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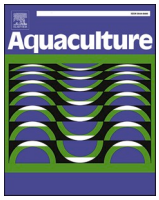

Genome-wide association analyses reveal genotype-by-environment interactions of growth and organ weights in gilthead seabream (*Sparus aurata*)

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ABSTRACT

Gilthead seabream is one of the most important fish species for Mediterranean aquaculture. More than 80% of the genetically improved fingerlings of this species originate from a single country, Greece. A previous study revealed moderate genotype-by-environment (G x E) interactions for growth traits and organ weights between commercial production sites in Greece and Spain. The main objectives of this study were to identify QTLs and genes associated with growth traits and organ weights in gilthead seabream and to identify the biological processes and pathways controlling these traits under different temperature environments. For growth traits, we found fourteen genome-wide suggestive SNP effects in Spain. A strong peak between 1.97 Mb to 4.69 Mb on chromosome (chr) 22 was shared among three growth traits (harvest weight, fillet weight and thermal growth coefficient). Fourteen genome-wide suggestive SNP effects were also identified in Greece. However, none overlap with those found in Spain. Two SNPs on chr5 (chr5:5729959, chr5:2336903) and two SNPs on chr6 (chr6:1957732, chr6:3659811) were associated with all three growth traits. For organ weights, a total of 15 SNPs were associated above the suggestive threshold in Spain. Four SNPs (chr22:1966940, chr22:6094383, chr22:67838, chr15:16798259) are shared between viscera weight and liver weight, while one SNP (chr22:67838) is shared among viscera, liver and heart weight. Interestingly, three of these four SNPs (chr22:1966940, chr22:67838, and chr15:16798259) also overlap with significant SNPs associated with growth traits. None of the SNPs associated with viscera weight, liver weight and heart weight are shared with Greece. GO and KEGG functional enrichment analyses showed that light absorption and ECM-receptor interaction are significantly enriched with growth traits QTLs in Spain but not in Greece. For organ weights, response to stimulus is the most prominent process in Spain, while cell adhesion, and regulation of phosphorylation are more prominent in Greece. Genomic architectures for growth traits and organ weights differ between environments. These findings not only explain part of the G x E interactions, but also give insight into how genetics determines differences in how environmental conditions affect growth and organ weights.

1. Introduction

Gilthead seabream is a domesticated fish species in the Sparidae family. The domestication of gilthead seabream started in the 1970's (Sola et al., 2007). Selective breeding, however, was not initiated until the mid-1990's (Knibb et al., 1997). Gilthead seabream has developed into one of the main aquaculture finfish species in Europe, with a total number of 228,000 tons in 2018 (FAO, 2020), ranking fourth in European aquaculture production. Although the species is farmed over a wide geographic area from the North Atlantic Ocean to the Red Sea, over 80% of the genetically improved fingerlings are derived from Greece (Janssen et al., 2015).

Genotype by environment (G x E) interactions for economic traits have been widely reported across different rearing environments in aquaculture species. For example, in rainbow trout (*Oncorhynchus mykiss*), Sae-Lim et al. (2013) found significant G x E interaction in

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response to variation in environmental factors such salinity with a genetic correlation between 0.19 and 0.48 for harvest weight between fresh and brackish water. Gonzalez et al. (2022) found similar genetic correlations in Atlantic salmon (*Salmo salar*) reared in seawater and freshwater. In Nile tilapia, Mengistu et al. (2020) estimated a genetic correlation of 0.81 between growth in hypoxic and normoxic environments. Given the wide area in which gilthead seabream are cultured, it is likely that G x E interactions are important in gilthead seabream aquaculture. Elalfy et al. (2021) investigate G x E interaction for harvest weight and growth for gilthead seabream reared in sea cage and estuary cage and found evidence for moderate G x E. A previous study in gilthead seabrean (Gulzari et al., 2022), showed moderate G x E interactions for harvest weight (0.45 ± 0.11), fillet weight (0.49 ± 0.12) and liver weight (0.61 ± 0.11) but a weak G x E interaction for heart weight (0.76 ± 0.11) in two distinct commercial production sites, one in south of Greece (Galaxidi Marine Farm) and another in southeast of Spain (Cudomar Farm).

As growth traits directly affect production and profit, there is a substantial endeavour to decipher the genomic architecture of growth traits. In fish species, the somatotropic axis (growth hormone, insulin-like growth factor and associated carrier proteins) plays a key role in regulation of growth (reviewed by De-Santis and Jerry (2007)). Several studies have identified QTLs for growth-related traits in fish species such as Atlantic salmon (Gutierrez et al., 2012; Tsai et al., 2015), rainbow trout (Salem et al., 2018; Ali et al., 2020), tilapia (Yoshida and Yáñez, 2021; Yu et al., 2021a, 2021b) and carp (Guo et al., 2022; Huang et al., 2020; Wang et al., 2021). In gilthead seabream, Loukovitis et al. (2011) performed the first QTL study to investigate the genetic basis of body weight and sex determination in gilthead seabream. They further identified novel growth-related and sex QTLs by increasing the number of informative microsatellite markers from 9 to 74 (Loukovitis et al., 2012). Although such studies may potentially inform marker assisted breeding, the low number of informative markers limits applicability. The differences in marker/trait associations in diverse environments that underly G x E interactions have received little attention in gilthead seabream and other aquaculture species.

Investigating the relative growth of various organs provides important information on correlations with physiological conditions. Organ weights are indicative of health, as diseases can affect the weight of internal organs (Kumar et al., 2014). On the other hand, organ weight is strongly corelated with growth traits. For example, harvest weight has strong positive genetic correlations with liver weight (0.68), heart weight (0.92) and viscera weight (0.80) in gilthead seabream (Gulzari et al., 2022). Very few studies have investigated QTL for organ weight, and most are in mice and chicken (Fawcett et al., 2008; Leamy et al., 2002; Neuschl et al., 2007; Moreira et al., 2019). Consequently, the genomic basis of organ weight, and G x E determining these traits, in aquaculture species remains unknown.

In this study, the experiment described in Gulzari et al. (2022) was used to identify QTL for growth-related traits (harvest weight, thermal growth coefficient and fillet weight) and organ weights (viscera weight, liver weight and heart weight) in Gilthead seabream. Specifically, this study investigated the genomic architecture of G x E interaction, between two different production sites, in Greece and Spain, that were stocked with progeny from the same breeding population. Here we extend the study of Gulzari et al. (2022) by applying genomics to address the key objectives of the present study: (1) discover QTLs associated with growth and organ weight in Spanish and Greek farms, respectively, (2) reveal underlying biological processes and pathways related to G x E interactions.

2. Material and methods

2.1. Study population

The fish in this study were derived from the selective breeding program at Galaxidi Marine Farm, located in the Gulf of Corinth, Greece (38° 21′ 06.6" N 22° 23′18.8″E). We used progeny from a total of 33 male and 20 female gilthead seabream parents in a single group spawning tank and collected fertilized eggs on a single day. We raised the fingerlings together minimize variation in common environmental effects. At an average weight of 3 g, one batch of 99,000 juveniles was stocked in a sea cage at the Galaxidi farm, in the gulf of Corinth, and the other batch of 84,605 juveniles was transferred and then stocked at the Cudomar farm, in the Mediterranean Sea (38°25′12" N 0°20′51" W) southeast Spain. The fish in both locations were fed with the same commercial diet after reaching an average weight of 100 g. The feed was provided once a day. The feed was provided once a day. The amount of feed given per kilogram of biomass was recorded throughout the grow-out period. During the grow-out period, both farms fed the fish with the same commercial diet and adjusted feeding levels according to biomass at different stages (Gulzari et al., 2022). The management conditions were kept as similar as possible, and the physical environment was considered the main difference in treatment between the two production farms. We recorded water temperature daily, and dissolved oxygen, salinity, and pH were monthly at the two farms during the grow-out period (Table 1).

After the grow-out period (465 days in Greece, and 500 days in Spain), we measured production traits and organ weights. Prior to sampling we euthanized fish with a mortal dose of 0.03 mL/L clove oil in an oxygenated tank. A random sample of fish was taken from the same sea cage. In total, we measured harvest weight (HW) and viscera weight (VW) using an electronic balance accurate to 0.5 g on 998 fish in Greece and 945 fish in Spain. Fin clips from all fish were collected, preserved in 75% ethanol, and stored at 4 °C until DNA extraction. Using a scale accurate to 0.001 g, we separated and measured liver weight (LW) and heart weight (HeW). The gutted fish were filleted, and we recorded the fillet weight for one side only and estimated total fillet weight (FW) by multiplying it by two. Measurement procedures were standardized between the two farms, except for fillet weight, which was skin-off in the Greece and skin-on in Spain. Further details on phenotyping are found in Gulzari et al. (2022). We calculated the Thermal growth coefficient (TGC) according to the formula of (Mayer et al., 2012):

$$\mathbf{TGC} = \left[\frac{\sqrt[2/3]{\mathbf{harvestweight}} - \sqrt[2/3]{\mathbf{stockingweight}}}{(\mathbf{T}\ \mathbf{x}\ \mathbf{t})}\right] \times \mathbf{1000} \quad (1)$$

where **T** is the average water temperature and **t** is the number of days during grow-out period.

2.2. DNA extraction, genotyping and quality control

Identigen (Dublin, Ireland) isolated genomic DNA from the fin clips and genotyped them using the MedFish array, which contains 30 K SNP markers distributed across the gilthead seabream reference genome (Peñaloza et al., 2021). Before SNP filtering, only samples with quality control values ≥ 0.82 and call rate per samples ≥ 97% were retained before SNP filtering as recommended by the Axiom® Analysis Suite (version 5.1) developed by Affymetrix.

We further filtered the data using Plink v1.9 (Purcell et al., 2007)

Table 1
Average environmental and management conditions in Greece and Spain.

Variable	Greek farm	Spanish farm
Overall water temperature (°C)	20.5	21.4
Summer water temperature (°C)	25.1	26.1
Autumn water temperature(°C)	20.0	21.7
Winter water temperature (°C)	14.3	15.2
Spring water temperature (°C)	16.5	17.7
Total degree days from stocking until harvest (°C)	3830	4630
Dissolved oxygen (mg/L)	5.58	8.19
Salinity (‰)	39.0	37.3

with the following parameters: SNPs with minor allele frequency (MAF) lower than 5%, missing call rates exceeding 10% were removed. The distribution over the genome and density of SNPs that passed filtering were visualized using the CMplot R package (Yin et al., 2021).

2.3. Descriptive statistics, population structure and linkage disequilibrium

Phenotypic statistics (means, standard errors and *t*-tests) for HW, TGC, FW, VW, LW and HeW of 1901 fish were calculate using R version 4.0.2. Before conducting a genome-wide association study (GWAS), we assessed external influencing factors such as sampling date in the linear model (lm function in R) using the stepwise regression algorithm, to choose the best model by AIC value as described (Yu et al., 2021a, 2021b). Because gilthead seabream are protandrous (García Hernández et al., 2020), all fish were functional males at the first two years, and sex was not included as a factor. After fitting the linear model, we identified and excluded observations with residuals >3.5 standard deviations from the subsequent association analysis.

To characterize population stratification, e.g. through family relationships, principal component analysis (PCA) was performed using Plink v1.9 and visualized by the scatterplot3d R package (Ligges and Mächler, 2002). Furthermore, high linkage disequilibrium (LD) between markers may limit resolution for the genome-wide association study (Newell et al., 2011). We estimated LD decay using Plink v1.9 with the default option except "–ld-window-r2 0", which means not to filter any SNPs based on squared correlations.

2.4. Univariate linear mix model for GWAS

To identify SNPs associated with growth-related traits, we performed a genome-wide association study using the univariate linear mixed model option "-lmm", implemented in Gemma (Zhou and Stephens, 2012):

$$\boldsymbol{y} = \boldsymbol{W\alpha} + \boldsymbol{x\beta} + \boldsymbol{K\mu} + \boldsymbol{\varepsilon} \quad (2)$$

where $\boldsymbol{y}$ is a vector of n individuals with phenotypes for each trait (HW, TGC, FW, VW, LW and HeW); $\boldsymbol{W}$ is an n x 6 matrix of fixed effects (including sampling date and top five PCs to correct population structure); $\boldsymbol{\alpha}$ is a vector of corresponding coefficients; $\boldsymbol{x}$ is a vector of the marker genotypes (0,1,2) and $\boldsymbol{\beta}$ is the additive genetic effect of the marker; $\boldsymbol{K}$ is the genomic kinship matrix based on standardized matrix method in Gemma; $\boldsymbol{\mu}$ is a vector of random animal effects; $\boldsymbol{\varepsilon}$ is an vector of random residuals. Here, we incorporated the top five PCs accounting for population structure as fixed effects, and kinship as a random effect in the mixed model. Several studies have demonstrated that combining PCs and kinship performs better in detecting false positive association signals compared to using only PCs or kinship (Hoffman, 2013; Lu et al., 2016; Neves et al., 2012). The estimated *p*-values for the genetic effects are based on the Wald tests. We visualized Manhattan and quantile-quantile (Q-Q) plots using the R package qqman (Turner, 2014). We calculated the genomic control λ (also known as inflation factor) in order to assess the presence of population stratification, values of $\lambda_{GC} < 1.05$ are generally considered good (Price et al., 2010).

2.5. QTL identification

Due to the overly conservative nature of Bonferroni correction, we used the SimpleM method, based on the effective number of independent tests (Gao et al., 2010) to calculate the suggestive and genome-wide significance thresholds, and considered SNPs above the suggestive threshold as candidate QTLs. For each QTL, the genetic effect ($\boldsymbol{\beta}$) of the leading SNP (with lowest *P*-value) was used to estimate the proportion of genetic variance explained by this peak per the formula below:

$$\mathbf{Vg}\% = \frac{\mathbf{2p(1-p)\beta^2}}{\boldsymbol{\sigma}_{\mathbf{a}}^{\mathbf{2}}} \times \mathbf{100} \quad (3)$$

where p is the minor allele frequency of the target SNP and $\boldsymbol{\sigma}_{\mathbf{a}}^{\mathbf{2}}$ is the total genetic variance.

Candidate genes located within a window of 50 kb up- and 50 kb down-stream of the SNPs for each QTL were identified using SnpEff v5.1 (Cingolani et al., 2012), based on the latest gilthead seabream genome reference (GCF_900880675.1). Due to the limited information on the gilthead seabream genome annotation, we explored additional information by comparing to homologous genes in zebrafish (*Danio rerio*). Therefore, we used BLASTP searches against *D. rerio*) proteins, based on genome build GCF_000002035.6_GRCz11. We regarded genes as homologous if they had best hits with an expected value less than 1E-6 (Yandell et al., 2008; Xu et al., 2016).

2.6. Gene ontology (GO) and KEGG enrichment analysis

Based on the candidate genes overlapping GWAS peaks, we used Gene Ontology and KEGG enrichment in Metascape (Zhou et al., 2019) and KOBAS-i (Bu et al., 2021) for functional assessment of GWAS results. We considered terms as enriched if they had a minimum of 2 genes overlapping with the input gene list, a *p*-value <0.05, and an enrichment factor > 1.5 (the ratio between the observed counts and the counts expected by chance). The significant GO and KEGG pathways were visualized with the ggplot2 R package (Wickham et al., 2016).

3. Results

3.1. SNP informativeness and distribution

In total we genotyped 1920 fish (979 from the Greek farm and 941 from the Spanish farm) for 32,359 SNPs. We determined the informativeness of the SNP array by calculating the minor allele frequency (MAF). The results showed that most SNPs belong to the common (MAF ≥0.3) and intermediate (0.1 ≤MAF < 0.3) minor allele frequency groups, at 48.2% and 40.5%, respectively (Fig. 1a). After quality control using the Axiom® analysis Suite and Plink, we retained 1901 individuals and 29,111 variants for subsequent analyses. The distribution of SNPs across the genome was plotted on the gilthead seabream genome reference, showing an even distribution with some clusters of higher density across all chromosomes (chr) (Fig. 1b). On average, there is one SNP per 28.63 kb. The highest number of SNPs (1555) is on chr6, while the lowest number of SNPs (1085) is on chr24.

3.2. Analysis of population structure and LD decay

The top 5 principal components explained 70.04% of the genetic variance of all individuals from both farms combined. According to the PCA plot of the first three principal components, a clear population stratification was observed (Fig. 2a). We corrected this bias by including the top 5 principal components in the GWAS model. Physical linkage of loci can result in linkage disequilibrium (LD). Characterizing LD is useful in GWAS studies because it determines the resolution at which we can expect to resolve loci. The LD decay plot (Fig. 2b) shows that average r^2 drops to population background level within 50 kb. Overall, LD is low at the SNP density applied in this study.

3.3. Descriptive statistics

934 samples from Spain and 967 samples from Greece, for growth-related traits (HW, TGC, FW) and organ weights (VW, LW, HeW) were collected. As shown in Table 2, fish from the Spanish farm have overall mean HW, TGC, FW of 412.31 g, 11.38 ($g^{2/3}$ x $°C^{-1}$ × 1000) and 179.6 g, respectively, while average measurements were 371.44 g, 12.74 ($g^{2/3}$ x

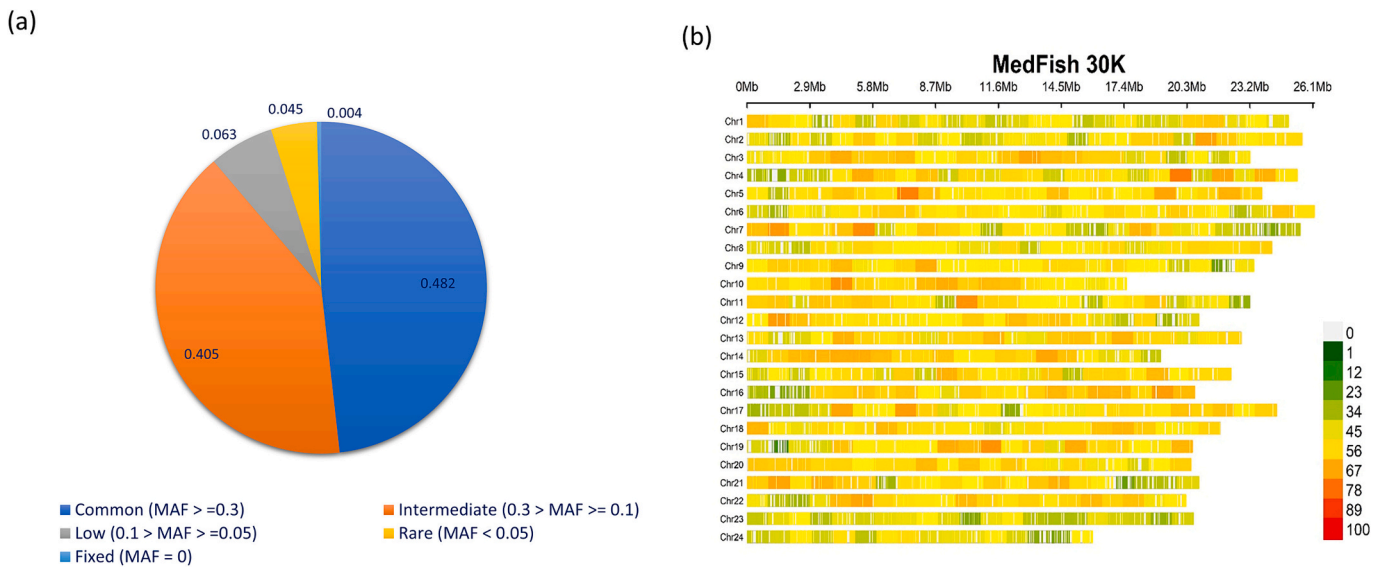

Fig. 1. (a) Informativeness and (b) genomic distribution of SNPs used in this study.

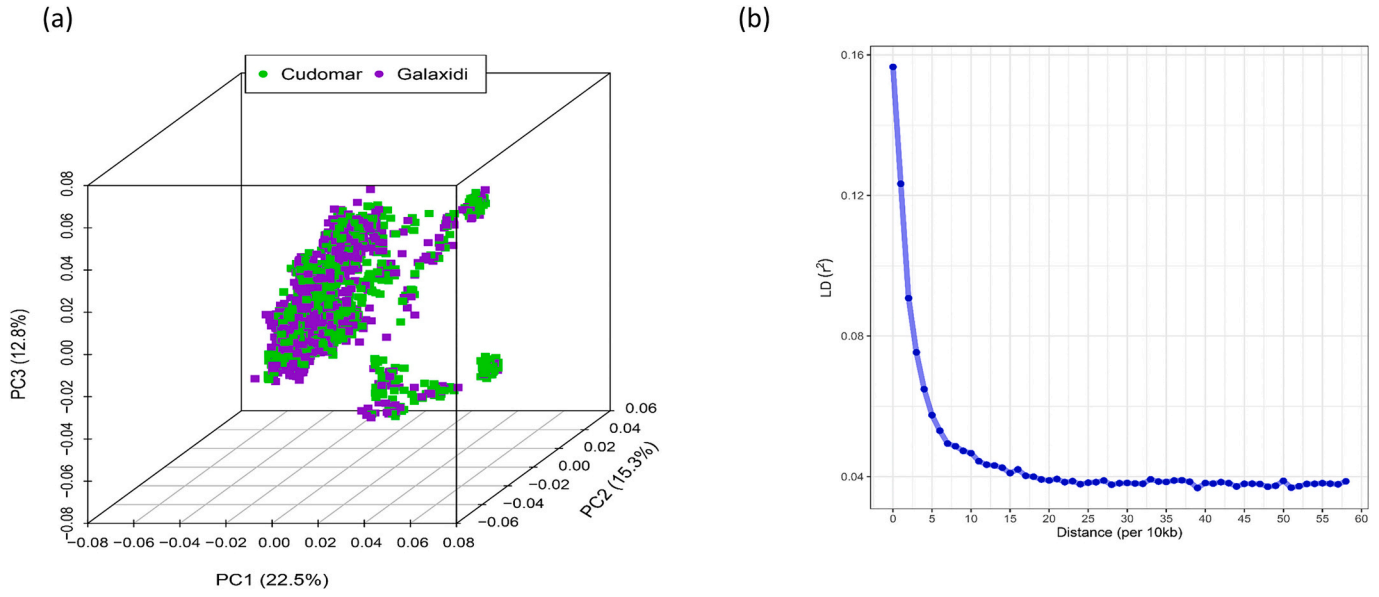

Fig. 2. (a) Principle component plot for all individuals across Spanish and Greek farms and (b) estimation of linkage disequilibrium decay using r^2 between pairs of SNPs within 600 kb of each other.

Table 2
Descriptive statistics of study traits in two farms.

Trait	Farm	Total number	Outliers	Mean	se	t-value	Effect of farm (*P* value)
Harvest weight (g)	Spain	934	0	412.31	2.29	13.15	< 2.2e-16
	Greece	967	1	371.44	2.1		
TGC ($g^{2/3}$ x $°C^{-1}$ × 1000)	Spain	934	13	11.38	0.04	−20.06	< 2.2e-16
	Greece	967	9	12.74	0.05		
Fillet weight (g)	Spain	934	3	179.6	0.57	46.10	< 2.2e-16
	Greece	967	3	116.78	0.37		
Viscera weight (g)	Spain	934	4	29.31	0.22	6.68	3.214E-11
	Greece	967	2	27.17	0.23		
Liver weight (g)	Spain	934	4	6.78	0.07	37.38	< 2.2e-16
	Greece	967	4	3.88	0.04		
Heart weight (g)	Spain	934	11	0.48	0.003	22.56	< 2.2e-16
	Greece	967	11	0.38	0.003		

Note: the mean is calculated after removing outliers.

$^{\circ}C^{-1} \times 1000$) and 116.78 g in the Greek farm. Fillet weight showed the highest difference between two farms. For internal organ weights, liver weight showed the largest difference between two farm locations, followed by heart height. Overall, all six traits were significantly different between the two farm locations.

3.4. QTLs for growth traits and organ weights

We applied GWAS to detect loci associated with growth and organ weights. A total of fourteen SNPs were found significantly or suggestively associated with growth traits in Spain (Fig. 3a). Seven (AX-384467133, AX-326257091, AX-326255828, AX-326250505, AX-384487970, AX-326258252, AX-326271807) out of fourteen SNPs were located on chr22, of which the highest peak, which included 3 SNPs between 1.97 Mb to 4.69 Mb, were shared among TGC, HW and FW. The leading SNP (AX-384467133, chr22: 1966940) for HW explained 6.2% additive genetic variation. The leading SNP (AX-384467133, chr22:1966940) for TGC explained 5.3% additive genetic variation. The leading SNP (AX-326257091, chr22: 4686464) for FW explained 6.9% additive genetic variation. Four candidate SNPs (chr22: 4686464, chr22:3468915, chr22:1966940 and chr11:10245719) were shared among HW, TGC and FW traits. In addition, genomic control factor (λ_{GC}) was close to 1 (1.005, 1.005, 0.995 for HW, TGC and FW, Supplementary Fig. 1a), which suggest population stratification is not affecting the association results.

In the Greek farm, we observed 14 suggestive or significant SNPs that were physically close to growth-related traits (Fig. 3b). However, none of those 14 SNPs was the same as those found for the Spanish farm. Two suggestive peaks, containing two SNPs on chr5 (chr5:5729959, chr5:2336903) and two SNPs on chr6 (chr6:1957732, chr6:3659811), were found to be associated with all three traits. The leading SNPs (AX-383652807, chr5:2336903 for HW, AX-383658435, chr5:5729959 for TGC, AX-383716149, chr6:3659811 for FW) explained 8.69%, 8.05% and 10.05% of the additive genetic variation respectively after PC correction. Furthermore, eight out of fourteen SNPs are located on chr5 and chr6. The inflation factors were 0.981, 0.991 and 1.014 for HW, TGC and FW respectively (Supplementary Fig. 1b), which suggests the absence of population stratification.

For organ weights, we found a total of 15 and 17 SNPs above the suggestive threshold in Spain (Fig. 4a) and Greece (Fig. 4b), respectively and QQ-plot in Supplementary Fig. 2a and Supplementary Fig. 2b. However, similar to the growth-related traits, none of these SNPs were shared between the farms. In Spain, the leading SNPs associated with viscera weight and liver weight are on the same chromosome, but different location (chr24:7543571 and chr24: 2799809). Four SNPs (chr22:1966940, chr22:6094383, chr22:67838, chr15:16798259) overlap between viscera weight and liver weight, while one SNP (chr22:67838) was shared among viscera, liver and heart weight. In Greece, none of the SNPs associated with viscera weight, liver weight and heart weight are shared between traits. Additionally, fewer candidate SNPs were found in the association analyses for Greece compared to Spain, which either reflects less genetic variation, or that the traits are

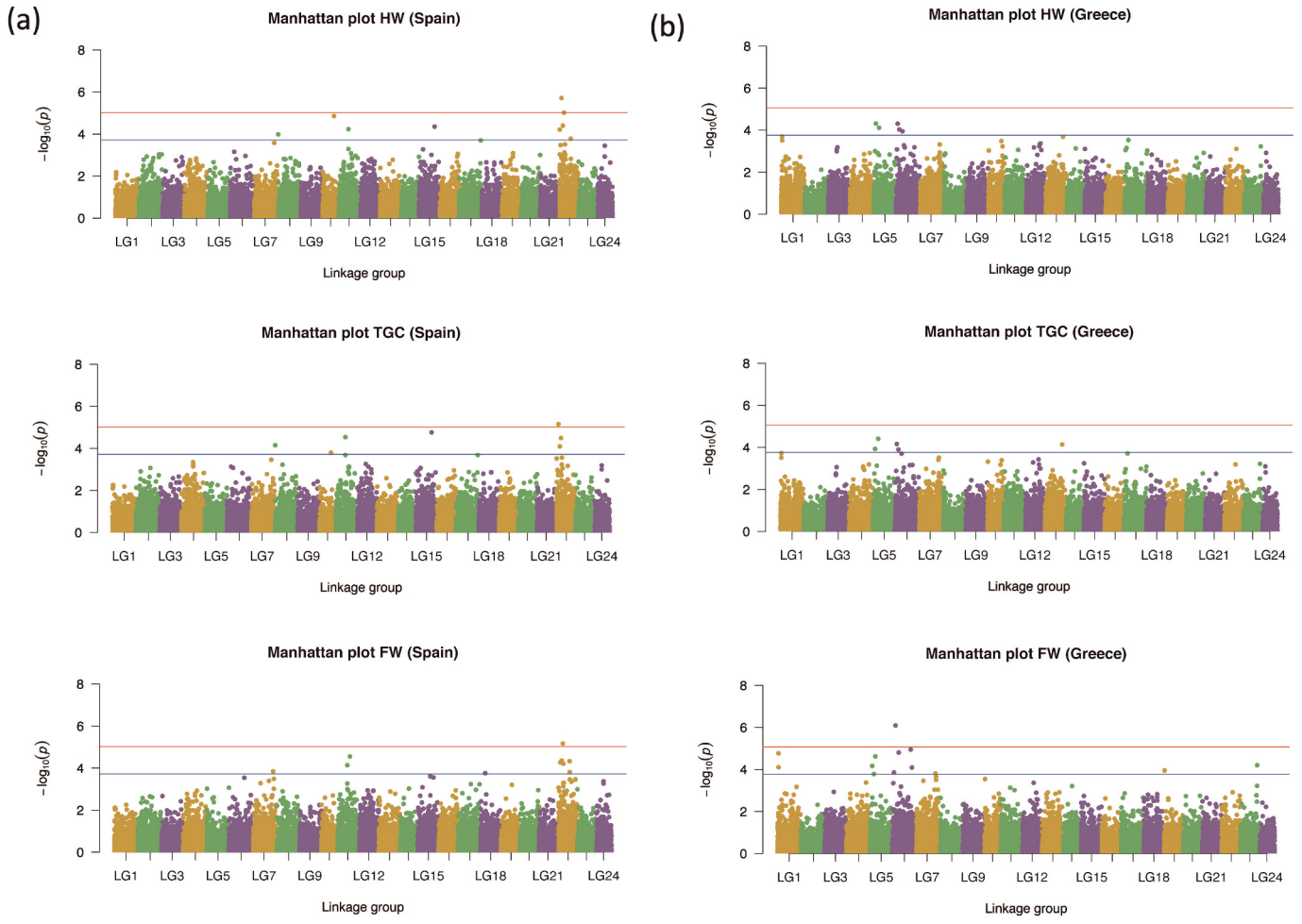

Fig. 3. (a) Manhattan plots for HW, TGC, FW for Spain farm. (b) Manhattan plots for HW, TGC, FW for the Greece farm. The orange and blue horizontal line represent the genome-wide significance (5.07) and suggestive threshold value (3.77), respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

more polygenic in Greece.

3.5. Candidate genes in the QTL regions

A summary of all candidate genes in those QTL regions and their functions is shown in Table 3. In total, we found eighteen genes associated with those 14 SNPs that showed significant or suggestive associations with growth traits in the Spanish farm. The highest peak for HW and TGC is located in an intron of *znf292* on chr22, and for FW it is located in an intron of *lama4* on chr22. Other candidate genes on chr22 are *usp18*, *mcl1b*, *si*, and *mapre3b*. Candidate genes on chr11 are *ncanb* and *mob3c*, while the remaining candidate genes are located on other chromosomes. The functional annotation for these candidate genes suggests involvement of laminin complex, tissue development, brain morphogenesis, and apoptotic signalling pathways.

For the Greek farm, we identified sixteen genes close to those 14 SNPs that exceeded the suggestive threshold for growth traits (Table 4). Candidate genomic locations were found mostly on chr5 and chr6. Two peaks completely overlap for HW and TGC. Genes located near these peaks are *traf2a*, *tnc, zfr* on chr5 and *uqcc1*, *gdf5* and two non-coding genes (LOC115583312, LOC115583301) on ch6. Other candidate genes are *hmgcra* on chr5, *gnai2b* and *si* on chr6, while the remainder of genes were found on other chromosomes.

For organ weights, the Spanish farm revealed sixteen candidate genes near the fifteen associated SNPs (as shown in Supplementary Table 1). The only SNP shared between VW, LW, and HeW (chr22:67838) is in an intergenic region, between genes *usp18* and *mcl1b*. For the Greek farm, twenty candidate genes were identified for three traits (VW, LW, HeW) (Supplementary Table 2). No shared SNPs were found for these three traits. Interestingly, candidate SNPs for growth traits and organ weights, were found to overlap including SNPs located in *ncan* (chr11:10245719), *znf292* (chr22:1966940) and *zfr* (chr5:5729959) suggesting a functional relationship between growth and organ weight.

3.6. GO and KEGG enrichment analyses

Since the number of genes associated with any of the traits was limited, all candidate genes resulting from the GWAS analysis were included to investigate functional enrichments. In total, 59 genes were included in the gene set enrichment. Overall, twelve GO terms were significantly enriched including cell adhesion (GO:0007155, $P < 0.001$), light absorption (GO:0016037, $P < 0.001$), muscle attachment (GO:0016203, $P = 0.007$), and regulation of phosphorylation (GO:0042325, $P = 0.014$) (Fig. 5a, Supplementary Table 3). Based on the KEGG pathway database, eight KEGG pathways were significantly enriched (Fig. 5b, Supplementary Table 4), including ECM-receptor interaction (dre04512, $P < 0.001$), and progesterone-mediated oocyte maturation (dre04914, $P = 0.011$).

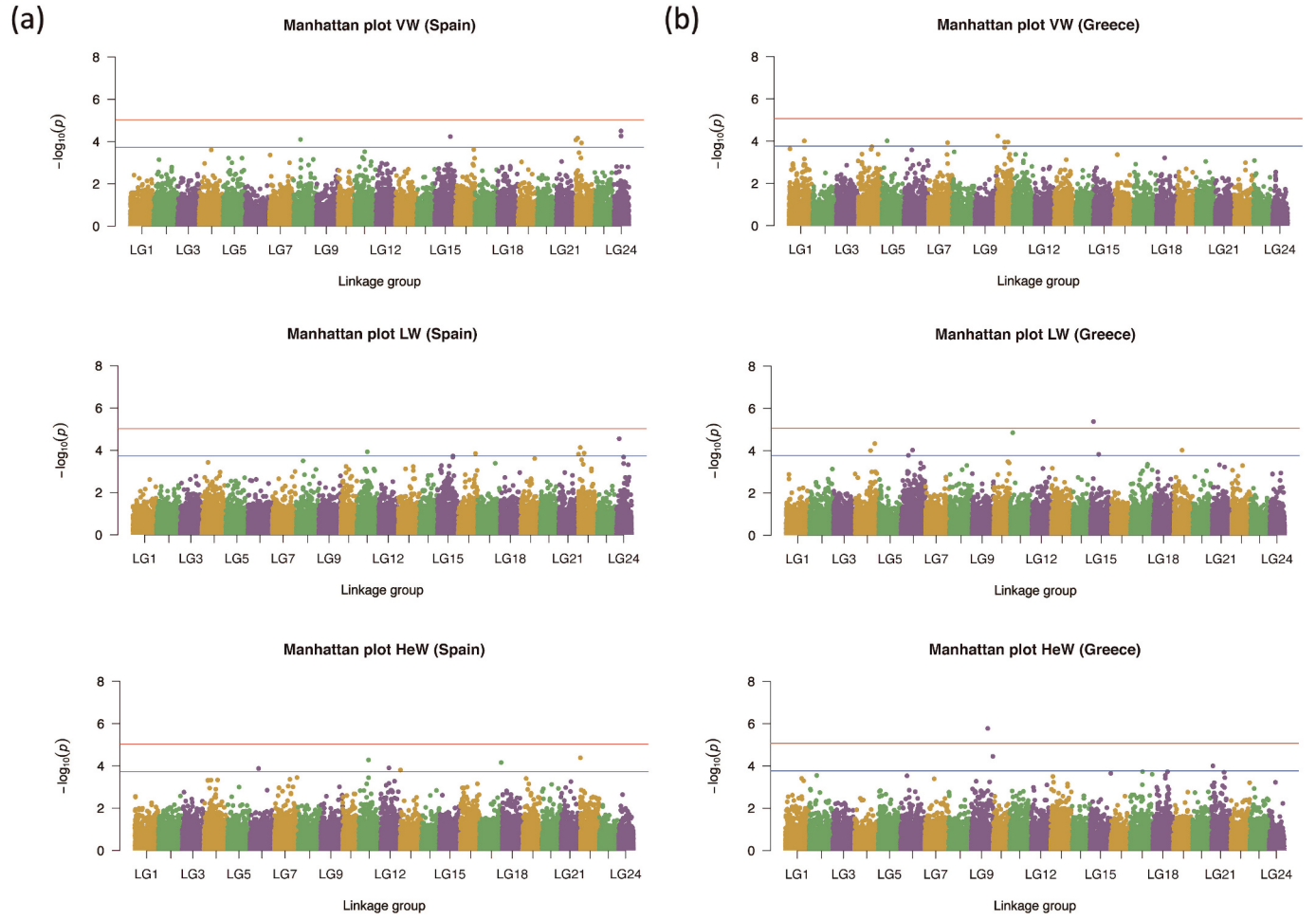

Fig. 4. (a) Manhattan plots for viscera weight (VW), liver weight (LW), heart weight (HeW) for Spanish farm. (b) Manhattan plots for VW, LW, HeW for Greek farm. The orange and blue horizontal line represent the genome-wide significance (5.07) and suggestive threshold value (3.77), respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Candidate genes for growth traits in the Spain.

chr	rs	ps	candidate genes	protein production description
7	AX-325704272	21,263,574	Intron (*kaznb*)	kazrin, periplakin interacting protein b
8	AX-383841266	819	Intergenic (chr_start; *lamb1a*)	laminin, beta 1a
10	AX-325829954	12,200,425	Intron (*LOC115589357*)	immunoglobulin-like fold
11	AX-325864416	10,245,719	Intron (*ncanb*)	neurocan b
11	AX-384019726	13,046,556	5_prime_UTR (*mob3c*)	MOB kinase activator 3C
15	AX-384213348	16,798,259	Intergenic (*LOC562831*; *cfap46*)	gamma-aminobutyric acid receptor subunit pi-like; cilia and flagella associated protein 46
18	AX-326147214	4,995,960	Intron (*glra1*)	glycine receptor, alpha 1
22	AX-384467133	1,966,940	Intron (*znf292b*)	zinc finger protein 292b
22	AX-326257091	4,686,464	Intron (*lama4*)	laminin, alpha 4
22	AX-326255828	3,468,915	Intergenic (*LOC115574565*; *trnav-cac-5*)	noncoding RNA; noncoding RNA
22	AX-326250505	67,838	Intergenic (*usp18*; *mcl1b*)	ubiquitin specific peptidase 18; MCL1 apoptosis regulator, BCL2 family member b
22	AX-384487970	11,920,943	Intergenic (*si*; *LOC115574686*)	sucrase-isomaltase; Noncoding RNA
22	AX-326258252	5,128,085	Exon (*trnad-guc-25*)	noncoding RNA
22	AX-326271807	12,048,260	Intron (*mapre3b*)	microtubule-associated protein, RP/EB family, member 3b

Note: 'chr' represents the chromosome number, 'rs' denotes the single nucleotide polymorphism (SNP) ID, and 'ps' indicates the position on the genome.

Table 4
Candidate genes above suggestive association with growth traits in Greece.

chr	rs	ps	candidate genes	protein product description
1	AX-325365571	931,499	Intron (*LOC559196*)	NACHT, LRR and PYD domains-containing protein 12-like
1	AX-325365525	940,207	Intron (*LOC559196*)	NACHT, LRR and PYD domains-containing protein 12-like
5	AX-383652807	2,336,903	Intergenic (*traf2a*; *tnc*)	TNF receptor-associated factor 2a; tenascin Cb
5	AX-325573574	4,200,955	Exon (*hmgcra*)	3-hydroxy-3-methylglutaryl-CoA reductase a
5	AX-383658435	5,729,959	Intron (*zfr*)	zinc finger RNA binding protein
6	AX-325618171	1,957,732	Intergenic (*uqcc1*; *gdf5*)	ubiquinol-cytochrome *c* reductase complex assembly factor 1; growth differentiation factor 5
6	AX-383716149	3,659,811	noncoding	–
6	AX-383722376	7,054,131	noncoding	–
6	AX-383767540	19,911,726	Intron (*si*)	sucrase-isomaltase
6	AX-383767025	21,248,353	Intron (*gnai2b*)	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 2b
7	AX-383834295	20,411,653	Intron (*cntn3a.2*)	contactin 3a, tandem duplicate 2
13	AX-384125859	18,533,562	Intergenic (*msantd2 - robo3*)	Myb/SANT DNA binding domain containing 2; roundabout, axon guidance receptor, homolog 3
19	AX-383049895	495,763	5′_primer UTR (*LOC110438316*)	zinc finger MYM-type protein 1-like
23	AX-326325158	17,713,731	Exon (*zgc:136410*)	rhamnose binding lectin-like precursor

Note: 'chr' represents the chromosome number, 'rs' denotes the single nucleotide polymorphism (SNP) ID, and 'ps' indicates the position on the genome.

4. Discussion

In this study, we analysed data from two commercial production farms in two different countries to investigate the effect of temperature on growth traits and organ weights. We observed variations in environmental parameters, particularly dissolved oxygen and salinity. Given that the fish originated from the same broodstock, this presented an opportunity to investigate the interaction between genetic variation and environmental conditions (G x E).

Historical temperature data shows that the marine environments of Greece are generally warmer than the Mediterranean coast of Spain, which should make Greece a more favorable environment for growth (Besson et al., 2016). Couto et al. (2008) showed that the growth rate of gilthead seabream was higher when reared at 25 than at 18 °C. However, in practice, the temperature we measured at the specific Greek farm was 0.9 to 1.7 °C lower than that at the Spanish farm during the experiment. This is because the Greek farm is located at the Gulf of Corinth, where cold water flows down from the mountains in winter. Therefore, we observed consistently higher average daily water temperatures at the Spanish farm compared to the Greek farm, and a significant difference in cumulative degree days. Furthermore, the dissolved oxygen at the Spanish farm was also higher than that at the Greek farm. Salinity at the Greek farm is on average 1.7 ‰ higher than that at the Spanish farm, which is consistent with a previous study (Soukissian et al., 2017).

Our previous study identified moderate genotype-by-environment interactions (G x E) for growth traits with a genetic correlation ranging from 0.43 to 0.49 (See Supplementary Table 5, Table 6 and Table 7, adopted from the previous results in (Gulzari et al., 2022). These values are usually considered moderate genotype reranking. When G x E leads to a reranking of genotypes, the genetically improved animals that perform well in one environment may not perform as expected in another, even though they have the same genetics. If moderate to strong G x E is observed, it is possible different genomic architecture is associated with growth traits, a similar pattern was observed in one of our previous studies (Yu et al., 2022). A high genetic correlation suggests a greater degree of shared genomic architecture associated with target traits, whereas a low genetic correlation indicates differences in the genomic architecture linked to these traits. For instance, the genetic correlations for Harvest weight, TGC and Fillet weight across the production environments of Greece and Spain were 0.45, 0.43 and 0.49. For these traits, no shared significant SNPs were found across farms. By contrast, the genetic correlations for viscera weight, liver weight and heart weight were 0.62, 0.61 and 0.76, and for these traits shared significant SNPs across farms were observed. A similar pattern has been observed in human studies. When comparing the genomic loci found the same SNPs in the intelligence and education data sets, it is consistent with the finding of a strong genetic correlation (0.81) between each of the two phenotypes (Hill et al., 2019). Functional annotation and pathway analysis of G × E genes underlying biological mechanisms respond to changes in their environment, such as in rice and cattle (Zhao et al., 2011; Braz et al., 2021). Similarly, in this study several biological

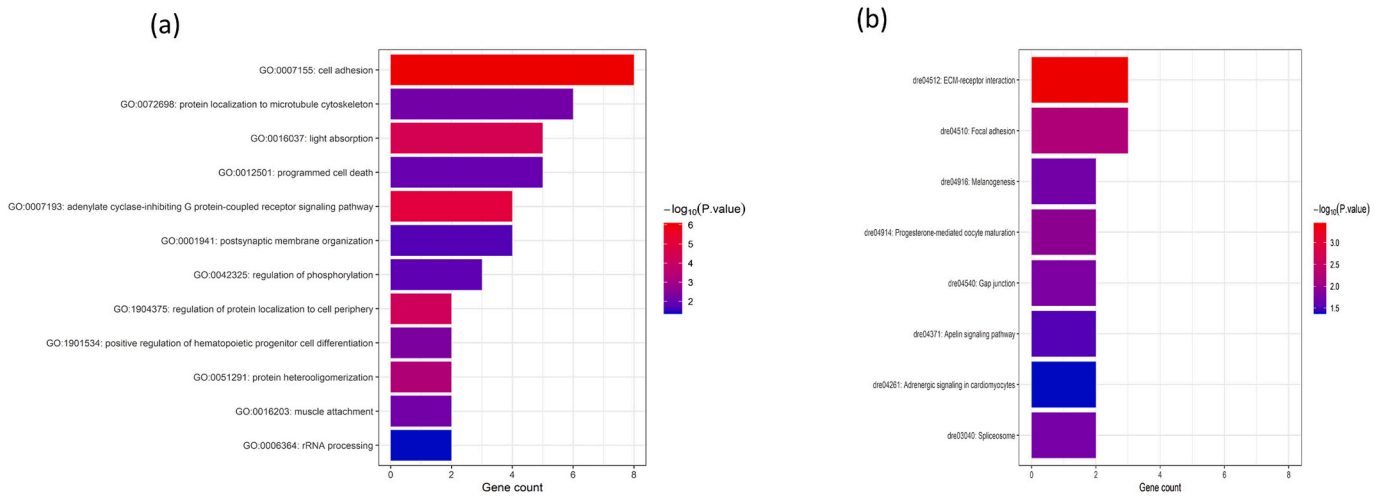

Fig. 5. (a) GO and (b) KEGG significant enrichment results by using all candidate genes for growth-related traits and organ weights.

pathways were uncoverd affected by G × E that likely drive environmental adaptation.

Our results reveal several genes associated with growth traits under two different temperature regimes. For the Spanish farm, we found eighteen genes above the suggestive threshold with the most significant association on chr22 at a genomic region containing genes *znf292* and *lama4*. In rat *znf292,* has been identified as an enhancer of growth hormone expression (Lipkin et al., 1993) while *lama4* has been shown to be expressed in the human basement membrane of skeletal muscles (Knöll et al., 2007). It was observed that the candidate genes were enriched for functions related to cell adhesion, light absorption, and ECM-receptor interaction. Light (in intensity, quality, and photoperiod) is an interesting characteristic in the aquaculture environment and was found to affect fish growth rate (Sumpter, 1992). Cell adhesion is known to interact with growth factor receptors (Ivaska and Heino, 2010). And the significance of light has been relatively overlooked in the farming of gilthead seabream. Interestingly, extracellular matrix (ECM) receptor is known to play an important role in several stress responses. For instance, Asakawa et al. (2019) reported that ECM-receptor was significantly activated after heat stress treatment in Yamame (*Oncorhynchus masou)*. Another study showed that extracellular matrix (ECM)-receptor was an enriched pathway in response to salinity stress in a hybrid tilapia (Su et al., 2020). These results reflect that growth and response to external stimuli were identified in the Spanish farm and it may be worthwhile to investigate the role of light further.

For the Greek farm, the enrichment analysis showed that focal adhesion, muscle attachment and regulation of phosphorylation were significantly enriched in QTL regions for growth-related traits in Greece. Genes located near these peaks are *traf2a*, *tnc*, *zfr* on chr5, and *uqcc1*, *gdf5* on chr6. Gene *traf2a,* are from *traf* gene family, which has been shown to participate in the immune system and apoptotic processes (Nie et al., 2022). Tenascin-C has been shown to regulate the activity of growth factors, such as epidermal growth factor (EGF)-dependent cell growth (Bradshaw, 2014). *Gdf5* has been shown to be involved in skeletal system, while *zfr* has been shown to be involved in multicellular organism development annotated by ZFIN database. This result may reflect the genetic architecture associated with growth-related metabolism. In general, these genes appear to be better understood in terms of their involvement in growth and development across various temperature conditions, rather than having a distinct function specifically related to temperature or heat stress.

For organ weights, the significantly enriched processes were mitochondrial promoter sequence-specific DNA binding (GO:0001018), 1-phosphatidylinositol binding (GO:0005545), light absorption (GO:0016037), cellular response to light stimulus (GO:0071482) for the Spanish farm. *Ddx49,* located within the highest peak for liver weight, is involved in rRNA processing. Recently, it was shown that *ddx49* is a key gene for muscle growth in Indian carp (Mohindra et al., 2022). *Polrmt,* associated with liver weight in our study, has been reported to regulate mitochondrial and energy metabolism (Yu et al., 2021b). *Usp18,* which encodes ubiquitin-specific peptidase 18, indeed plays significant roles in various biological functions during cell and organ development (Honke et al., 2016). By contrast, in Greece, we found twenty genes associated with organ weight that were connected in cell-cell adhesion (GO:0098609) and cytoskeleton-dependent cytokinesis (GO:0061640). *Erbb4b* plays a critical role in cardiac development, cardiomyocyte proliferation, and homeostasis and function of heart in human (Wadugu and Kühn, 2012). Overall, these candidate genes have functions that may explain their role in the observed differences in organ weights.

We also found an overlapping GWAS peaks (Fig. 3a) between traits within farms. *Znf292b* a growth hormone-dependent transcription factor (Lee et al., 2016) was found to regulate both organ weight and growth traits in Spain. The overlap between growth traits and organ weights within farms is expected, as bigger fish have relatively large organs. Considering the genetic correlation between growth and organ weight (Gulzari et al., 2022), a shared genomic architecture between growth and organ weight traits is likely, and our results confirm that organ weights and general growth traits share GWAS peaks. Furthermore, we observed a lack of overlapping GWAS peaks between sites most likely the result of a strong G x E for growth traits, although we cannot exclude that this is because of a low statistical power of the GWAS.

While a higher temperature may not necessarily induce severe stress in fish, pathways associated with environmental stimuli may still be involved. Although we initially assumed that temperature (like over 26 °C in the Spanish farm) might be the primary stressor, we cannot definitively establish a direct relationship. Interestingly, based on functional enrichment results in Greece, we did not detect any processes related to responses to stimuli or stress. This observation could suggest that gilthead seabream grow relatively better under conditions at this location.

5. Conclusions

Although shared genomic architecture for growth traits and organ weights is evident between the two sites, there are different genes involved that may explain the G x E interactions between geographic locations. For growth-related traits, the functional enrichment confirms that light absorption and ECM-receptor interaction processes are important in Spain, with muscle attachment and regulation of phosphorylation in Greece. For organ weight traits, we find organ growth and

development processes involved at both farms, while response to stimulus is prominent in Spain, and cell adhesion and regulation of phosphorylation in Greece. Overall, these findings not only explain part of the G x E interaction, but also give a genetic insight on how environmental differences affect overall growth and organ weights. This study, therefore, provides insights that can inform future phenotyping and directed breeding towards improved resilience in aquaculture.

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CRediT authorship contribution statement

Xiaofei Yu: Writing – original draft, Writing – review & editing, Formal analysis, Data curation, Methodology. **John W.M. Bastiaansen:** Writing – review & editing, Conceptualization, Data curation, Funding acquisition. **Benan Gulzari:** Writing – review & editing, Formal analysis, Data curation. **Mark Camara:** Writing – review & editing, Conceptualization, Investigation. **Han A. Mulder:** Writing – review & editing, Conceptualization, Investigation. **Hans Komen:** Writing – review & editing, Supervision, Conceptualization, Project administration. **Martien A.M. Groenen:** Writing – review & editing, Supervision, Conceptualization, Resources. **Hendrik-Jan Megens:** Writing – original draft, Writing – review & editing, Supervision, Formal analysis, Investigation.

Declaration of competing interest

Wageningen University & Research advises Galaxidi Marine Farm on their gilthead seabream breeding program.

Data availability

Data will be made available on request.

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References

Ali, A., Al-Tobasei, R., Lourenco, D., Leeds, T., Kenney, B., Salem, M., 2020. Genome-wide identification of loci associated with growth in rainbow trout. BMC Genomics 21, 1–16. https://doi.org/10.1186/s12864-020-6617-x.

Asakawa, S., Kraitavin, W., Yoshitake, K., Igarashi, Y., Mitsuyama, S., Kinoshita, S., Kambayashi, D., Watabe, S., 2019. Transcriptome analysis of yamame (Oncorhynchus masou) in normal conditions after heat stress. Biology 8, 1–15. https://doi.org/10.3390/biology8020021.

Besson, M., Vandeputte, M., van Arendonk, J.A.M., Aubin, J., de Boer, I.J.M., Quillet, E., Komen, H., 2016. Influence of water temperature on the economic value of growth rate in fish farming: the case of sea bass (Dicentrarchus labrax) cage farming in the Mediterranean. Aquaculture 462, 47–55. https://doi.org/10.1016/j.aquaculture.2016.04.030.

Bradshaw, A.D., 2014. Regulation of cell behavior by extracellular proteins. In: Principles of Tissue Engineering. Elsevier, pp. 279–290.

Braz, C.U., Rowan, T.N., Schnabel, R.D., Decker, J.E., 2021. Genome-wide association analyses identify genotype-by-environment interactions of growth traits in Simmental cattle. Sci. Rep. 11, 13335. https://doi.org/10.1038/s41598-021-92455-x.

Bu, D., Luo, H., Huo, P., Wang, Z., Zhang, S., He, Z., Wu, Y., Zhao, L., Liu, J., Guo, J., 2021. KOBAS-i: intelligent prioritization and exploratory visualization of biological functions for gene enrichment analysis. Nucleic Acids Res. 49, W317–W325.

Cingolani, P., Platts, A., Wang, L.L., Coon, M., Nguyen, T., Wang, L., Land, S.J., Lu, X., Ruden, D.M., 2012. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly 6, 80–92.

Couto, A., Enes, P., Peres, H., Oliva-Teles, A., 2008. Effect of water temperature and dietary starch on growth and metabolic utilization of diets in gilthead sea bream (Sparus aurata) juveniles. Comp. Biochem. Physiol. -Part A Mol. Integr. Physiol. 151, 45–50. https://doi.org/10.1016/j.cbpa.2008.05.013.

De-Santis, C., Jerry, D.R., 2007. Candidate growth genes in finfish - where should we be looking? Aquaculture 272, 22–38. https://doi.org/10.1016/j.aquaculture.2007.08.036.

Elalfy, I., Shin, H.S., Negrín-Báez, D., Navarro, A., Zamorano, M.J., Manchado, M., Afonso, J.M., 2021. Genetic parameters for quality traits by non-invasive methods and their G x E interactions in ocean cages and estuaries on gilthead seabream (Sparus aurata). Aquaculture 537. https://doi.org/10.1016/j.aquaculture.2021.736462.

FAO, 2020. The State of World Fisheries and Aquaculture 2020 (Sustainability in Action). Rome.

Fawcett, G.L., Roseman, C.C., Jarvis, J.P., Wang, B., Wolf, J.B., Cheverud, J.M., 2008. Genetic architecture of adiposity and organ weight using combined generation QTL analysis. Obesity 16, 1861–1868. https://doi.org/10.1038/oby.2008.300.

Gao, X., Becker, L.C., Becker, D.M., Starmer, J.D., Province, M.A., 2010. Avoiding the high Bonferroni penalty in genome-wide association studies. Genetic Epidemiology 34, 100–105.

García Hernández, M.P., Cabas, I., Rodenas, M.C., Arizcun, M., Chaves-Pozo, E., Power, D.M., García Ayala, A., 2020. 17α-ethynylestradiol prevents the natural male-to-female sex change in gilthead seabream (Sparus aurata L.). Sci. Rep. 10, 1–15. https://doi.org/10.1038/s41598-020-76902-9.

Gonzalez, C., Gallardo-Hidalgo, J., Yáñez, J.M., 2022. Genotype-by-environment interaction for growth in seawater and freshwater in Atlantic salmon (Salmo salar). Aquaculture 548, 1–5. https://doi.org/10.1016/j.aquaculture.2021.737674.

Gulzari, B., Komen, H., Nammula, V.R., Bastiaansen, J.W.M., 2022. Genetic parameters and genotype by environment interaction for production traits and organ weights of gilthead seabream (Sparus aurata) reared in sea cages. Aquaculture 548, 737555. https://doi.org/10.1016/j.aquaculture.2021.737555.

Guo, J., Wang, A., Mao, S., Xu, X., Li, J., Shen, Y., 2022. Construction of high-density genetic linkage map and QTL mapping for growth performance in black carp (Mylopharyngodon piceus). Aquaculture 549, 737799. https://doi.org/10.1016/j.aquaculture.2021.737799.

Gutierrez, A.P., Lubieniecki, K.P., Davidson, E.A., Lien, S., Kent, M.P., Fukui, S., Withler, R.E., Swift, B., Davidson, W.S., 2012. Genetic mapping of quantitative trait loci (QTL) for body-weight in Atlantic salmon (Salmo salar) using a 6.5K SNP array. Aquaculture 358–359, 61–70. https://doi.org/10.1016/j.aquaculture.2012.06.017.

Hill, W.D., Marioni, R.E., Maghzian, O., Ritchie, S.J., Hagenaars, S.P., McIntosh, A.M., Gale, C.R., Davies, G., Deary, I.J., 2019. A combined analysis of genetically correlated traits identifies 187 loci and a role for neurogenesis and myelination in intelligence. Mol. Psychiatry 24, 169–181.

Hoffman, G.E., 2013. Correcting for population structure and kinship using the linear mixed model: theory and extensions. PloS One 8. https://doi.org/10.1371/journal.pone.0075707.

Honke, N., Shaabani, N., Zhang, D.E., Hardt, C., Lang, K.S., 2016. Multiple functions of USP18. Cell Death and Disease 7, 1–9. https://doi.org/10.1038/cddis.2016.326.

Huang, X., Jiang, Y., Zhang, W., Cheng, Y., Wang, Y., Ma, X., Duan, Y., Xia, L., Chen, Y., Wu, N., Shi, M., Xia, X.Q., 2020. Construction of a high-density genetic map and mapping of growth related QTLs in the grass carp (Ctenopharyngodon idellus). BMC Genomics 21, 1–12. https://doi.org/10.1186/s12864-020-6730-x.

Ivaska, J., Heino, J., 2010. Interplay between cell adhesion and growth factor receptors: from the plasma membrane to the endosomes. Cell Tissue Res. 339, 111–120. https://doi.org/10.1007/s00441-009-0857-z.

Janssen, K., Chavanne, H., Berentsen, P., Komen, H., 2015. Gilthead seabream (Sparus aurata)–current status of selective breeding in Europe. In: Abstract Book of the International Symposium on Genetics in Aquaculture XII. Citeseer, Santiago de Compostela, Spain.

Knibb, W., Gorshkova, G., Gorshkov, S., 1997. Selection for growth in the gilthead seabream, Sparus auratai *L. Israeli*. Journal of Aquaculture 49, 57–66.

Knöll, R., Postel, R., Wang, J., Krätzner, R., Hennecke, G., Vacaru, A.M., Vakeel, P., Schubert, C., Murthy, K., Rana, B.K., 2007. Laminin-α4 and integrin-linked kinase mutations cause human cardiomyopathy via simultaneous defects in cardiomyocytes and endothelial cells. Circulation 116, 515–525.

Kumar, V., Abbas, A.K., Fausto, N., Aster, J.C., 2014. Robbins and Cotran Pathologic Basis of Disease, Professional Edition e-Book. Elsevier Health Sciences.

Leamy, L.J., Pomp, D., Eisen, E.J., Cheverud, J.M., 2002. Pleiotropy of quantitative trait loci for organ weights and limb bone lengths in mice. Physiol. Genomics 2002, 21–29. https://doi.org/10.1152/physiolgenomics.00018.2002.

Lee, J.H., Song, S.Y., Kim, M.S., Yoo, N.J., Lee, S.H., 2016. Frameshift mutations of a tumor suppressor gene ZNF292 in gastric and colorectal cancers with high microsatellite instability. Apmis 124, 556–560. https://doi.org/10.1111/apm.12545.

Ligges, U., Mächler, M., 2002. Scatterplot3d-an r Package for Visualizing Multivariate Data. Technical Report.
Lipkin, S.M., Näär, A.M., Kalla, K.A., Sack, R.A., Rosenfeld, M.G., 1993. Identification of a novel zinc finger protein binding a conserved element critical for Pit-1-dependent growth hormone gene expression. Genes Dev. 7, 1674–1687.
Loukovitis, D., Sarropoulou, E., Tsigenopoulos, C.S., Batargias, C., Magoulas, A., Apostolidis, A.P., Chatziplis, D., Kotoulas, G., 2011. Quantitative trait loci involved in sex determination and body growth in the gilthead sea bream (Sparus aurata L.) through targeted genome scan. PLoS ONE 6. https://doi.org/10.1371/journal.pone.0016599.
Loukovitis, D., Sarropoulou, E., Batargias, C., Apostolidis, A.P., Kotoulas, G., Tsigenopoulos, C.S., Chatziplis, D., 2012. Quantitative trait loci for body growth and sex determination in the hermaphrodite teleost fish Sparus aurata L. Anim. Genet. 43, 753–759. https://doi.org/10.1111/j.1365-2052.2012.02346.x.
Lu, K., Xiao, Z., Jian, H., Peng, L., Qu, C., Fu, M., He, B., Tie, L., Liang, Y., Xu, X., Li, J., 2016. A combination of genome-wide association and transcriptome analysis reveals candidate genes controlling harvest index-related traits in Brassica napus. Sci. Rep. 6, 1–13. https://doi.org/10.1038/srep36452.
Mayer, P., Estruch, V.D., Jover, M., 2012. A two-stage growth model for gilthead sea bream (Sparus aurata) based on the thermal growth coefficient. Aquaculture 358–359, 6–13. https://doi.org/10.1016/j.aquaculture.2012.06.016.
Mengistu, S.B., Mulder, H.A., Benzie, J.A.H., Khaw, H.L., Megens, H.-J., Trinh, T.Q., Komen, H., 2020. Genotype by environment interaction between aerated and non-aerated ponds and the impact of aeration on genetic parameters in Nile tilapia (Oreochromis niloticus). Aquaculture 529, 735704. https://doi.org/10.1016/j.aquaculture.2020.735704.
Mohindra, V., Chowdhury, L.M., Chauhan, N., Maurya, R.K., Jena, J.K., 2022. Transcriptome analysis revealed hub genes for muscle growth in Indian major carp, Catla catla (Hamilton, 1822). Genomics 114, 110393.
Moreira, G.C.M., Salvian, M., Boschiero, C., Cesar, A.S.M., Reecy, J.M., Godoy, T.F., Ledur, M.C., Garrick, D., Mourão, G.B., Coutinho, L.L., 2019. Genome-wide association scan for QTL and their positional candidate genes associated with internal organ traits in chickens. BMC Genomics 20, 1–15. https://doi.org/10.1186/s12864-019-6040-3.
Neuschl, C., Brockmann, G.A., Knott, S.A., 2007. Multiple-trait QTL mapping for body and organ weights in a cross between NMRI8 and DBA/2 mice. Genet. Res. 89, 47–59. https://doi.org/10.1017/S001667230700852X.
Neves, H.H.R., Carvalheiro, R., Queiroz, S.A., 2012. A comparison of statistical methods for genomic selection in a mice population. BMC Genet. 13, 1–24. https://doi.org/10.1186/1471-2156-13-100.
Newell, M.A., Cook, D., Tinker, N.A., Jannink, J.-L., 2011. Population structure and linkage disequilibrium in oat (Avena sativa L.): implications for genome-wide association studies. Theor. Appl. Genet. 122, 623–632.
Nie, Z., Wang, B., Zhang, Z., Jia, Z., Xu, R., Wang, H., Zhou, W., Gong, Y., 2022. Genome-wide identification of the traf gene family in yellow catfish (Pelteobagrus fulvidraco) and analysis of their expression in response to bacterial challenge. J. Fish Biol. 0–1 https://doi.org/10.1111/jfb.15126.
Peñaloza, C., Manousaki, T., Franch, R., Tsakogiannis, A., Sonesson, A.K., Aslam, M.L., Allal, F., Bargelloni, L., Houston, R.D., Tsigenopoulos, C.S., 2021. Development and testing of a combined species SNP array for the European seabass (Dicentrarchus labrax) and gilthead seabream (Sparus aurata). Genomics 113, 2096–2107. https://doi.org/10.1016/j.ygeno.2021.04.038.
Price, A.L., Zaitlen, N.A., Reich, D., Patterson, N., 2010. New approaches to population stratification in genome-wide association studies. Nat. Rev. Genet. 11, 459–463. https://doi.org/10.1038/nrg2813.
Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., De Bakker, P.I.W., Daly, M.J., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81, 559–575.
Sae-Lim, P., Kause, A., Mulder, H.A., Martin, K.E., Barfoot, A.J., Parsons, J.E., Davidson, J., Rexroad, C.E., Van Arendonk, J.A.M., Komen, H., 2013. Genotype-by-environment interaction of growth traits in rainbow trout (Oncorhynchus mykiss): a continental scale study. J. Anim. Sci. 91, 5572–5581. https://doi.org/10.2527/jas.2012-5949.
Salem, M., Al-Tobasei, R., Ali, A., Lourenco, D., Gao, G., Palti, Y., Kenney, B., Leeds, T.D., 2018. Genome-wide association analysis with a 50K transcribed gene SNP-chip identifies QTL affecting muscle yield in rainbow trout. Front. Genet. 387.
Sola, L., Moretti, A., Crosetti, D., Karaiskou, N., Magoulas, A., Rossi, A.R., et al., 2007. Gilthead Seabream—*Sparus aurata*. Genet. Impact Aquac. Act. Nativ. Popul 47.
Soukissian, T.H., Denaxa, D., Karathanasi, F., Prospathopoulos, A., Sarantakos, K., Iona, A., Georgantas, K., Mavrakos, S., 2017. Marine renewable energy in the Mediterranean Sea: status and perspectives. Energies 10. https://doi.org/10.3390/en10101512.
Su, H., Ma, D., Zhu, H., Liu, Z., Gao, F., 2020. Transcriptomic response to three osmotic stresses in gills of hybrid tilapia (Oreochromis mossambicus female × O. Urolepis hornorum male). BMC Genomics 21, 1–20. https://doi.org/10.1186/s12864-020-6512-5.
Sumpter, J.P., 1992. Control of growth of rainbow trout (Oncorhynchus mykiss). Aquaculture 100, 299–320.
Tsai, H.Y., Hamilton, A., Guy, D.R., Tinch, A.E., Bishop, S.C., Houston, R.D., 2015. The genetic architecture of growth and fillet traits in farmed Atlantic salmon (Salmo salar). BMC Genet. 16 https://doi.org/10.1186/s12863-015-0215-y.
Turner, S.D., 2014. Qqman: an R package for visualizing GWAS results using QQ and Manhattan plots. Biorxiv 5165.
Wadugu, B., Kühn, B., 2012. The role of neuregulin/ErbB2/ErbB4 signaling in the heart with special focus on effects on cardiomyocyte proliferation. Am. J. Physiol. Heart Circ. Physiol. 302, 2139–2147. https://doi.org/10.1152/ajpheart.00063.2012.
Wang, L., Jia, S., Zhang, Y., Jiang, S., Chen, Y., Chen, J., Yu, M., Zhang, L., Qiao, Z., Li, X., 2021. Construction of three high-density genetic linkage maps and dynamic qtl mapping of growth traits in yellow river carp (Cyprinus carpio haematopterus). Curr. Issues Mol. Biol. 43, 2276–2288. https://doi.org/10.3390/cimb43030160.
Wickham, H., Chang, W., Wickham, M.H., 2016. Package "ggplot2." Creat. elegant data Vis. using Gramm. Graph. Version 2, pp. 1–189.
Xu, M., Jiang, L., Shen, K.N., Wu, C., He, G., Hsiao, C., 2016. Transcriptome response to copper heavy metal stress in hard-shelled mussel (Mytilus coruscus). Genom. Data 7, 152–154. https://doi.org/10.1016/j.gdata.2015.12.010.
Yandell, M., Moore, B., Salas, F., Mungall, C., MacBride, A., White, C., Reese, M.G., 2008. Genome-wide analysis of human disease alleles reveals that their locations are correlated in paralogous proteins. PLoS Comput. Biol. 4 https://doi.org/10.1371/journal.pcbi.1000218.
Yin, L., Zhang, H., Tang, Z., Xu, J., Yin, D., Zhang, Z., Yuan, X., Zhu, M., Zhao, S., Li, X., 2021. rMVP: a memory-efficient, visualization-enhanced, and parallel-accelerated tool for genome-wide association study. Genom. Proteom. Bioinforma. 19 (4), 619–628.
Yoshida, G.M., Yáñez, J.M., 2021. Multi-trait GWAS using imputed high-density genotypes from whole-genome sequencing identifies genes associated with body traits in Nile tilapia. BMC Genomics 22, 1–13. https://doi.org/10.1186/s12864-020-07341-z.
Yu, X., Megens, H.J., Mengistu, S.B., Bastiaansen, J.W.M., Mulder, H.A., Benzie, J.A.H., Groenen, M.A.M., Komen, H., 2021a. Genome-wide association analysis of adaptation to oxygen stress in Nile tilapia (Oreochromis niloticus). BMC Genomics 22, 1–13. https://doi.org/10.1186/s12864-021-07486-5.
Yu, H.J., Xiao, G.L., Zhao, Y.Y., Wang, X.X., Lan, R., 2021b. Targeting mitochondrial metabolism and RNA polymerase POLRMT to overcome multidrug resistance in cancer. Front. Chem. 9, 1–7. https://doi.org/10.3389/fchem.2021.775226.
Yu, X., Mengistu, S.B., Mulder, H.A., Palstra, A.P., Benzie, J.A.H., Trinh, T.Q., Groenen, M.A.M., Komen, H., Megens, H.-J., 2022. Quantitative trait loci controlling swimming performance and their effect on growth in Nile tilapia (Oreochromis niloticus). Aquaculture 560, 738522.
Zhao, K., Tung, C.-W., Eizenga, G.C., Wright, M.H., Ali, M.L., Price, A.H., Norton, G.J., Islam, M.R., Reynolds, A., Mezey, J., 2011. Genome-wide association mapping reveals a rich genetic architecture of complex traits in Oryza sativa. Nat. Commun. 2, 467.
Zhou, X., Stephens, M., 2012. Genome-wide efficient mixed-model analysis for association studies. Nat. Genet. 44, 821–824.
Zhou, Y., Zhou, B., Pache, L., Chang, M., Khodabakhshi, A.H., Tanaseichuk, O., Benner, C., Chanda, S.K., 2019. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. Nat. Commun. 10, 1–10.