Aquaculture Reports 24 (2022) 101178

Contents lists available at ScienceDirect

Aquaculture Reports

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

# Genome Wide Association (GWAS) Analysis and genomic heritability for parasite resistance and growth in European seabass

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ARTICLE INFO

*Keywords:*
European seabass (*Dicentrarchus labrax*)
*Lernanthropus kroyeri*
Growth
Body weight
MedFish SNP-array
GWAS

ABSTRACT

There is an increasing demand for the breeding programs to focus on resistance against diseases since the treatments or vaccinations increase production costs and, in some cases, they are not available at all. Most of the studies focus on virus and bacterial diseases, but limited information is available for parasitic diseases in European seabass. A sample of infected fish (985) with *Lernanthropus kroyeri* and their parents were genotyped using the recently developed MedFISH SNP array. The sample was selected (selective genotyping) in order to capture the genetic variation of the resistance against the *Lernanthropus kroyeri.* Body weight was recorded at different growth stages in the population. The genetic parameters were estimated for body weight and parasite count, using Restricted Estimation Maximum Likelihood (REML) methods utilizing a Genomic Relationship Matrix (GRM). Additionally, univariate GWAS, multitrait GWAS and Regional Heritability Mapping (RHM) were performed in order to identify genomic areas related to host resistance and growth. A moderate genomic heritability was detected for growth at sea cage (0.39) and for weight at 2, 4, 6 months after the fish were transferred to the sea cage (0.46–0.41). A moderate to low genomic correlation between growth (body weight/growth at the sea cage) and parasites was observed (0.36–0.21). GWAS results indicated two putative QTL affecting host resistance to *Lernanthropus kroyeri* count, each one of them explaining approximately 2% of the phenotypic variation.

## 1. Introduction

The European seabass (*Dicentrarchus labrax*) was the first marine non-salmonid species which was cultured for commercial consumption in Europe, while nowadays it is one of the most important species cultured in the Mediterranean Sea (Antonelli et al., 2016). Additionally, the total production reached approximately 212,977 tons in 2019 with a total value at first sale exceeding 1064.9 million euros (APROMAR, 2020). The increasing fish production favored the spread of infection diseases between farmed and wild populations (Antonelli et al., 2012; Whittington et al., 2002) and, therefore, the necessity to improve disease resistance and robustness in fish is becoming rapidly popular in breeding programs as a selection objective (Chavanne et al., 2016; Rauw, 2016). In European seabass, recent studies have concentrated on resistance against viruses such as VNN (Viral Nervous Necrosis) (Griot et al., 2021; Palaiokostas et al., 2018), since high mortality has been reported in infected fish (Palaiokostas et al., 2018), however, parasitic diseases can also be the reason for high mortalities and additionally could lead to low-quality of the final product, as well as, to afflict the welfare of the fish. Consequently, high economic losses can be observed due to

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https://doi.org/10.1016/j.aqrep.2022.101178
Received 28 December 2021; Received in revised form 15 April 2022; Accepted 16 May 2022

parasitic infestation (Fioravanti eal., 2020; Aly et al., 2020; Antonelli et al., 2012; Golomazou et al., 2014). *Lernanthropus* de Blain ville, 1822, is a genus of copepods that parasite on several host species. In the Mediterranean Sea, *L. kroyeri* affects the European seabass reared in the sea cages (Sethi et al., 2018; Yardimci and Pekmezci, 2012). The parasite attaches on the gills of the fish influencing the breathing process when large number of parasites are attached. Generally, infestation increases when fish are raised in warm waters (Cabral et al., 1984), and leads to high economic losses (Sobhana, 2009). Indeed, such high mortality (87%) was recorded in the first year of the trials for parasite resistance (Papapetrou et al., 2021), from which trials the selected sample of this study was drawn.

Nevertheless, for some diseases or parasite infestations, treatments have an increasing cost while, in others, vaccines are not available yet (Athanasopoulou et al., 2009; Čolak et al., 2021; Tokşen et al., 2010). In such cases, selective breeding to increase resistance can be considered a powerful tool to improve productivity, reduce treatment cost and ameliorate farmed fish welfare (Aslam et al., 2020). However, the effectiveness of the selection of candidates with better resistance to parasite infestation, is influenced by the heritability of the trait (Aslam et al., 2020). The first approach to interpret the inheritance of the *L. kroyeri* count, as well as, that of another parasite (*Diplectanum aequans*) in European seabass, has been attempted by Papapetrou et al. (2021) when approximately 1576 fish that were alive at the end of the trial [from the total 2425 participated in the trial initially (36% mortality)], were used to investigate the heritability of parasite count, using Restricted Estimation Maximum Likelihood (REML) methods that were utilizing the pedigree relationship matrix of the population. The heritability of the parasite count was estimated at 0.28 for *L. kroyeri* and 0.2 for *D. aequans*.

Apart from the Best Linear Unbiased Prediction (BLUP) approach (Henderson, 1977), the use of genome-wide association study (GWAS) analysis provided generally superior results in aquaculture with the identification of QTL affecting disease resistance (Houston et al., 2020). In Atlantic salmon, studying the resistance against Amebic gill disease (AGD), two suggestive QTL were identified on chromosome 18, (Robledo et al., 2018), while for the salmon louse, putative SNPs affecting the parasite number were detected in chromosomes 9 and 22 (Houston et al., 2014). Apart from testing the significance of each SNP individually using GWAS, genomic selection (GS) can be applied, in which all the available genomic information is exploited, since the relationship matrix is estimated based on the genotypes instead of the pedigree information. Generally, the advantages in the application of GS are the increasing genetic gain, the higher accuracy of selection and the smaller generation intervals that can be achieved (Hayes et al., 2009; Hickey et al., 2017; Meuwissen et al., 2013, 2001; Palaiokostas and Houston, 2017).

The aim of the study was to investigate the genomic heritability of parasite count for *L. kroyeri* using a selective sample, the genomic versus phenotypic correlation between *L. kroyeri*, growth at sea cages and body weight after six months in the sea cages and, moreover, if possible, to identify QTL affecting the above traits through GWAS and applying the recently developed 30 K MedFISH SNP array (Peñaloza et al., 2020) in European seabass.

## 2. Materials and methods

### 2.1. Selective breeding of fish

Initially, 2425 European sea bass (*D. labrax*) juveniles, originating from a commercial breeding program, were individually pit-tagged, fin-clipped and challenged with the copepod *L. kroyeri* through natural cohabitation in an environment heavily infested with the parasite (Sagiada site) [the trial is more extensively described in Papapetrou et al. (2021)]. From this population, 1576 fish were found alive in the sea cage at the end of the trial (Papapetrou et al., 2021) and were utilized to apply a selective genotyping scheme, where a subgroup of fish (495) was selected based on the discordant Estimated Breeding Values (EBVs) which were estimated for *L. kroyeri* using the BLUP approach (approximately 50% of the selected sample). Thus, fish with the lowest [i.e 248 fish (preferable, low parasite count)] and the highest [i.e. 247 fish (non-preferable, high parasite count)] EBVs were selected. The rest of the fish of the selected subgroup [i.e. 490 fish (approximately 50% of the rest of the selected sample)] were selected based on the within family variation of parasite count of *L. kroyeri*. These 490 fish belong to 34 families (out of the total 92) with the larger within family variance for parasite count. The within family variance of the parasite count ranged from 19.4 to 325.6 while the family average parasite count was 28.9 (19.6–44.3) and the range of offspring per family was between 9 and 19. The above selective genotyping methods were used in order to capture both the within and the between family variation of the parasite count in our genotyping population. Selective genotyping is a method generally used for the selection of individuals from a population in order to reduce the cost of QTL (Chatziplis and Haley, 2000; McClure et al., 2012; Palombo et al., 2018; Xing and Xing, 2009) detection studies without adverse effects on the power of detection of the study. Finally, the selected 985 fish and their 91 parents (58 sires and 33 dams), were successfully genotyped with the 30 K Affymetrix MedFISH SNP-array (Peñaloza et al., 2020).

### 2.2. Data collection

More details concerning the parasite cohabitation trials are available in Papapetrou et al. (2021). Parasites of *L. kroyeri* on the gills of the fish, at the end of the trial, and body weight at different growth stages were recorded. The parasite resistance trait was defined according to the parasite count (PC), which was found on each individual fish six months after the transportation to the sea cage. Since all fish were infected by *L. kroyeri*, counts of parasite was analyzed as a quantitative variable. However, a log-transformation of the variable PC, using the formula ln (PC + 25) (lnPC) (Bishop et al., 1996; Vagenas et al., 2007), was also used in order to correct for any skewness of the distribution (Supplementary material 1, Fig. 1).

Focusing on growth traits, body weight was initially measured before transferring the fish to the sea cage (Weight 1). Thereafter, body weight was also measured after two months (W2M), four months (W4M), and

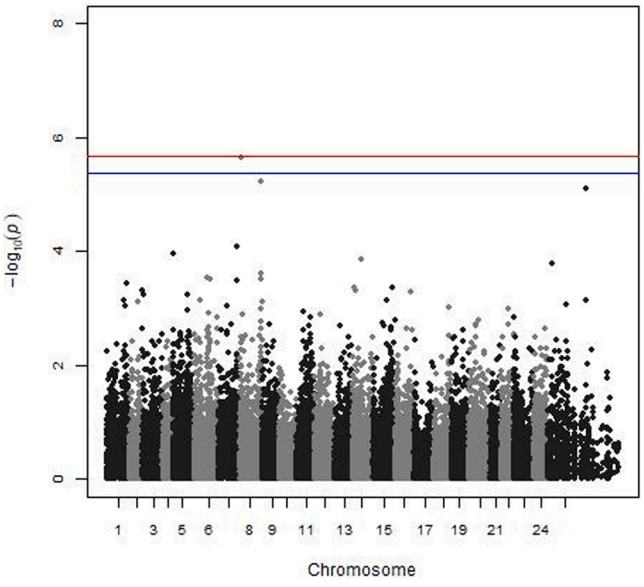


**Fig. 1.** Manhattan plot from GWAS analysis for *Lernanthropus kroyeri*(PC). Red line illustrates the threshold for a = 0.05 after bonferroni correction and blue line for a = 0.1.

six months (W6M) after the placement of the fish in the sea cages. In order to estimate the absolute growth in the sea cage (GAS), the difference between W6M and the weight before the fish were transferred in the sea cage was calculated.

### 2.3. Quality control

Quality control was performed in 30K MedFISH SNP array (Peñaloza et al., 2020), using PLINK software (Purcell et al., 2007). Genomic data were filtered based on the following criteria: SNP were removed when call rate was lower than 90%, moreover, SNP with Minor Allele Frequency (MAF) lower than 0.05 and deviation from the Hardy-Weinberg equilibrium (HWE) lower than $10^{-6}$ were also removed.

### 2.4. Estimation of genomic heritability and correlations

Genomic heritability of parasite count, body weight and growth in the sea cage, along with their genomic/phenotypic correlations between them in the selected sample, were estimated using Restricted Estimation of Maximum Likelihood method. A multivariate animal model utilizing the W2M, W4M, W6M, *L. kroyeri* count (PC) and GAS was used in order to estimate genomic heritability and genomic parameters:

$$\boldsymbol{Y} = \mu + \boldsymbol{Zu} + \boldsymbol{e}$$

where $Y$ corresponds to the matrix of W6M, *L. kroyeri* (PC) and GAS, the $\mu$ is the mean of the above traits, $Z$ is the incidence matrix, $u$ is the additive genetic effect utilizing the GRM and it is illustrated as $\sim N(0, G\sigma_a^2)$ ($G$ is the GRM and $\sigma_a^2$ is the additive variance). Finally, the $e$ is the residual. All the analyzes were performed using AIREMLF90 (Aguilar et al., 2014). For the *L. kroyeri* transformed Parasite Count (as lnPC), the same model was used, but instead of all the variables (5), all the possible pairs between the lnPC and body weight or growth were used due to convergence issues of the multivariate model.

### 2.5. Linkage disequilibrium

The linkage disequilibrium was estimated in genomic data using the pairwise correlation ($r^2$) between all pairs of SNPs in each chromosome (Vallejo et al., 2018; Wall and Pritchard, 2003), using PLINK software (Purcell et al., 2007). The average of pairwise correlation of all pairs of each chromosome (Vallejo et al., 2018) was calculated using R (R Core Team, 2020). The linkage disequilibrium estimation was performed, only in chromosomes 1-24, while chr25 was not included in the analysis, since it included all the unstructured SNP (unlinked-unmapped SNP).

### 2.6. GWAS

#### 2.6.1. Univariate GWAS for parasite resistance

A univariate animal model using parasite count for *L. kroyeri* (PC) as a depended variable and a univariate animal model using log transformation of the parasite count (lnPC) as a depended variable, were performed. In both models, the genomic relationship matrix among candidates was used in order to estimate the polygenic component which was fitted as a random effect in the analysis. No fixed effect was present and, therefore, none was fitted in both models. All GWAS analyses were performed using GEMMA (Zhou and Stephens, 2012). The proportion of phenotypic variance (PVE) explained by a given SNP was estimated as described in Shim et al. (2015).

#### 2.6.2. Multitrait GWAS for growth

A multitrait linear animal model was utilized to identify association between SNPs and growth in European seabass using GEMMA software (Zhou and Stephens, 2014). In the model, the weight after 2, 4 and 6 months at the sea cages (W2M, W4M and W6M, resp.) combined with the growth at the sea cage (GAS), were used. In addition, Mahalanobis distance was estimated between the three body weights (W2M, W4M and W6M) and growth at sea cages (GAS) in order to remove fish which deviated from the multivariate normality, thus, fish with p-value less than 0.01 were removed from the dataset (Shim et al., 2015). The above analysis was performed in R software (R Core Team, 2020).

### 2.7. Bonferroni correction

Haplotype blocks of the genomic data were constructed using Haploview (Barrett et al., 2005) in the genotyped population. SNPs in sequence were considered a haplotype block if their D' value was higher than 0.98, which is evidence of strong Linkage Disequilibrium between the SNPs (Gabriel et al., 2002), while the SNPs in chr25 did not participate in the present analysis since they were considered unstructured.

An alternative Bonferroni correction was used for statistical significance inference, in which, instead of correcting based on the total number of SNPs, the number of the independent SNPs (not included in haplotypes blocks) combined with the total number of haplotype blocks were used to perform the correction (Nyholt, 2004). The SNPs in chr25 were added to the independent SNPs.

### 2.8. Regional heritability mapping for disease resistance

Regional heritability mapping for *L. kroyeri* count (PC) and for their lnPC, using a 20 SNP size region with no overlapping, was performed (Caballero et al., 2015; Riggio et al., 2013). Thus, the genome panel was split into 1297 non-overlapping windows of 20 consecutive SNPs and the windows which included SNPs from two chromosomes were removed from the analysis, thus 1275 remained. Then, the following model was used with REACTA software (Cebamanos et al., 2014):

$$Y = \mu + Zu_i + Zu_{(-i)} + e$$

where $Y$ is the vector of PC or lnPC, $\mu$ is the mean, $u_i$ is the additive genetic effect for the window i following $\sim N(0, G_i\sigma_{ai}^2)$ ($G_i$ is the regional genomic relationship matrix of the window i using only SNPs in the region and $\sigma_{ai}^2$ is the additive variance of the window i), $u_{(-i)}$ is the additive genetic effect of the rest genome (without the window i), using the GRM, and it is described as $\sim N(0, G\sigma_{a(-i)}^2)$ ($G$ is the GRM and $\sigma_{a(-i)}^2$ is the additive variance), $Z$ is the incidence matrix and $e$ is the random residual. The window/region ranged from 1 to 1275.

The Bonferroni correction was used (Bonferroni, 1936) for statistical significance inference using the total number of regions (1275 regions) instead of the total number of SNPs. The Likelihood Ratio Test (LRT) was used to assess the significance of the effect of each of the 20 SNP size window. The genome-wide significance threshold ($a = 0.05$ and $a = 0.1$) after Bonferroni correction was 3.92157E-05 and 7.84314E-05 and the -log10($p$) was 4.40 and 4.10, respectively. Finally, the unstructured SNP in chr25 were excluded from the analysis.

## 3. Results

For the selectively genotyped sample, the average number of *L. kroyeri* attached to the gills of the fish was 25.04 ± 13.26, ranging

**Table 1**
Descriptive statistics of the phenotypes.

| | Weight1 (g) | W2M (g) | W4M (g) | W6M (g) | PC | GAS (g) |
|---|---|---|---|---|---|---|
| **Average ± SD** | 19.4 ± 4.42 | 53.5 ± 10.85 | 117.7 ± 23.80 | 174.7 ± 38.60 | 25.4 ± 13.13 | 155.3 ± 36.93 |
| **Number of fish** | 984 | 982 | 984 | 975 | 985 | 974 |
| **Max** | 34.4 | 82.00 | 179.00 | 290.00 | 84.00 | 266.70 |
| **Min** | 8.0 | 22.00 | 26.00 | 40.00 | 1.00 | 25.30 |

from 1 to 84 parasites (Table 1). The estimated genomic heritability of *L. kroyeri* count for PC was 0.71 while for lnPC was 0.75 (Tables 2, 3), which was overestimated using the selecting strategy of the sample. Moreover, the genomic heritability of the body weight in the studied subpopulation at 6 months in the sea cage (W6M) was 0.41 and for growth at sea (GAS) the heritability was estimated at 0.39. For body weight at 2 and 4 months in the sea cage, the heritability was 0.46 and 0.43, respectively. The genomic and phenotypic correlation between the measurements of body weight was high (0.71–0.96, Table 2). Growth at sea shows higher genomic and phenotypic correlation with the last two measurements of body weight since it was estimated using them.

A low genomic correlation between body weight (W6M) and parasite count was found (0.23). However, moderate genomic correlations with the body weight at W2M and W4M (equal to 0.36 and 0.33) and low estimations for the phenotypic correlations (equal to 0.25 and 0.21) with the *L. kroyeri* (PC) were observed (Table 2). In addition, the genomic correlation between growth at sea (GAS) and PC was also low, i.e. 0.21. Similarly, the respective phenotypic correlations between them were also low (0.18–0.08) (Table 2). In Table 3, using the transformed *L. kroyeri* (lnPC), showed approximately similar estimates in terms of genomic and phenotypic correlations with the Parasite Count (PC).

In terms of the number of SNP utilized in the study, from the 30K Affymetrix MedFISH SNP-array (Peñaloza et al., 2020), 26,821 SNPs remained after quality control for further analyses. The average whole-genome linkage disequilibrium was 0.07, while the average LD per chromosome ranged from 0.029 (chr15) to 0.33 (chr21) (Table 5). Using 26,821 SNPs, 2141 haplotype blocks were constructed including 4975 SNPs (data not shown). Thus, the Bonferroni correction was calculated using all the individual 21,846 SNP (which were not used in the construction of haplotype blocks) and the 2141 blocks that were constructed (thus 23,987 SNPs and haplotypes instead of 26,821 SNPs).

Two putative QTL affecting *L. kroyeri* parasite count were detected in chr8 (LG7) which explained 2.06% and 1.89% of the phenotypic variance, respectively (Fig. 1). These QTL were also identified when the lnPC was used as the variable, explaining 2.07% and 1.95% of the phenotypic variance (Fig. 2). The average parasite count for *L. kroyeri* per genotype for AX-373127007 and AX-373218583 is illustrated in Table 4. Different average parasite count was found on the gills per fish genotype; the parasites count of genotype CC for SNP AX-373127007 was 20.77 while the parasites count of genotype TT was 26.86. Focusing on SNP AX-373218583, average parasite count for the CC genotype was 20.77 while for genotype TT it was 33.23.

When a multitrait GWAS was performed (using in the model W2M, W4M, W6M and GAS), a QTL (AX-172292596) was identified in chr14 (LG13) but a lack of trailing from the remaining SNP was noted (Fig. 3), as was the case in the Manhattan plots in Figs. 1 and 2. Moreover, a suggestive QTL (AX-172277062) in chr15 (LG14) affecting the above bodyweight and growth traits, was also identified.

Regarding Regional Heritability Mapping analysis, the results are illustrated on Figs. 4 and 5 for the *L. kroyeri* as PC and as lnPC,

**Table 3**
Genomic parameters using bivariate models between lnPC and body weight or growth. Standard error is in parenthesis.

| | Heritability | Genetic correlation | Phenotypic correlation |
|---|---|---|---|
| W2M | 0.46 (0.05) | 0.36 (0.09) | 0.24 (0.04) |
| W4M | 0.45 (0.05) | 0.33(0.09) | 0.21 (0.04) |
| W6M | 0.41 (0.05) | 0.24 (0.1) | 0.12 (0.04) |
| GAS | 0.38 (0.05) | 0.19 (0.1) | 0.09 (0.04) |
| lnPC | 0.75 (0.04) | | |

**Table 4**
Descriptive statistic of the putative QTL affecting the host resistance against *Lernanthropus kroyeri*.

| SNP | AX-373127007 | | AX-373218583 | |
|---|---|---|---|---|
| Genotypes | Average *L. kroyeri* count (PC) | Number of Offspring | Average *L. kroyeri* count (PC) | Number of Offspring |
| CC | 20.77 | 59 | 21.33 | 387 |
| CT | 23.54 | 346 | 26.24 | 450 |
| TT | 26.86 | 574 | 33.23 | 147 |

**Table 2**
Genomic heritability (bold) on the diagonal and genomic (blue)/phenotypic (green) correlations of the traits above and below the diagonal, respectively. Standard error is in parenthesis.

| Ratio | W6M | *L.kroyeri*(PC) | GAS |
|---|---|---|---|
| **W6M** | **0.41 (0.05)** | 0.23 (0.1) | 0.99 (0.0) |
| ***L. kroyeri*(PC)** | 0.12 (0.04) | **0.71 (0.04)** | 0.19 (0.1) |
| **GAS** | 0.99 (0.0) | 0.08 (0.08) | **0.39 (0.05)** |

| | W2M | W4M | W6M | *L. kroyeri* (PC) | GAS |
|---|---|---|---|---|---|
| **W2M** | **0.46 (0.05)** | 0.92 (0.02) | 0.81 (0.05) | 0.36 (0.09) | 0.55 (0.05) |
| **W4M** | 0.82 (0.01) | **0.43 (0.05)** | 0.96 (0.01) | 0.33 (0.09) | 0.94 (0.2) |
| **W6M** | 0.71 (0.02) | 0.89 (0.01) | **0.41 (0.05)** | 0.25 (0.04) | 0.99 (0.00) |
| **PC** | 0.25 (0.04) | 0.21 (0.04) | 0.12 (0.04) | **0.72 (0.04)** | 0.21 (0.10) |
| **GAS** | 0.67 (0.02) | 0.87 (0.01) | 0.99 (0.00) | 0.08 (0.04) | **0.39 (0.05)** |

**Table 5**
Linkage disequilibrium per chromosome.

| LG | Chr | Number of SNPs | Average LD |
|---|---|---|---|
| LG1A | 1 | 1249 | 0.036 |
| LG1B | 2 | 842 | 0.041 |
| LG2 | 3 | 1107 | 0.039 |
| LG3 | 4 | 642 | 0.061 |
| LG4 | 5 | 1206 | 0.040 |
| LG5 | 6 | 1310 | 0.034 |
| LG6 | 7 | 1232 | 0.049 |
| LG7 | 8 | 1243 | 0.049 |
| LG8 | 9 | 1042 | 0.041 |
| LG9 | 10 | 1034 | 0.054 |
| LG10 | 11 | 1078 | 0.036 |
| LG11 | 12 | 1200 | 0.035 |
| LG12 | 13 | 1059 | 0.039 |
| LG13 | 14 | 1251 | 0.330 |
| LG14 | 15 | 1297 | 0.029 |
| LG15 | 16 | 1144 | 0.330 |
| LG16 | 17 | 1080 | 0.048 |
| LG17 | 18 | 1098 | 0.041 |
| LG19 | 19 | 1109 | 0.034 |
| LG20 | 20 | 1198 | 0.035 |
| LG24 | 21 | 719 | 0.330 |
| LG18–21 | 22 | 765 | 0.042 |
| LG22–25 | 23 | 1131 | 0.040 |
| LGX | 24 | 898 | 0.041 |
| UN | 25 | 887 | |
| | | 26,821 | 0.070 |

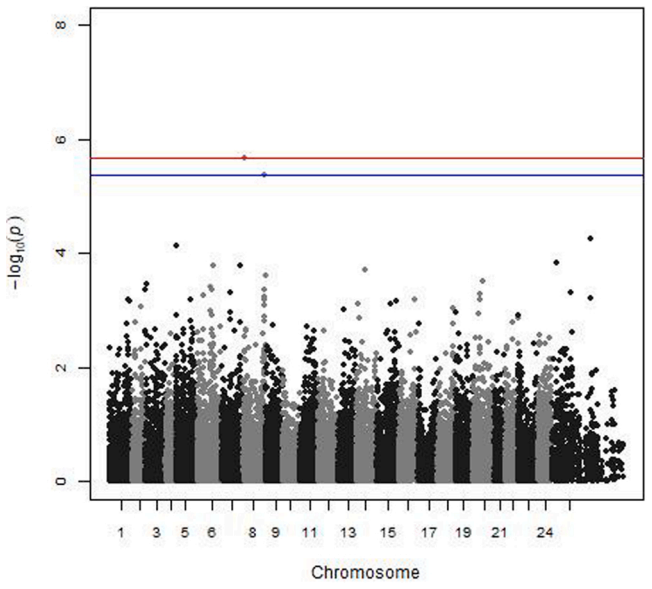


**Fig. 2.** Manhattan plot from GWAS analysis for log transformation of *Lernanthropus kroyeri* count [lnPC]. Red line illustrates the threshold for a = 0.05 after bonferroni correction and blue line for a = 0.1.

respectively. The highest peak appeared in chromosomes 10, 5 and 2 using PC or lnPC while in chr8, in which two putative QTL were revealed during the univariate GWAS, there was no trailing at all. However, no statistically significant association between 20 SNP-size regions and parasite count of *L. kroyeri* (PC) nor with its lnPC, was observed.

## 4. Discussion

A selective genotyping with the recently developed 30K MedFISH SNP array (Peñaloza et al., 2020) was performed into a population of the European seabass, to improve our knowledge on the species resistance against *L. kroyeri* and also on growth. Genomic heritability and genomic/phenotypic correlation between parasites and growth were estimated, while a first approach to identify QTLs affecting growth and host resistance was performed in our selected sample.

### 4.1. Estimation of genomic heritability and genomic parameters

Ectoparasite infections from *Caligus rogercresseyi*, *Lepeophtheirus salmonis*, *Ichthyophthirius multifiliis, Neoparamoeba perurans* have been studied in the Atlantic salmon, providing evidence of moderate heritability in host resistance (from 0.20 to 0.36) (English et al., 2019; Gjerde et al., 2011; Lhorente et al., 2012; Lira et al., 2020; Robledo et al., 2018; Slinger et al., 2021; Taylor et al., 2007). In Mediterranean species, such as the European seabass, limited information is available for the genetic architecture of the host resistance against any parasite including the *L. kroyeri* parasite. Papapetrou et al. (2021) estimated a low heritability of *L. kroyeri* (0.28), using the total population (n = 1576) and a pedigree relationship matrix. In our study, a selectively genotyped sample of this population was analyzed utilizing a Genomic Relationship Matrix (GRM) and the genomic heritability of *L. kroyeri* was estimated (0.71 and 0.75 for PC and lnPC, respectively). This estimate of heritability was considerably higher than the estimate using the traditional pedigree approach (utilizing the Pedigree Relationship Matrix (PRM)) with the whole data set, since a selective sample was used to estimate it in the present study. However, such difference between the heritability estimates could be justifiable since the sample analyzed in our study was selected in such a way in order to capture most of the variation for parasite count (PC or lnPC) and maximize the differences between individuals and sibs, and hence, affect both genetic and phenotypic variation of the trait in the sample. Discordant EBVs (estimated using BLUP approach) and high within family variance of *L. kroyeri* count were the selection criteria for the selective genotyping method. Consequently, the overestimation of heritability of *L. kroyeri* using GRM is probably caused by both the sample size and the selective methods used to draw a sample for genotyping. Indeed, when the genetic heritability (based on the pedigree relationship matrix) was estimated only in the genotypic sample [0.75, (Supplementary material 1)], it was found similar to the genomic heritability (0.71 or 0.75). In the present study, the estimation of the heritability, using pedigree (Supplementary material 1) or genomic relationship matrix (Tables 2 and 3), shows that selecting a sample using the variance between the families as well as the discordant EBVs, can increase the intensity of the selection improvement even using a low intensity selection strategy (i.e., selecting 985 fish from 1576), and not as representative estimate for the resistance to *L. kroyeri* in the European seabass.

Focusing on growth, studies provided evidence that the range of heritability for body weight was 0.47–0.63 in European seabass (Chatziplis et al., 2020; Dupont-Nivet et al., 2008; Ferrari et al., 2016; Saillant et al., 2009; Vandeputte et al., 2016; Volckaert et al., 2012). The heritability of the body weight in the studied selected sample at 6 months in the sea cage (W6M) was estimated using the Genomic Relationship Matrix (0.41), and it was found to be similar to the genetic heritability (estimated using PRM) of the whole population (0.34 or 0.42 depending on the model used, Papapetrou et al., 2021). The higher increase of heritability was noted for the body weight in studied sample at 4 months in the sea cage (W4M), in which the genomic heritability, was 0.43 (Table 2) or 0.45 (Table 3), while in Papapetrou et al. (2021), using pedigree relationship matrix as well as 1576 fish, the heritability was 0.28. For growth at sea (GAS), the heritability was estimated 0.39 (Table 2) or 0.38 (Table 3), using the GRM, while in the whole population of the parasite trials (Papapetrou et al., 2021) it was found to be 0.29 and 0.43 (in the two trials for *L. kroyeri* and *D. aequans*, respectively).

The genomic correlation between body weight (W6M) and *L. kroyeri* count was similar and low independently of the utilized relationship matrix (0.25 or 0.24 using GRM and 0.22 using PRM). However, the genomic/genetic correlation between growth at sea (GAS) and *L. kroyeri* showed a small increase, being 0.21 (Table 2) or 0.19 (Table 3) using the

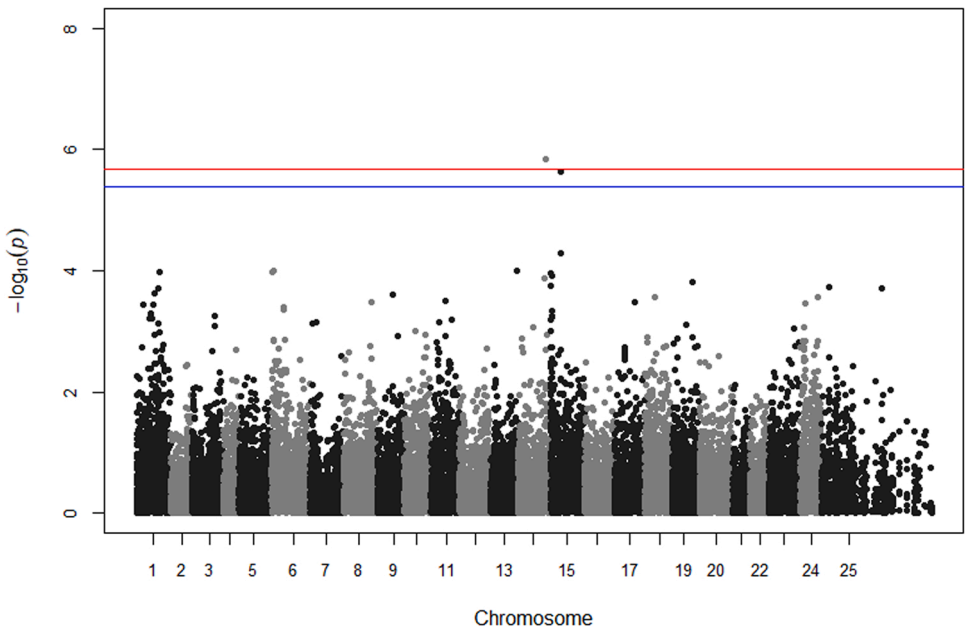


**Fig. 3.** Manhattan plot from multitrait GWAS analysis for *growth (*W2M, W4M, W6M and GAS). Red line illustrates the threshold for a = 0.05 after bonferroni correction and blue line for a = 0.1.

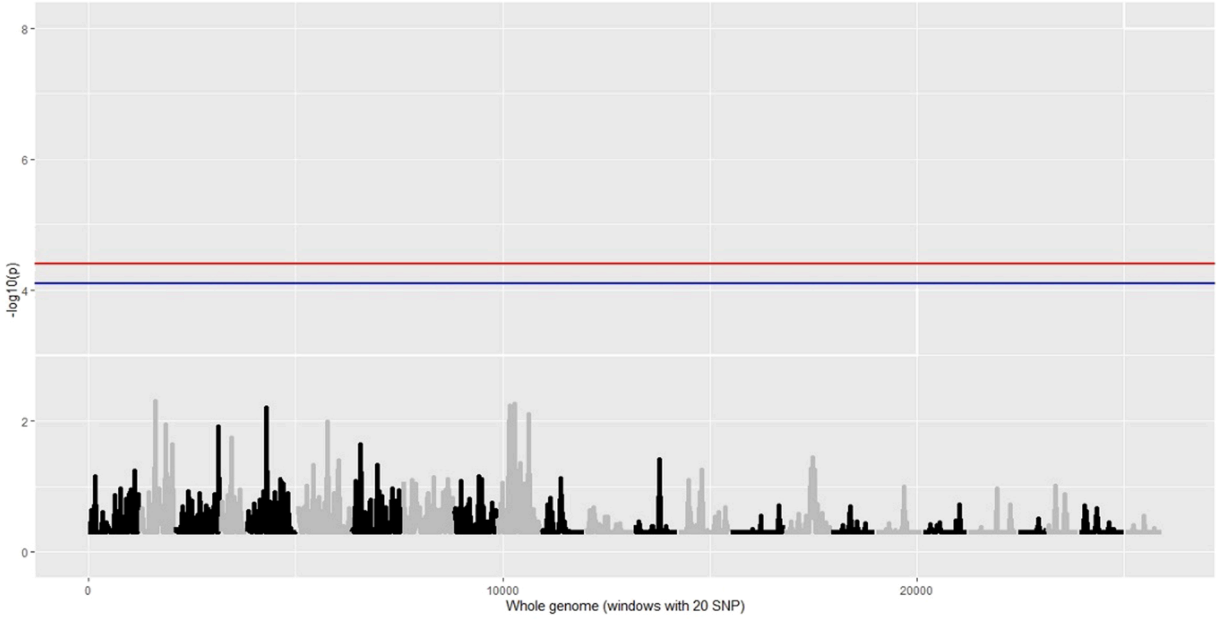


**Fig. 4.** Regional heritability mapping for *Lernanthropus kroyeri* (PC).The red (initial value 0.05) and blue (initial value 0.1) lines illustrate the threshold lines after Bonferroni correction.

GRM and 0.09 using the PRM. A small increase of the genomic/genetic correlation was noted between PC and W4M since the genomic estimation was 0.33 and using pedigree it was 0.28 (Papapetrou et al., 2021).

Generally, focusing on the genomic correlations in the present study along with genetic correlation in Papapetrou et al. (2021) between body weight and parasite count, showed a high genetic correlation with tagging weight (0.40, Papapetrou et al., 2021), followed by moderate for W2M (0.36, Tables 2 and 3) and W4M (0.33 in Tables 2 and 3, 0.28 in Papapetrou et al., 2021) and, finally, a low estimation in W6M (0.25 in Table 2, 0.24 in Table 3, 0.22 in Papapetrou et al., 2021).

Nevertheless, when the Breeding Values of the *L. kroyeri*, body weight and growth were estimated (Supplementary material 1) in the selective sample using genomic relationship matrix (GEBVs) and pedigree relationship matrix (EBVs) along with the accuracy of estimation, a high Pearson correlation between them was noted (0.91–0.92, Supplementary material 1, Table 2).

### 4.2. GWAS analysis

Although there are studies providing QTL or suggestive QTL affecting disease resistance, i.e. against Amebic Gill Disease (*Neoparamoeba perurans)*, a large part of phenotypic variation in Atlantic salmon (i.e. 4–11.6%) (Robledo et al., 2018) remains to be explained. Evidence that a possible effect comes from a polygenic structure has been suspected in some cases (i.e. Salmon louse (*Lepeophtheirus salmonis*)

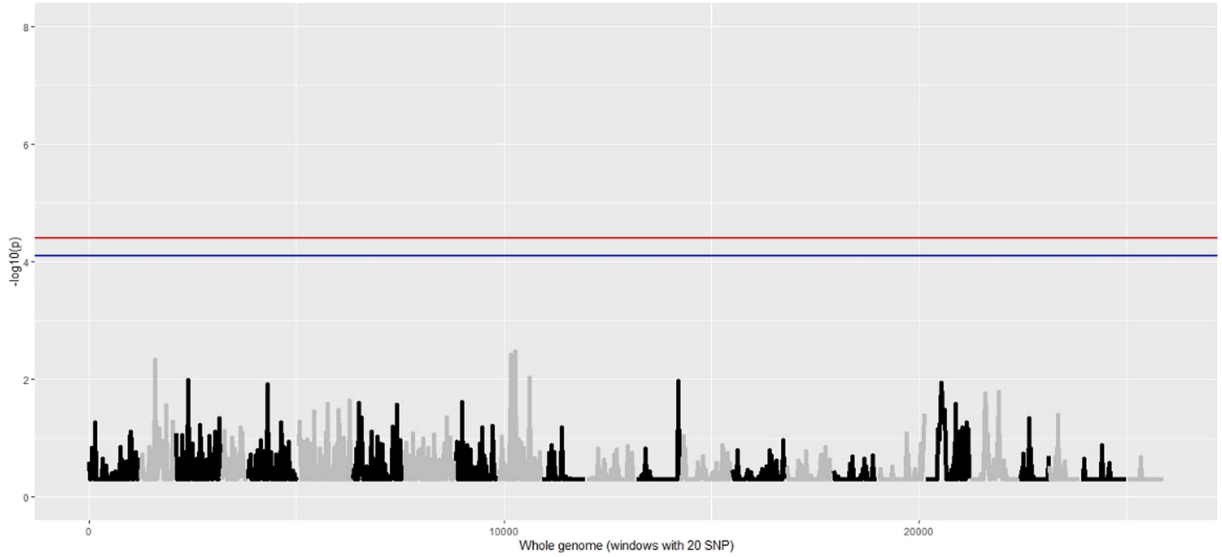


**Fig. 5.** Regional heritability mapping for *Lernanthropus kroyeri* (lnPC).The red (initial value 0.05) and blue (initial value 0.1) lines illustrate the threshold lines after Bonferroni correction.

(Houston et al., 2014), *C. rogercresseyi* (Cáceres et al., 2021)), since many QTL (regions) explaining only a small part of the total genetic variance for the trait (1%) were detected (Cáceres et al., 2021). In the present study, increasing LOD scores from SNPs which can possibly affect the host resistance against *L. kroyeri*, were detected in chromosomes 6, 8, 20 and in chr25 (unstructured chromosome). However, only two putative QTL affecting the resistance against *L. kroyeri* (AX-373127007, AX-373218583) were detected in chromosome 8 (LG7), explaining approximately 2% of the phenotypic variation each. Table 3 illustrates the average number of parasite counts for the AX-373127007 and AX-373218583, where a large difference among the genotypes was found, i.e. the average number of parasite counts in genotype CC of AX-373127007 was 20.77 while the respective value in genotype TT was 26.86 parasites.

Regarding growth performance, even though there is a lack of increasing LOD scores from SNPs, a significant QTL was detected in chr14. QTL affecting body weight in early growth stages (318 DPH–378 DPH) have been also found in another population of European seabass (Oikonomou et al., 2022) using a multitrait GWAS and a different SNP array (57K Affymetrix array, Griot et al., 2021).

### 4.3. Linkage disequilibrium (LD) and Regional heritability mapping (RHM)

Generally, linkage disequilibrium influences the power of the QTL detection, when association analysis is performed, and the accuracy of the genomic selection (Kijas et al., 2019; Niu et al., 2016; Slatkin, 2008). Using a 57K SNP array in rainbow trout and a 50KSNP array in Nile tilapia, the linkage disequilibrium, estimated as the pairwise correlation between SNP, ranged from 0.21 to 0.44 and from 0.04 to 0.08, respectively (Vallejo et al., 2018; Yoshida et al., 2019). However, using a different 57K Affymetrix SNP array (Griot et al., 2021) in another population of European seabass, the average LG in the whole genome was 0.036 (Oikonomou et al., 2022). In the present study, using the 30K MedFish array (Peñaloza et al., 2020) in European seabass, which is considered to be a medium density SNP chip, an average linkage disequilibrium of 0.07 was observed in our sample. Applying Regional Heritability Mapping in a 20 SNP-sizes region, did not provide any significant results when the variables of *L. kroyeri* count (PC and lnPC) were analyzed. Those findings had probably been influenced by the low LD across the chromosomes and the low number of haplotype blocks that were constructed (2141 haplotype blocks were constructed using 4975 SNPs, data not shown), since a large distance between SNPs existed and, consequently, no significant regions were detected.

### 4.4. Breeding strategies for L. kroyeri

Nevertheless, the identification of QTL related to the studied parasite is of great importance since any classical selection method must be based on sib or progeny testing for the genetic evaluation of the selection candidates. Such selection method provides lower selection accuracy and increases the generation interval with negative effects on selection response. Thus, using the two QTL affecting the host resistance against *L. kroyeri* through MAS (Marker Assisted Selection), can increase the selection accuracy for the candidates and an improved genetic gain can be achieved.

Moreover, the preliminary results of the genetic and genomic evaluation are very promising concerning the use of a SNP array in breeding programs. Ranking correlations between classically (via the pedigree) Estimated Breeding Values (EBV) and Genomic Estimated Breeding Values (GEBV) were very high (0.92).

Genomic selection methods can drastically reduce the very laborious and costly measurement of Parasite Count, which would only be taken into a training population once every few generations compared to the necessity of measurements for Parasite Count in every generation, in sibs of the selection candidates, when using a pedigree-based selection method. In addition, any classical selection method must focus on sib or progeny testing for the genetic evaluation of the selection candidates, which has a negative effect on the accuracy and the generation interval of the selection and, consequently, on the selection response for parasite resistance traits.

## 5. Conclusions

The selective genotyping method, applied in our initial population, captures both within and between family variation for *Lernanthropus kroyeri* count on the gills of European seabass, thus a high genomic heritability was found studying the host resistance against the parasite, while it assisted for two putative QTL to be detected, explaining approximately 2% of the phenotypic variation each. In addition, a QTL affecting growth was identified using body weight at 2, 4 and 6 months and also growth at sea cage.

The newly developed SNP array (30K MedFISH SNP array, Peñaloza et al., 2020) showed a substantial improvement of prediction accuracy

in breeding values. Despite the low re-ranking of animals with GEBVs compared to EBVs and the high correlations between GEBVs and EBVs, the gain in accuracy is expected to significantly increase the response to selection. These preliminary results are highly favorable for future application of genomic selection in fish farming for resistance and other production traits, as well. However, the true prediction accuracy, before any application of Genomic Selection, should be confirmed with an experimental response to selection in terms of a broader study.

## CRediT authorship contribution statement

**Stavroula Oikonomou:** Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. **Zoi Kazlari:** Data curation, Formal analysis. **Maria Papapetrou:** Data curation. **Kantham Papanna:** Investigation, Methodology. **Leonidas Papaharisis:** Validation, Resources, Funding acquisition, Supervision. **Tereza Manousaki:** Data curation, Investigation. **Dimitrios Loukovitis:** Resources. **Lefteris Kottaras:** Resources. **Arkadios Dimitroglou:** Resources. **Evgenia Gourzioti:** Data curation. **Charalampos Pagonis:** Data curation. **Andreas Kostandis:** Data curation. **Costas S. Tsigenopoulos:** Project administration, Funding acquisition, Investigation, Conceptualization, Investigation, Methodology, Supervision. **Dimitrios Chatziplis:** Methodology, Validation, Funding acquisition, Conceptualization, Writing – review & editing, Investigation, Supervision.

## Declaration of Competing Interest

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

## Acknowledgments

This study was funded by the EU Project Perform Fish, European Union (H2020, Grant agreement no. 727610). The authors would also like to thank IdentiGEN Ltd. (Dublin, Ireland) for providing the genotyping services.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2022.101178.

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