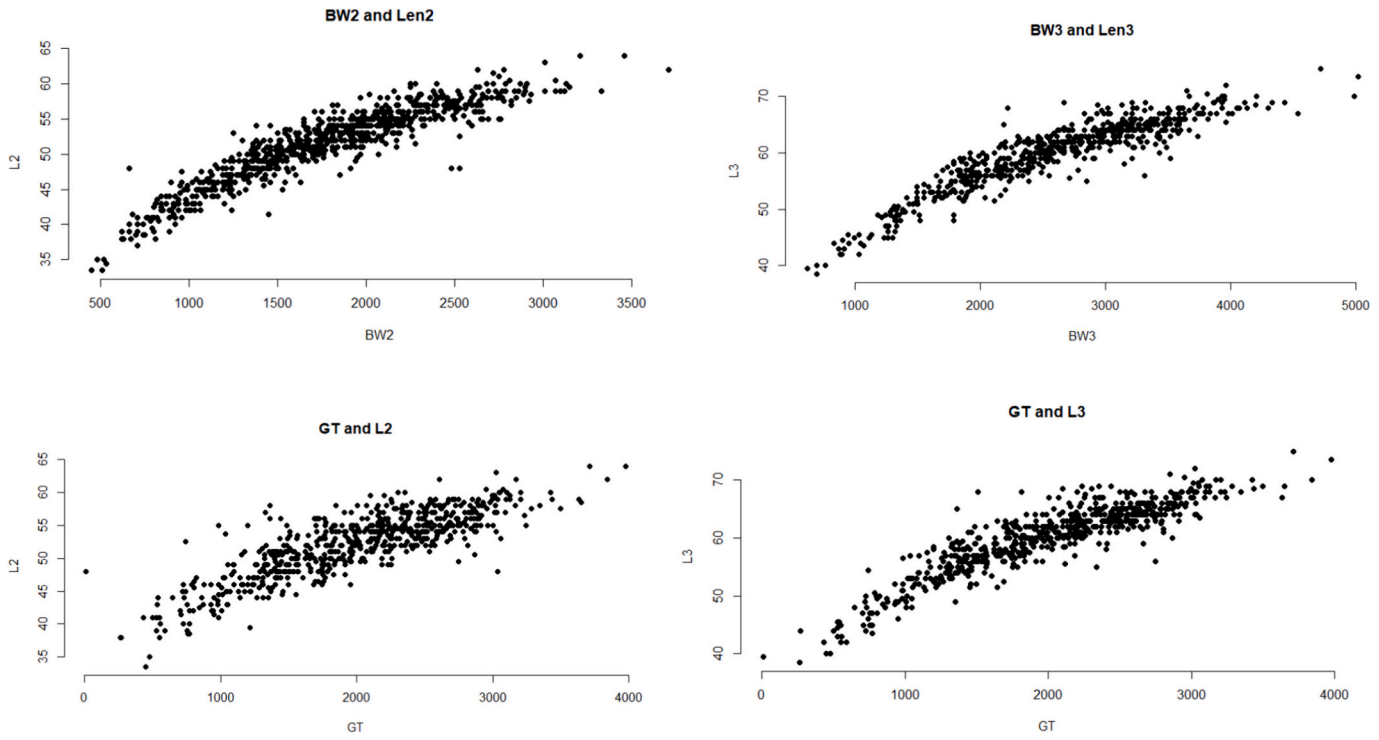

Fig. 1. (a) Scatter plot between the BW2 and the L2. (b) Scatter plot between the BW3 and the L3. (c) Scatter plot between the GT and the L2. (d) Scatter plot between the GT and the L3.

Table 2
Number of SNPs, haplotype blocks and number of SNPs per Haplotype block per chromosome.

CHR	Total number of SNP	Total number of Haplotype blocks	Total number of SNPs per Haplotype block	Average number of SNPs per Haplotype block	Average size of Haplotype block (KB)
1	233	19	54	2.84	119.66
2	217	11	39	3.55	277.37
3	218	18	70	3.89	180.59
4	192	13	42	3.23	170.27
5	238	22	70	3.18	186.96
6	239	22	77	3.50	149.12
7	183	10	35	3.50	62.63
8	214	14	40	2.86	89.13
9	198	16	49	3.06	152.27
10	216	20	74	3.70	227.32
11	242	20	68	3.40	210.18
12	200	10	37	3.70	232.36
13	170	15	47	3.13	172.71
14	194	13	53	4.08	296.36
15	218	16	46	2.88	70.90
16	169	14	42	2.80	133.76
17	190	15	60	4.00	345.62
18	158	9	28	3.11	164.97
19	174	14	44	3.14	211.85
20	169	9	23	2.56	114.58
21	161	13	39	3.00	147.19
22	121	9	21	2.33	109.22
23	126	5	17	3.40	221.54
24	133	0	0	0.00	0.00
Total	4573	327	1075	–	–
Average	**190.54**	**13.63**	**44.79**	**3.12**	**168.61**

information in meagre, a comparison between the species that are phylogenetically closer to meagre, such as the European sea bass (Papadogiannis et al., 2023) was selected to be presented.

In the aquaculture industry, the reproduction of meagre is mainly conducted through mass spawning, and it is necessary to identify the parental pair for each offspring. In order to achieve parental identification in meagre, microsatellite markers were first used (Nousias et al., 2020; Vallecillos et al., 2021, 2022, 2023). In these studies, a range of successful parentage assignment rates for offspring in single families was achieved between 87.5 % and 97.8 %. However, the parental assignment in the current study was conducted using SNP markers and 92.8 % of the offspring were distributed into families (male and female), which is within the above range. Nousias et al. (2020) found 20 families using five females and seven males as breeders and Vallecillos et al. (2021) found the same number of families using four males and five females as breeders. In our case, a slightly higher number of families (23) were discovered using six males and six females as breeders. An unequal contribution of the broodstock was noted in our study since some males and females participated in a higher percentage in family structure compared to other males and females. To elaborate, one out of six males contributed 46.7 % of the offspring while one out of six females contributed 36.3 % of the offspring. The same pattern appeared in Vallecillos et al. (2021) and Nousias et al. (2020). Vallecillos et al. (2021) reported that one out of five females which were used as breeders produced 45.3 % of the offspring in cages and 55.5 % in tank while one out of four males, that were used as breeders, contributed 59.5 % of the offspring in cages and 63.8 % in tank. Nousias et al. (2020), reported that two out of five females, which were used as breeders, produced 30.17 % and 37.07 % of the offspring in batch 1 respectively, while in batch 2, two out of five females produced 26.84 % and 35.53 % of the offspring. Focusing on the males, in batch 1, one out of six males contributed 52.87 % of the offspring and in batch 2 one out of six males contributed 47.11 %. Apart from the meagre, an unequal contribution of the available broodstock in the studied population was also reported in dusky kob (*Argyrosomus japonicus*) by Jenkins et al. (2020). In the latter study, a mass spawning protocol took place as well as in Nousias et al. (2020) and Vallecillos et al. (2021, 2023). Focusing in meagre, a pattern which was noticed is that in all the studies in meagre, one male was responsible for more than the half of the offspring studied. This could be a potential problem for the future generations because of inbreeding and

Table 3
Number of offspring per parent and per family along with the percentage of the participation of each family in the studied population.

Parents	No parent	F 852	F 895	F 898	F 903	F 905	F 906	Total
No parent	58 (7.16 %)							58 (7.16 %)
M 894		1 (0.12 %)	35 (4.32 %)	8 (0.99 %)	12 (1.48 %)	11 (1.36 %)		67 (8.24 %)
M 896			11 (1.36 %)	40 (4.94 %)	251 (30.9 %)	74 (9.14 %)		376 (46.42 %)
M 900		69 (8.52 %)			3 (0.37 %)	3 (0.37 %)	2 (0.25 %)	77 (9.51 %)
M 901		1 (0.12 %)		2 (0.25 %)	5 (0.62 %)			8 (0.99 %)
M 902			127 (15.68 %)	14 (1.73 %)	23 (2.84 %)	50 (6.17 %)	8 (0.99 %)	222 (27.41 %)
M 907				1 (0.12 %)		1 (0.12 %)		2 (0.25 %)
Total	58 (7.16 %)	71 (8.77 %)	173 (21.36 %)	65 (8.02 %)	294 (36.3 %)	139 (17.16 %)	10 (1.23 %)	810

Table 4
Phenotypic variation explained by the top significant or non-significant SNPs for growth in meagre.

Trait	Chromosome	SNP (name)	Position (bp)	*P*-value	-log(*p*-value)	PVE (%)
G2	17	1173820_327	3,583,658	0.0000048*	5.317*	3.25
GT	13	1052601_67	15,135,921	0.0000457	4.340	2.61
GT	17	1205106_249	16,014,171	0.0000598	4.223	2.53

* indicates statistically significant association.

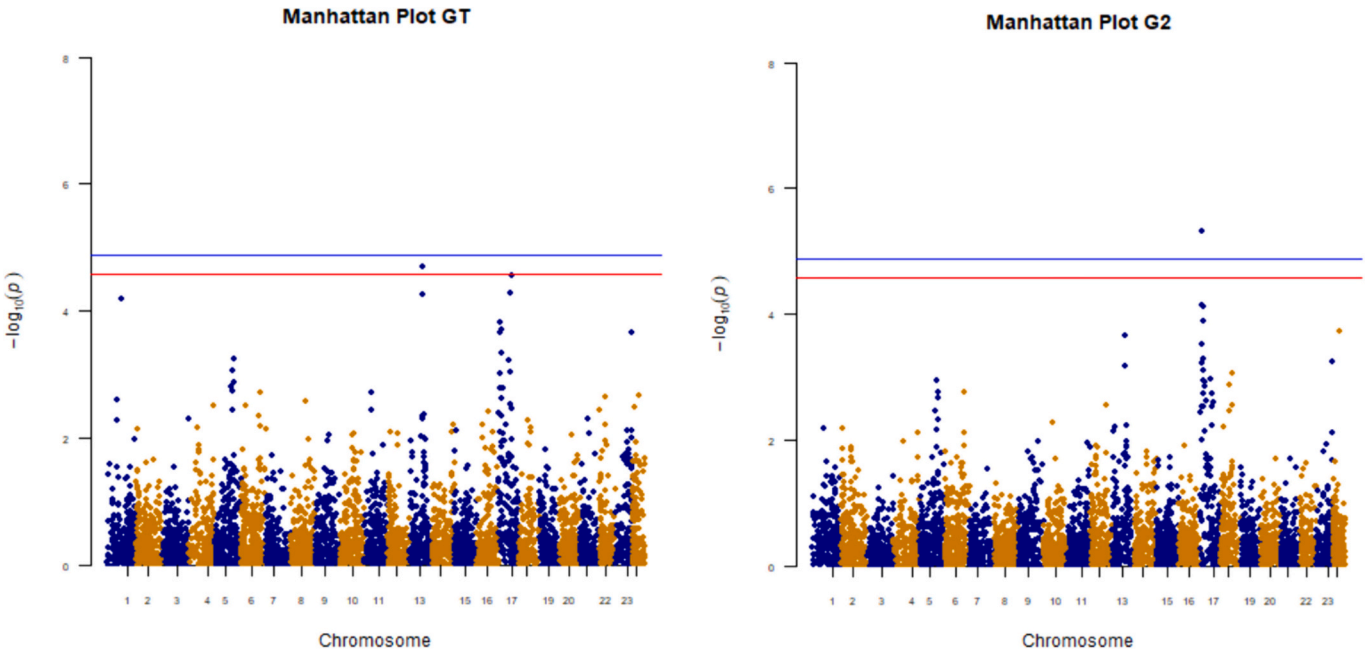

Fig. 2. Manhattan plot for GT (in left) and G2 (in right). The blue (initial value 0.05) and the red line (initial value 0.1) illustrate the threshold after the alternative Bonferroni correction (at the genomic level). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

it should be under consideration.

In the European sea bass, parentage assignment using SNP markers, coming from the RAD sequencing approach, has been done by Palaiokostas et al. (2015, 2018) using a different software. Palaiokostas et al. (2018), assigned 1293 offspring (out of the 1538) into 140 full-sib families using 48 sires and 17 dams which is approximately 84 % of the offspring. Faggion et al. (2019), also assigned all the 927 offspring into families (60 sires and 9 dams). In our case, a higher parentage assignment was noted compared to Palaiokostas et al. (2018) while it was lower compared to Faggion et al. (2019). Apart from the parentage assignment, an important difference between the meagre and European sea bass is the number of the breeders which are used. In the latter species, a larger number of breeders is available per matting while in meagre the majority of the studies used a range of 9 (Vallecillos et al., 2021; Nousias et al., 2020) to 12 (in our study). This could be explained by the fact that meagre is a species which recently received a rising interest since it was introduced into the late 1990s (Duncan et al., 2013) while the European sea bass is one of the important Mediterranean

Table 5
Candidate genes linked to the significant SNPs.

SNP	Gene	Gene Name	Gene Description	Gene start	Gene end	Gene relative to the SNP
1173820_327	AregG03052	*ATP8B1*	*ATPase aminophospholipid transporter class I type 8B member 1*	3,483,609	3,515,825	Upstream of the SNP
1173820_327	AregG03053	*TCF4*	*TRANSCRIPTION FACTOR 4*	3,703,912	3,774,184	Downstream of the SNP
1205106_249	AregG19594	*RABGAP1*	*RAB GTPase activating protein 1*	15,951,282	16,018,889	Within intron
1052601_67	AregG04530	*EPB41L2*	*Erythrocyte membrane protein band 4.1-like 2*	15,097,851	15,145,938	Within intron

Table 6
Heritability (h^2) and genetic/phenotypic correlation for studied phenotypes using the pedigree relationship matrix (PRM). Heritability of the traits are on the diagonal in black bold; genetic and phenotypic correlations between the traits are above and below the diagonal, respectively. Standard errors, of the estimated parameters, are illustrated in the parentheses.

	BW1	BW2	L2	BW3	L3	GT	G2	G1
BW1	**0.17**	0.60	0.56	0.40	0.29	0.37	0.18	0.58
	(0.08)	(0.70)	(0.50)	(0.65)	(0.71)	(0.60)	(0.74)	(0.76)
BW2	0.53	**0.49***	0.82	0.98*	0.87*	0.98*	0.85	**
	(0.04)	**(0.16)**	(0.44)	(0.18)	(0.37)	(0.17)	(0.43)	
L2	0.42	0.85	**0.52***	0.88*	0.98*	0.8*	0.86*	0.82
	(0.05)	(0.02)	**(0.18)**	(0.27)	(0.25)	(0.29)	(0.39)	(0.41)
BW3	0.39	0.89	0.82	**0.60***	0.91*	**	**	0.98*
	(0.05)	(0.01)	(0.02)	**(0.21)**	(0.30)			(0.14)
L3	0.30	0.80	0.90	0.87	**0.53***	0.91*	0.91*	0.89*
	(0.06)	(0.02)	(0.01)	(0.02)	**(0.20)**	(0.43)	(0.36)	(0.22)
GT	0.33	0.92	0.84	**	0.88	**0.69***	0.95*	0.98*
	(0.06)	(0.01)	(0.02)		(0.02)	**(0.22)**	(0.30)	(0.13)
G2	0.12	0.47	0.54	**	0.69	0.95	**0.52**	0.90*
	(0.06)	(0.06)	(0.06)		(0.04)	(0.02)	**(0.21)***	(0.24)
G1	0.43	**	0.86	0.90	0.82	0.98	0.49	**0.64***
	(0.04)		(0.02)	(0.01)	(0.03)	(0.01)	(0.06)	**(0.18)**

* statistically significant estimation.
** model did not converge.

Table 7
Heritability (h^2) and genomic/phenotypic correlation for studied phenotypes using the genomic relationship matrix (GRM). Heritability of the traits are on the diagonal in black bold; genomic and phenotypic correlations between the traits are above and below the diagonal, respectively. Standard errors, of the estimated parameters, are illustrated in the parentheses.

	BW1	BW2	L2	BW3	L3	GT	G2	G1
BW1	**0.27***	0.74*	0.65*	0.55*	0.48*	0.45*	0.06	0.64*
	(0.05)	(0.07)	(0.09)	(0.1)	(0.13)	(0.06)	(0.18)	(0.09)
BW2	0.58	**0.34***	0.87	0.94*	0.82*	0.95*	**	**
	(0.03)	**(0.05)**	(0.03)*	(0.02)	(0.05)	(0.02)		
L2	0.47	0.86	**0.37***	0.83*	0.96*	0.84*	0.72*	0.88*
	(0.03)	(0.01)	**(0.06)**	(0.05)	(0.01)	(0.04)	(0.1)	(0.03)
BW3	0.44	0.87	0.81	**0.41***	0.87*	**	0.89*	0.95*
	(0.04)	(0.01)	(0.01)	**(0.06)**	(0.04)		(0.04)	(0.01)
L3	0.36	0.79	0.89	0.87	**0.39***	0.89*	0.81*	0.86*
	(0.04)	(0.01)	(0.009)	(0.01)	**(0.06)**	(0.03)	(0.07)	(0.04)
GT	0.35	0.88	0.81	**	0.87	**0.45***	0.88*	0.94*
	(0.04)	(0.01)	(0.01)		(0.01)	**(0.06)**	(0.04)	(0.02)
G2	0.11	**	0.42	0.76	0.62	0.78	**0.34***	0.71*
	(0.04)		(0.03)	(0.02)	(0.03)	(0.01)	**(0.06)**	(0.1)
G1	0.44	**	0.87	0.88	0.81	0.86	0.38	**0.41**
	(0.03)		(0.01)	(0.01)	(0.01)	(0.01)	(0.04)	**(0.05)***

* statistically significant estimation.
** model did not converge.

species for the aquaculture industry (Palaiokostas et al., 2018) and it has been farmed from 1970 (Janssen et al., 2017). Due to the lack of available breeders and their unequal contribution to offspring, the inbreeding must be under control in the future generations.

Moving on to the haplotype blocks constructed as described in Gabriel et al. (2002), a large number of those blocks consisted of a small number of SNP per chromosome. In this study, 4573 SNPs were used in order to find the haplotype blocks and nearly a quarter of them (1075 SNPs) participated in 327 blocks. Studying the European sea bass, using a medium density SNP array (30 K MedFish, Peñaloza et al., 2021) a slightly lower percentage (18.54 %) of the SNPs participated in 2141 haplotype blocks (4975 out of 26,821 SNP, Oikonomou et al., 2022b). In this study, we had a very low-density coverage of SNPs, 23.5 % of SNPs participated in the haplotype blocks while in Oikonomou et al. (2022b), using a moderated density SNP array, 18.54 % of the SNPs participated in the haplotype blocks. The high percentage of the participating SNPs in

Table 8
Predicted ability of the breeding values (correlation between the breeding values and the corrected phenotype (BW2, BW3) or the phenotype (G2, GT) using the traditional pedigree approach and the genomic selection along with the *p*-value from the pairwise *t*-test.

Trait	Predicted ability Average (SD)		*P*-value from the pairwise *t*-test
	Traditional Pedigree approach (using the PRM, BLUP)	Genomic selection (using the GRM, GBLUP)	
BW2	0.62 (0.04)	0.67 (0.04)	0.000000511*
BW3	0.64 (0.05)	0.69 (0.04)	0.00000003*
GT	0.64 (0.03)	0.71 (0.03)	0.0000000000106*
G2	0.56 (0.05)	0.62 (0.05)	0.000000514*

* Statistically significant <0.05.

the haplotype blocks worked beneficially in our study since a high trailing load was noted in chromosome 17 analyzing GT and G2. The leading SNP (1173820_327) in chr 17 for the G2, participated in one of the haplotype blocks which also included 5 other SNPs (starting position 3,582,196 bp and ending position 4,323,855 bp in the chromosome). The leading SNP (1205106_249) in chr 17 for the GT, participated in one of the haplotype blocks which also included 5 other SNPs (starting position 15,770,707 bp and ending position 16,290,688 bp in the chromosome). On the other hand, the leading SNP (1052601_67) in chr 13 for the GT, participated in one of the haplotype blocks which included only 1 other SNP (starting position 15,133,262 bp and ending position 15,135,921 bp in the chromosome).

Nousias et al. (2022) reported a QTL related to body weight (2303 g) on LG15 explaining approximately 31 % of the phenotypic variation in meagre but when the polygenic component was fitted to the model, the

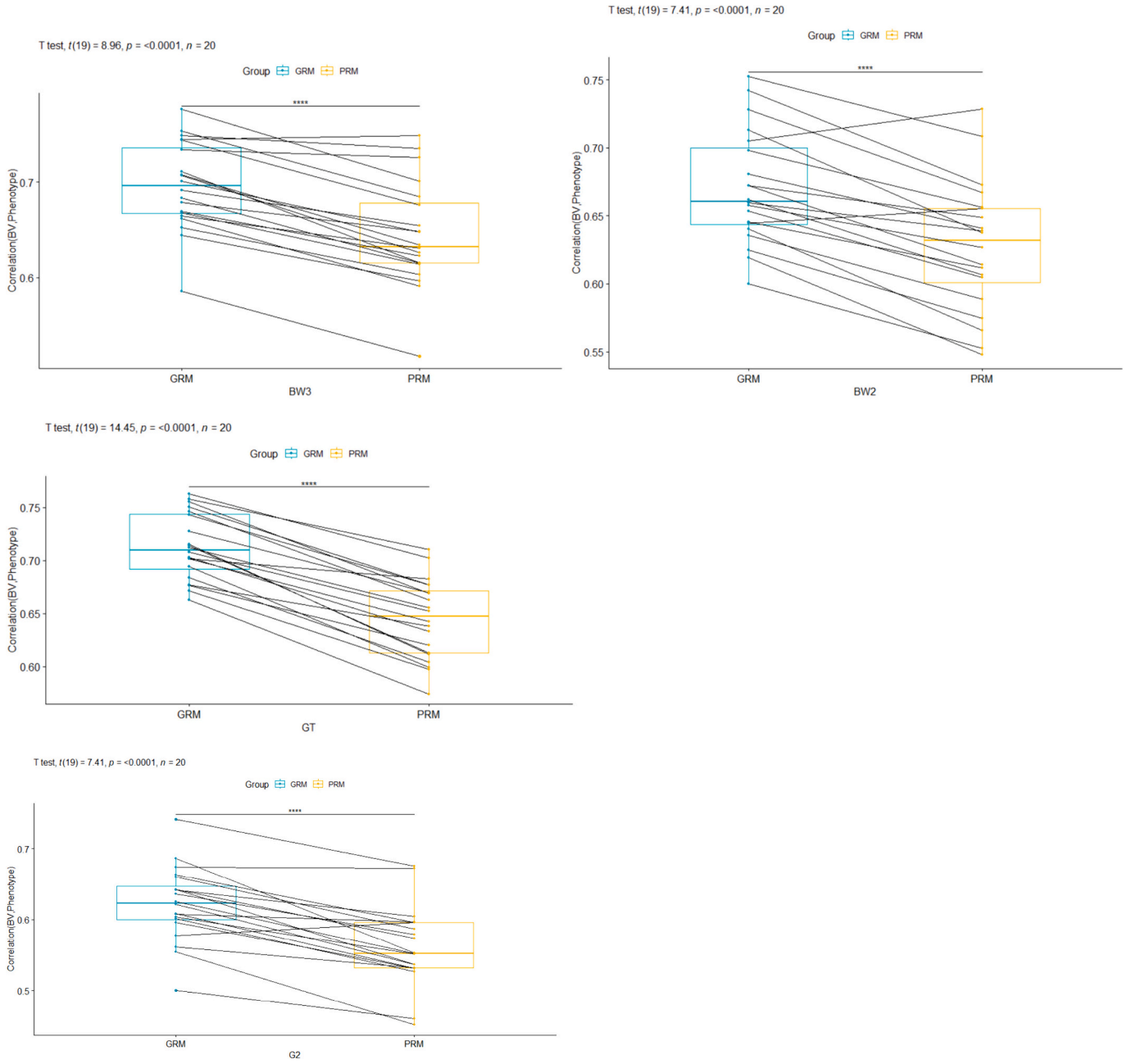

Fig. 3. Box-plots along with the *t*-test for the predicted ability of the breeding values as the correlation between the Predicted Breeding value and the corrected phenotype for the following phenotypes the BW3 (a), BW2 (b) or as the correlation between the Predicted Breeding value and the phenotype for the GT(c) and G2(d).

peak was minimized. Jackson and Rhode (2024), studied the dusky kob (*Argyrosomus japonicus*) and they detected 25 QTL linked to body weight, length and condition factor. The phenotypic variance explained by those 25 QTL ranged from 29.5 % to 9.3 %. In our study, no QTL were detected in any chromosomes associated with body length. Additionally, no QTL was found linked to the direct measurements of the body weight (using all population); however, when growth was considered (GT and G2), an association of SNPs was observed on chr 17 (Fig. 2a, b). This trailing is considered striking impressive based on the low density of the SNPs on this chromosome (190 SNPs). Analyzing growth at 770–978 DPH (G2), a QTL was identified in the same chromosome (17) which explains approximately 3.25 % of the PVE while analyzing the total growth (GT, 394–978 DPH) approximately 2.53 % of the PVE was explained by the detected QTL. A putative QTL was also identified for the total growth (GT) in chromosome 13 (using as threshold the <0.1 before alternative Bonferroni correction) without strong supportive evidence of nearby SNP markers (i.e. without trailing) (Fig. 2a). However, there were 170 SNPs on this chromosome with 47 of them distributed into haplotype blocks; thus, the lack of trailing could be a result of the low-density coverage of the chromosome, or it could be a false positive. Studying the European sea bass, using the 30 K MedFISH (Peñaloza et al., 2021), putative QTL were found associated with the body weight (multitrait GWAS), in Oikonomou et al. (2022b). Additionally, using the Thermo Fisher Axiom TM Seabass 57 k SNP Dlab-Chip, Griot et al., 2021) even though no QTL was revealed using the total population (using the univariate GWAS), when only one of the two batches (Batch 10) was analyzed, two statistically significant SNPs in chr 16 were detected. Their proportion of phenotypic variance ranged from 2.4 % to 2.9 % (Oikonomou et al., 2022a). In our case a slightly higher range of phenotypic explanation variation was found (2.53 % -3.25 %), analyzing only the growth. If the range of the explained phenotypic variation is low, the SNPs are not considered as major QTL. This is acceptable when studying traits such as body weight and growth, which are described as polygenic traits and are controlled by many loci, but their effect is small (Palaiokostas and Houston, 2017).

Regarding the genetic parameters, three studies were conducted in meagre to estimate the heritability for body weight and length at different growth stages. Nousias et al. (2020) reported the heritability for body weight (~2303 g) to be equal to 0.62 and for length (~62.04 cm) equal to 0.64. In our case, studying the BW3 (~2604.1 g) the heritability was very close to the above estimate, yet with higher standard errors using the PRM. The main difference was noted studying the L3 (~60 cm) in which the heritability was lower in our population. Vallecillos et al. (2021, 2023) used fish at the age of 549 DPH and estimated the heritability for body weight (average ~ 1065.5 g) and length (~39.15 cm) to range from 0.42 to 0.39 and from 0.38 to 0.34, respectively. In our study, the highest heritability among the body weight measurements (with a small difference using the PRM) was identified for BW2 (770 DPH), while the lowest heritability (this time with a high difference using the PRM) was estimated for the first record (BW1 at 394 DPH). Body length records were available only for the two last time points (i.e., for 770 and 978 DPH) and showed higher heritability estimates than body weight. Moreover, in the previous studies, the genetic correlation between body weight and length ranged slightly from 0.94 to 0.96 (Vallecillos et al., 2021, 2023; Nousias et al., 2020). In the present study, a lower range was found using the PRM with 0.82 for the BW2-L2 and 0.91 for the BW3-L3. While fitting the additive variance in the model using the GRM, the correlation between the body weight and length was found 0.87 for both pairs (at 770 and 978 DPH). The present findings in comparison with the trend of the scatter plots (Fig. 1) would suggest that body weight and length at later stages of meagre may not be strictly linear. Focusing on the high standard errors which were estimated for the heritability of the studied traits in the present study using the PRM, were also noted in the literature linked to meagre (i.e., Vallecillos et al., 2021, 2023; Nousias et al., 2020). Studying the weight trait, the standard error of the heritability ranged from 0.12 to 0.24 and for the length ranged from 0.12 to 0.22. Our standard errors were within the above ranges when the PRM was used for the heritability of the body weight, length and growth. The same pattern with high standard errors was also noted in other new and phylogenetically-close species in the aquaculture industry, such as the dusky kob (*Argyrosomus japonicus*), in which the heritability for weight was 0.46 ± 0.29 and for the standard length was 0.41 ± 0.27 (Rhode et al., 2023).

Since the growth and body weight are polygenic traits and no major QTL associated with them has been detected, it is more effective to use the genomic prediction in order to improve them in an animal breeding program (Robledo et al., 2018). A comparison between the predicted ability using the GRM and using the PRM has been done in the present study, analyzing body weight and growth. In the literature, the accuracy of prediction of the breeding values for four different body weight measurements corrected by the square root of the heritability, was higher using the GRM (it ranged from 0.62 to 0.54, Thermo Fisher Axiom TM Seabass 57 K SNP Dlab-Chip, Griot et al., 2021) than using the PRM (ranged from 0.54 to 0.42) in the European sea bass (Oikonomou et al., 2022a). Additionally, the predicted ability (as a correlation between the breeding values and the phenotypes) of the body weight (~174.7 g) at the early stages in infested European sea bass (Oikonomou et al., 2022c), was higher and equal to 0.41 using the GRM (30 K MedFISH array, (Peñaloza et al., 2021)) than using the PRM in which case it was equal to 0.38. The latter difference was statistically significant between the two methods (Oikonomou et al., 2022c). In the present study, the same pattern was detected, revealing the superiority of the use of the GRM. However, the predicted ability which was estimated for the body weight (0.62–0.69) independently of the relationship matrix was higher than the predicted ability which was estimated by Oikonomou et al. (2022c)(0.38–0.41). This could be explained by the different age of the fish and the family structure. In Oikonomou et al. (2022c), a balanced family structure was used, since individual mattings took place, families were kept in separate tanks and fish were pit-tagged before mixing (more information available in Oikonomou et al. (2022b) while in the present study the offspring originated from a mass spawning with the risk of having an unequal contribution of the parents. Based on the findings, for body weight and growth in meagre, it is more efficient to genetically improve by using the genomic selection (or a combination of genomic and pedigree relationship matrix) instead of using the traditional pedigree approach.

The use of growth as a selection criterion or when it is monitored under selection pressure in an applied breeding program could potentially increase size homogeneity in fish. In the aquaculture industry, meagre shows a large size heterogeneity during growth which increases the production cost because fish have to be size-sorted (sometimes more than once) and split into different sea-cages i.e., smaller and larger fish as it happened in the current study due to the high competition for feed and/or potential cannibalism. All these additional management practices are increasing operational costs and losses in aquaculture farming. For those reasons, growth, as a trait, should be considered as a selection criterion in an applied breeding program; furthermore, our findings on chromosome 17 (in which the QTL and the high trailing of the SNPs were detected) could provide more information on growth and should be further investigated.

Nevertheless, even though a small number of SNPs (4573 SNPs) were available in the present analysis, after a very restrictive quality control, a successful parentage assignment, a better performance using the genomic selection to predict the accuracy of the breeding values in case of phenotype absence and a QTL detection related to the later stages of growth were discovered.

Parentage assignment is considered as one of the main setbacks when using mass spawning for controlled reproduction of the broodstock in a breeding program; this is because we need to obtain the necessary pedigree for the estimation of the genetic parameters, the genetic evaluation of the broodstock, and the selection candidates. However, the use of microsatellite markers for parentage assignment is increasing the

operational cost of a breeding program; moreover, costs could be increased by a small amount to genotype all fish (broodstock and offspring) using Genotype-by-Sequencing (GBS) approaches or a low-density SNP panel in the future. The use of SNP markers from such methods could provide beneficial advantages in genetic evaluation of the broodstock and the selection candidates in terms of accuracy of the breeding values using a combination of traditional pedigree and genomic selection and/or pre-selection of selected candidates on detected QTL or direct genomic selection and the inbreeding.

5. Conclusions

For the first time, a genomic evaluation for body weight, length and growth estimates was performed in meagre *Argyrosomus regius*. Fin-clip were collected from fish which were coming from a mass-spawning event and used to genotyped with ddRAD-seq. The parentage assignment was performed using 300 homologous SNP markers, providing good results for an applied breeding program. Apart from that, genomic and genetic parameters were estimated between body weight, length and growth. Moderate to high heritability was detected using pedigree and genomic relationship matrices showing their polygenic nature as traits in the late stages. A high predicted ability was detected using only a low-density SNP compared to the pedigree approach, which can provide beneficial results in the genetic improvement of the species. A QTL associated with growth explaining approximately 3.25 % of the phenotypic variance was found and two genes TCF4 and ATP8B1, which participated in myogenesis, and weight loss, respectively, were found linked to it. As a conclusion, genotyping of fish only for 4000–5000 SNPs instead of microsatellite panels can provide not only the parentage assignment but also to use the combination pedigree and genomic relationship matrices to select the "best" for growth and the less inbred candidates.

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CRediT authorship contribution statement

Stavroula Oikonomou: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Tereza Manousaki:** Writing – original draft, Validation, Methodology, Investigation. **Antonio Vallecillos:** Writing – original draft, Validation, Methodology, Investigation. **Katerina Oikonomaki:** Writing – original draft, Validation, Methodology, Investigation. **Konstantinos Tzokas:** Writing – original draft, Validation, Resources, Methodology, Investigation. **Nikolaos Katribouzas:** Writing – original draft, Validation, Resources, Investigation, Conceptualization. **Costas Batargias:** Writing – review & editing, Writing – original draft, Validation, Resources, Investigation, Conceptualization. **Dimitrios Chatziplis:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Costas S. Tsigenopoulos:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Costas Tsigenopoulos reports financial support was provided by General Secretariat for Research and Innovation, Greece. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2024.741622.

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