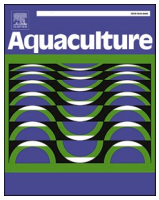
Aquaculture 590 (2024) 741023

Contents lists available at ScienceDirect

Aquaculture

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



Genome-wide association study and genomic prediction of resistance to summer mortality in Pacific oyster (*Crassostrea gigas*) using whole genome resequencing

Yong Chi [a], Hang Yang [a], Ben Yang [a], Chenyu Shi [a], Chengxun Xu [a], Shikai Liu [a], Qi Li [a,b,*]

[a] Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao 266003, China
[b] Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266237, China

ARTICLE INFO

Keywords:
Crassostrea gigas
Summer mortality
Heritability
GWAS
Genomic prediction

ABSTRACT

The Pacific oyster *Crassostrea gigas* is one of the most important farmed marine shellfish worldwide. However, massive mortality during the summer months caused significant economic losses to the *C. gigas* farming industry. Understanding the genetic architecture of resistance traits has been an ongoing research issue, and the incorporation of genomic information into breeding programs is expected to accelerate the process of genetic improvement. Genomic selection (GS), as a new approach of genetic improvement, has great potential for breeding new lines with adaptive advantages. In this study, we conducted a genome-wide association study (GWAS) for summer mortality resistance and estimated the accuracy of genomic predictions using different methods. The heritability of the *C. gigas* summer mortality resistance was low, with heritability of 0.108 ± 0.036, 0.161 ± 0.102 and 0.134 ± 0.043 for PBLUP, GBLUP and ssGBLUP, respectively. In addition, we detected 9,783,674 SNPs and used a mixed linear model to identify 18 significant SNPs associated with summer mortality resistance. The phenotypic variance explained (PVE) for these SNPs ranged from 8.25% to 10.14%. Based on these significantly related SNPs, nine significant candidate genes were identified (*TLR4*, *HEX*, *SLC22A8*, *PDE2A*, *HUWE1*, *SLMAP*, *RAD52*, *TBK1* and *RAPH1*). The prediction accuracy using PBLUP, GBLUP, ssGBLUP, and weight ssGBLUP (WssGBLUP) was 0.549 ± 0.026, 0.381 ± 0.074, 0.544 ± 0.026, and 0.869 ± 0.018, respectively. Therefore, WssGBLUP models are more suitable for genomic prediction of summer mortality resistance in *C. gigas*. Our results suggest a polygenic genetic architecture that provide new perspectives for studying candidate genes for resistance to summer mortality, which may facilitate the genetic improvement for resistance lines in *C. gigas*.

1. Introduction

The Pacific oyster (*Crassostrea gigas*) is one of the major marine bivalve shellfish and is of great interest to the aquaculture industry because of its rapid growth and environmental tolerance (Ruesink et al., 2005). In recent years, severe summer mortalities have been reported regularly in *C. gigas* aquaculture, which has resulted in significant economic losses to the oyster industry (Solomieu et al., 2015). Massive summer mortality of *C. gigas* has been recorded in several regions around the globe, including European countries, the United States, Japan and most recently in China (Koganezawa, 1974; Glude, 1975; Burge et al., 2006; Segarra et al., 2010; Cotter et al., 2010; Yang et al., 2021; Chi et al., 2021). Numerous studies have shown that mortality events are multifactorial, with age, microbiota, genetics and physiological status being important factors associated with mortality outbreaks (Alfaro et al., 2019). In the absence of effective methods to prevent or control disease outbreaks, increasing host resistance to mortality through selective breeding is the preferred option to improve summer survival (Dégremont et al., 2015). For oyster growers who rely on high yields for farm profitability, improving survival under field conditions is critical. Quantitative genetic analyses have estimated genetic parameters of summer mortality resistance and have shown the potential for selective breeding to improve summer survival in *C. gigas*, as they are heritable traits (Dégremont et al., 2007; Chi et al., 2022).

Traditional selective breeding techniques are inefficient for the genetic improvement of resistance traits due to differences in tolerance

* Corresponding author at: Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao, China.
E-mail address: qili66@ouc.edu.cn (Q. Li).

https://doi.org/10.1016/j.aquaculture.2024.741023
Received 14 February 2024; Received in revised form 18 April 2024; Accepted 28 April 2024
Available online 30 April 2024

among individuals within populations (Meuwissen et al., 2001; Blay et al., 2021). Currently, aquaculture breeding programs incorporate genomic information of animals for more accurate selection of candidates (Houston et al., 2020). In such programs, it is important that effective methods are used to clarify the genetic variation associated with disease resistance and to identify the corresponding candidate genes. This information can be used to conduct genome-wide association studies (GWAS) to identify genetic markers that are significantly associated with the target traits (Hayes and Goddard, 2010). In addition, SNP information can be used to implement genomic selection (GS). GS involves the estimation of genomic breeding values for selection candidates, with or without phenotypic information, through prediction equations using relevant reference populations with genotypes and phenotypes (Meuwissen et al., 2001; Hayes et al., 2009). When applying GS in genetic improvement program, various influencing factors need to be considered and the predictive accuracy of different GS models need to be compared, ultimately selecting the most appropriate GS approach for the target trait.

It has been postulated that the ability of oysters to avoid disease is dependent on their innate immune defense systems and, as such, efforts have been made over the last few years to investigate the molecular processes involved in oyster defense responses. Previous genome-wide expression profiling studies in resistant and susceptible oysters have highlighted reproduction, antioxidant defense, and immune as constitutive factors influencing the divergent phenotypes observed between these two groups (Fleury et al., 2010; Fleury and Huvet, 2012). Transcriptomic studies of multiple biparental oyster families with varying degrees of susceptibility to summer mortality have shown that resistant families consistently exhibited distinct expressions of genes associated with innate immune response and antiviral pathways (De Lorgeril et al., 2020; Chi et al., 2023). A recent study identified a QTL associated with OsHV-1 mortality resistance in a breeding population of *C. gigas*, which correlated with the expression of *IRF2* and *VIPERIN* (Divilov et al., 2023). However, the genetic architecture of summer mortality resistance may also differ due to differences in genetic and environmental background. Clearly, current studies are not sufficient to provide us with an understanding of the complex regulatory mechanisms of summer mortality resistance in *C. gigas*.

Our study aims to (1) detect SNPs and candidate genes associated with summer mortality resistance for *C. gigas* through GWAS, (2) compare the predictive accuracy of different models for GS in relation to summer mortality resistance. This study provides valuable data for further elucidation of genetic mechanisms and a preliminary exploration of the optimal genomics approach for summer mortality resistance in *C. gigas*, which will contribute to the future selection of resistant lines.

2. Materials and method

2.1. Experiment oysters

The *C. gigas* families used in this study were constructed following the scheme described in previous studies (Chi et al., 2022; Chi et al., 2023). Briefly, the 42 full-sib families were constructed in March 2021 at the Laizhou breeding base as part of the selective breeding program. After maturation, oyster gonads were dissected and fertilized by mixing sperm and eggs in a beaker, then incubated in a 100-L bucket. Larvae were cultured in the 100-L buckets according to the routine culture methods described previously (Chi et al., 2022). After a period of temporary rearing in ponds, spat were transported and cultivated in Rongcheng (37°11′N 122°48′E), Shandong province of China in May 2021.

2.2. Sample collection and field test

In March 2022, oysters from 42 families were transported from Rongcheng to the Laizhou breeding base. Gill tissues of 1330 oysters were sampled, and then each sampled oyster was numbered and labelled with a waterproof label. To force the oyster shell to open, we chose to anaesthetize the oysters with $MgCl_2$ (50 mg/mL) (Suquet et al., 2009; Zhai et al., 2021). A small number of gill filaments were then cut using fine scissors and preserved in 95% anhydrous ethanol. Previous studies have shown that this method of gill sampling does not result in oyster death or behavioral abnormalities and therefore has a negligible effect on oyster viability (Liu et al., 2021). Sampled oysters were not immediately deployed to the field but remain in the Laizhou breeding base. Under Laizhou breeding base, water quality, food rationing and temperature was controlled. The water temperature was 19–20 °C, salinity 30–32%, dissolved oxygen 8–9 mg/L, and pH 8.1–8.2. In June 2022, sampled oysters were placed in lantern nets and sent to Rongcheng to test for summer mortality resistance. Oysters in lantern nets were checked and counted every other month and dead oyster tags were recorded. The water temperature fluctuated between 22 °C and 26 °C during the whole experiment. Survival rate was recorded until September 2022. Oysters were grouped according to whether they survived or died, followed by DNA extraction.

2.3. Genotyping and quality control

DNA was extracted using phenol-chloroform method (Li et al., 2006) and used for whole-genome resequencing. All samples were sequenced on the BGI-T7 platform with 150 bp paired-end reads libraries. Raw reads were filtered using Fastp (Chen et al., 2018) and the clean reads were aligned to the *C. gigas* genome (cgigas_uk_roslin_v1) using the BWA (Li and Durbin, 2009). The alignment files were processed using software SAMtools (Li et al., 2009). The GATK software was used to discover and call SNPs (McKenna et al., 2010). The PLINK software was used for further filtering with the criteria of minor allele frequency (MAF) $>$ 0.05, SNP call rate $>$ 0.90 and individual call rate $>$ 0.80. Finally, a total of 9,783,674 SNPs passed the quality control procedure for further analyses.

2.4. Assessment of the population structure and LD

Principal Component Analysis (PCA) was performed on all samples using Plink (Purcell et al., 2007). SNPs were also pruned using PLINK to reduce the possible effect of linkage disequilibrium (LD). The retained unlinked sites were further used for the individual-based assignment test using the Admixture software (Alexander et al., 2009). To investigate the LD decay in resistance and susceptible populations, nonrandom associations among alleles at two or more loci were calculated using software PopLDdecay (Zhang et al., 2019).

2.5. GWAS analysis

In this study, GWAS analysis was carried out using GEMMA software with a linear mixed model (Zhou and Stephens, 2012). The GWAS model is shown below (Peng et al., 2021):

$$y = Xb + Sa + Ku + e$$

where y is the vector of observed phenotypes (binary survival); b is the vector of fixed effects (first 3 PCA values); a is a vector of SNP genotypes; u is the vector of additive genetic effects; e is the vector of residual error; the design matrices X, S, and K are provided to the corresponding vectors b, a, and μ.

A more modest Bonferroni approach was used in calculating genome-wide significance threshold (Zhong et al., 2017). The genome-wide significant threshold was set as 1/N (N is the total number of SNPs), and the chromosome-wide significant threshold was set as 1/n (n is the number of SNPs on chromosome) (Yang et al., 2022; Peñaloza et al., 2022).

The phenotypic variance explained (PVE) was assessed using the following formula (Teslovich et al., 2010):

$$PVE = \frac{2\widehat{\beta}^2 MAF(1-MAF)}{2\widehat{\beta}^2 MAF(1-MAF) + (se(\widehat{\beta}))^2 2NMAF(1-MAF)}$$

Where $\widehat{\beta}$ is the effect size of SNP, $se(\widehat{\beta})$ is the standard error of the tested SNP effect size, *MAF* is the minor allele frequency and *N* is the sample number.

2.6. Trait-related gene detection and functional annotation

Given the rapid decay of LD in this population, only genes containing significant SNPs were identified as candidates for resistance to summer mortality. In addition, all significant SNPs associated with summer mortality resistance were annotated using software ANNOVAR (Wang et al., 2010).

2.7. Heritability estimation and genomic prediction

Survival was considered a binary variable (1 = survivors, 0 = dead). Three methods (PBLUP, GBLUP and ssGBLUP) were used to estimating variance components and heritability (Campos-Montes et al., 2023). PBLUP, GBLUP and ssGBLUP use the pedigree-based kinship matrix A, the genome-based relationship matrix G, and a blend between the pedigree and genomic relationship matrix H, respectively. All these three matrixes were constructed using the BLUPF90 (Misztal et al., 2018). In matrix form, the mixed model by PBLUP was:

$$y = u + Zg + e$$

Where y represents the vector of observations (binary survival), u is the overall mean of phenotypes, Z is incidence matrices, g is the vector of animal additive genetic effects, $g \sim N(0, \sigma_g^2 A)$, where A is the pedigree-based relationship matrix, e is the vector of residual effects, $e \sim N(0, \sigma_e^2 I)$, where I is an identity matrix of appropriate order.

Heritability was calculated as follows:

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}$$

Where σ_g^2 is additive genetic variance components, and σ_e^2 is residual variance components.

GBLUP and ssGBLUP use the same model specification, replacing the A with the G matrix or the H matrix, respectively. For GBLUP, the G matrix was constructed as described by VanRaden (2008):

$$G = \frac{ZZ^{-1}}{2\sum_i^m p_i(1-p_i)}$$

Where Z is a matrix of centered genotypes, p_i is the MAF of SNP i, and m is the total number of SNP.

For ssGBLUP, the H matrix and the inverse of the H matrix was constructed as (Legarra et al., 2009; Aguilar et al., 2011; Christensen and Lund, 2010):

$$H = \begin{pmatrix} A_{11} + A_{22}A_{22}^{-1}(G - A_{22})A_{22}^{-1}A_{21} & A_{12}A_{22}^{-1}G \\ GA_{22}^{-1}A_{11} & G \end{pmatrix}$$

$$H^{-1} = A^{-1} + \begin{pmatrix} 0 & 0 \\ 0 & G^{-1} - A_{22}^{-1} \end{pmatrix}$$

Where the subscript 1 and 2 denotes ungenotyped and genotyped individuals, respectively.

To assess the potential of GS for summer mortality resistance, the predictive ability of GS was estimated by a five-fold cross-validation analysis with ten replications. GEBVs (EBVs) of summer mortality resistance for the validation population were predicted using four different methods (PBLUP, GBLUP, ssGBLUP and WssGBLUP). The expression of WssGBLUP is the same as ssGBLUP, with the key difference lying in the construction of the weighted G matrix. In WssGBLUP, the iterative algorithm is employed to generate the weighted G matrix, which is then used for estimating genomic breeding values (Wang et al., 2012). In the first iteration, weights were fixed to 1 which corresponds to the standard ssGBLUP, and we performed three iterations.

The predictive ability was calculated as described by Legarra et al. (2008):

$$\frac{|(G)EBV, y|}{\sqrt{h_{obs}^2}}$$

Where $|(G)EBV, y|$ is the Pearson correlation coefficient between the $(G)EBV$ and the phenotype y, h_{obs}^2 is the genomic based heritability.

3. Results

3.1. Oyster mortality

Prior to the field trial, no unusual mortality (<5%) was observed in oysters from the Laizhou breeding center. A total of 1270 oyster were used in the field trial. Summer survival rates varied from 20.00 to 85.25% among families, with a mean survival rate of 46.60% (Fig. 1A). Dead oysters were considered susceptible oysters and survivors were considered resistant oysters. In total, 279 DNA samples were collected from 137 susceptible oysters and 142 resistant oysters (Fig. 1B).

3.2. Data filtering and SNP density

Whole-genome resequencing yielded a total of 2141.09 Gb raw data, leaving 2123.75 Gb of clean data after removal of low-quality reads. The raw sequence reads have been submitted to the SRA database of NCBI with the accession number PRJNA1099940. Of these, 95.90% were successfully mapped to the reference genome. Additionally, the average sequencing depth for all samples was calculated to be 12.69×, with values ranging from 7.78× to 55.28× (Table S1). After quality control, 9,783,674 SNPs remained in the final GWAS analyses. These SNPs markers were observed to be uniformly distributed across all 10 chromosomes of *C. gigas* (Fig. 2A). The number of SNPs varied across chromosomes, ranging from 531,014 SNPs on Chromosome 9 to 1,274,687 SNPs on Chromosome 4. The average marker density across the genome was calculated to be 16,600.54 SNPs/Mb.

3.3. Analysis of population structure and LD

Resistant and susceptible oysters were evenly distributed according to PCA (Fig. 2B). The first three principal components explained 5.7%, 4.6% and 3.9% of the population structure and genetic relatedness, respectively. The cross-validation error decreases continuously as the value of k increased, suggesting the absence of discernible subpopulations in our study (Fig. 2C). At the genomic level, LD analyses showed extremely rapid decay (decaying by 50% within 100 bp) and showed similar patterns in the resistant and susceptible populations (Fig. 2D).

3.4. Genome-wide association analysis and candidate gene identification

To identify SNPs significantly associated with summer mortality resistance, GWAS analysis was performed, and the results were visualized using Manhattan and QQ plots (Fig. 3). A total of 18 SNPs were significantly associated with summer mortality resistance, mainly on seven chromosomes (Table 1). The minimum allele frequency of the 18 significant SNPs ranged from 0.061 to 0.301, and the PVE of these SNPs ranged from 8.25% to 10.14%. According to the relative positions of SNPs and gene loci, SNPs can be classified into three categories: intergenic region, exons region and introns region.

Among all the significant SNPs, a total of nine candidate genes were annotated, which are involved in a variety of biological processes,

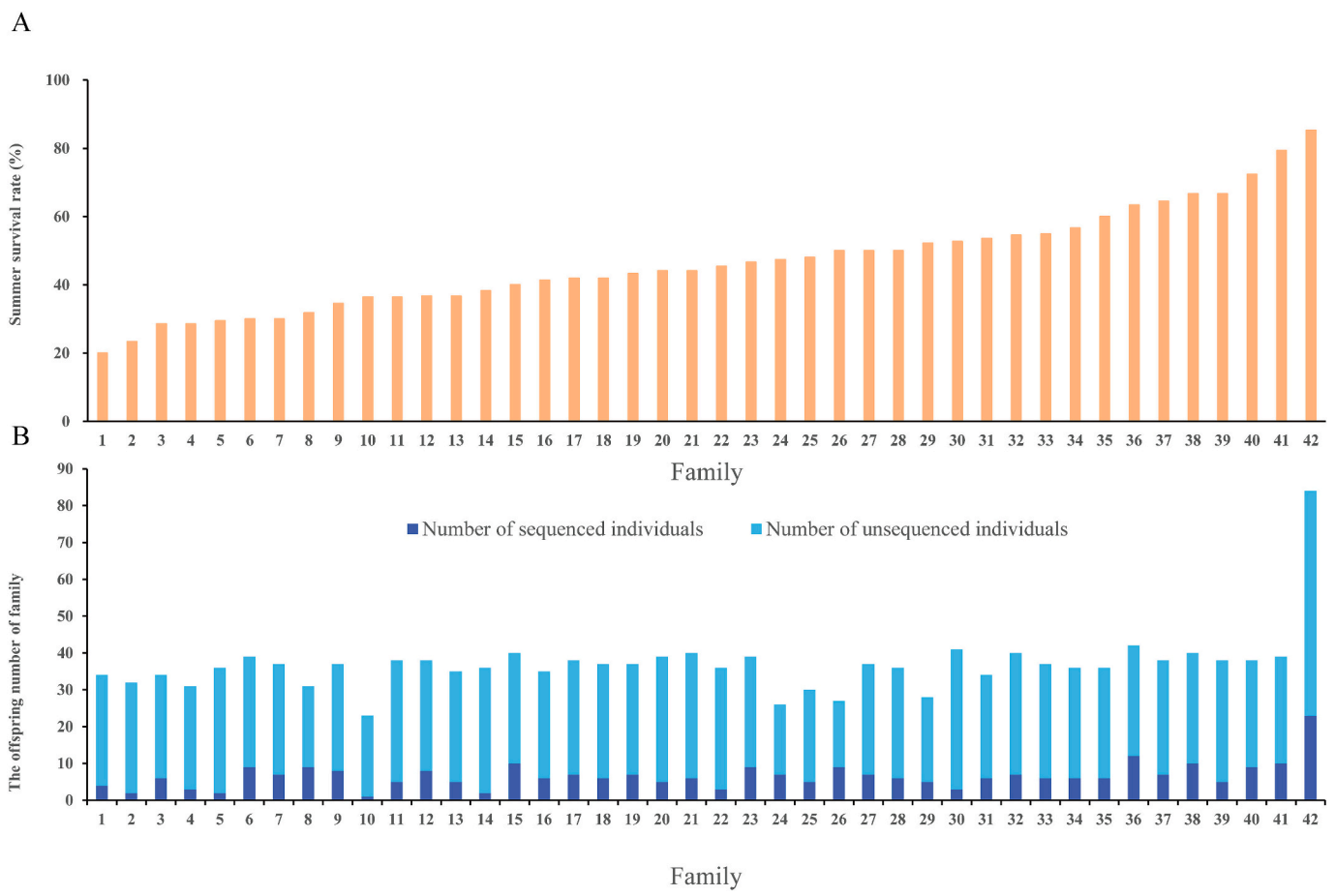


Fig. 1. The average survival rate of oyster families in test site (A). The oyster number of each family for sequenced individuals and unsequenced individuals (B).

including immune response (*TLR4*, *HUWE1* and *TBK1*), signaling (*PDE2A* and *SLMAP*), metabolism (*HEX* and *SLC22A8*) and DNA repair (*RAD52*) (Table 2).

3.5. Heritability estimates and genomic prediction

The estimates of heritability are summarized in Table 3. The heritability of the summer mortality resistance of *C. gigas* was low, with heritabilities of 0.108 ± 0.036, 0.161 ± 0.102 and 0.134 ± 0.043 for PBLUP, GBLUP and ssGBLUP, respectively.

Genomic prediction accuracy of summer mortality resistance in *C. gigas* under different models are summarized in Table 4. The correlation of breeding values for PBLUP, GBLUP, ssGBLUP and WssGBLUP were 0.220 ± 0.010, 0.153 ± 0.030, 0.219 ± 0.011 and 0.350 ± 0.007, respectively. The prediction accuracy of PBLUP, GBLUP, ssGBLUP and WssGBLUP for summer mortality resistance in *C. gigas* was 0.549 ± 0.026, 0.381 ± 0.074, 0.544 ± 0.026 and 0.869 ± 0.018, respectively.

4. Discussion

Frequent outbreaks of massive summer mortalities severely hamper the global oyster industry (Alfaro et al., 2019). This work was motivated by the desire to better elucidate the genetic architecture of summer mortality resistance, as well as to detect which genes may be associated with these traits. Ideally, oysters in lantern nets would be monitored daily for continuous survival phenotypes (e.g., time of death). However, the planting sites for this study were located in remote areas of the field. This made it impossible to travel to the site for extended periods of time at short intervals to make observations. Therefore, we used only binary traits for GWAS. Furthermore, the accuracy of genomic prediction was estimated using four different methods (PBLUP, GBLUP, ssGBLUP and WssGBLUP). Overall, this study not only enhance our comprehension of the intricate genetic basis of summer mortality resistance, but will also contribute to future GS of resistant *C. gigas* lines in selective breeding programs.

Population stratification is a bias that affects the effectiveness of GWAS, possibly due to differences in individual genetic structure (Price et al., 2006). In general, PCA is a widely used method for identifying differences in individual ancestry. In this study, PCA and population structure analysis did not reveal population genetic stratification, suggesting that the observed variation did not contain a subpopulation component. GWAS relied on LD, which is a non-random correlation between alleles that tends to reveal recombination of alleles, and which interacts with selection, mutation, and genetic drift in a complex manner (Slatkin, 2008). The results of LD analyses indicate that LD decline in *C. gigas* is very rapid, which is consistent with the results of a previous study (Yang et al., 2022). In this study, a total of 9,783,674 SNPs were detected, and the average SNP density was 16,600.54/Mb, which was higher than that of previous GWAS studies for *C. gigas* (Gutierrez et al., 2018; Yang et al., 2022; Liu et al., 2022). This indicates that the data quality of this study is reliable and provides a good basis for GWAS.

Heritability, a vital parameter in selective breeding, is typically defined as the proportion of phenotypic variation attributed to genotypic differences for a specific trait within a population (Falconer and Mackay, 1996). It quantifies the extent to which genetic factors contribute to the observed variation, aiding in the strategic improvement of traits through selective breeding programs (Visscher et al., 2008). In this study, heritability values were estimated using three different models. The heritability estimated using SNPs (GBLUP) was higher than that from REML analysis of pedigree data (PBLUP).

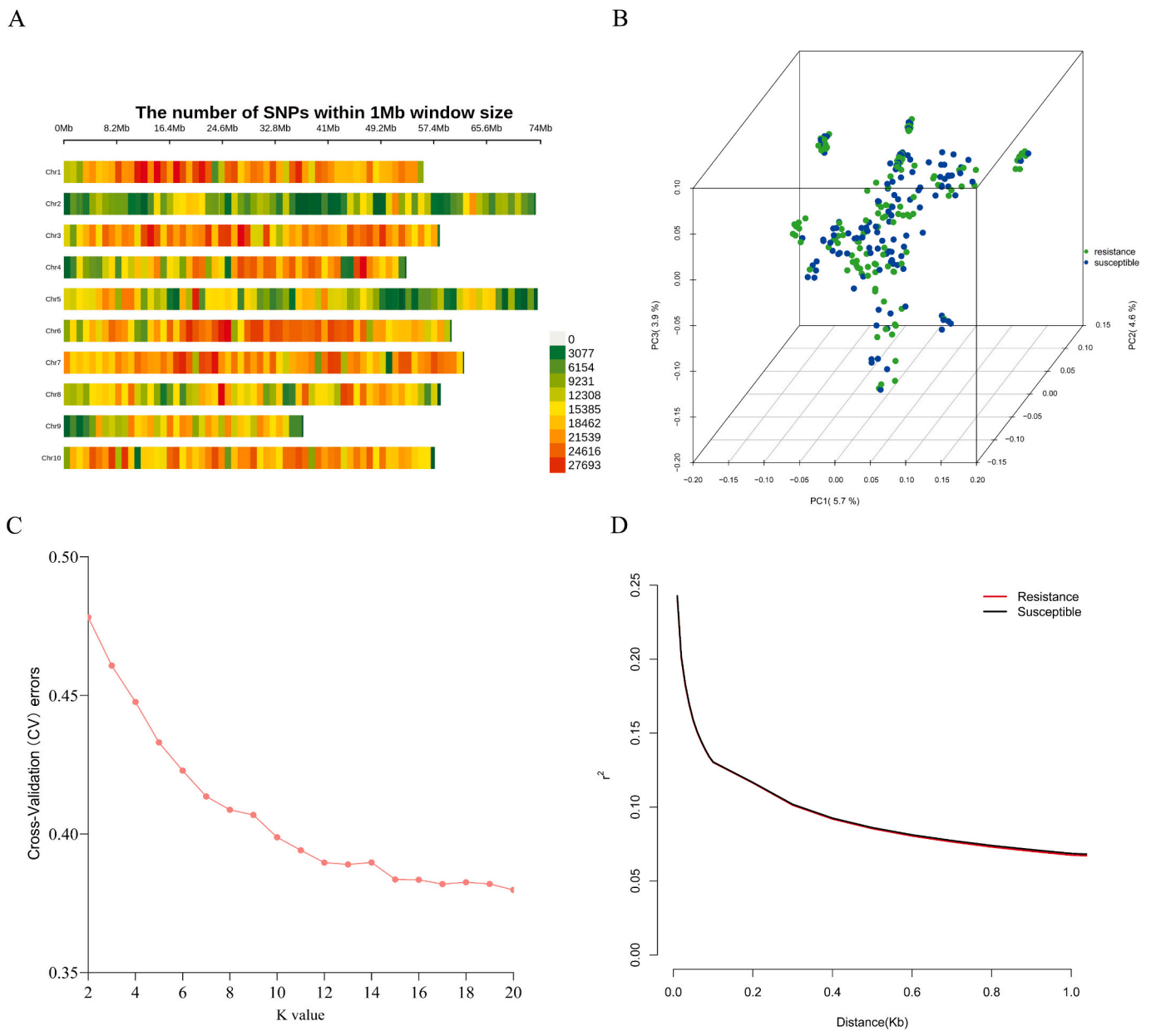


Fig. 2. SNP density plots across all chromosomes of *C. gigas* (A). Principal component analysis of 279 oysters (green dot: resistance oysters; blue dot: susceptible oysters) (B). The change of cross validation error at different K-value (C). Genome-wide linkage disequilibrium (LD) decay in resistance and susceptible groups (D). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Similarly, Gutierrez et al. (2020) found that heritability of OsHV-1 resistance estimated using genomic information (0.37 ± 0.05) was higher than the pedigree estimates (0.25 ± 0.05). Similar results were also seen in many other aquatic animals, such as shell shape and body weight in triangle sail mussel, *Hyriopsis cumingii* (Wang et al., 2022a), mantle colour in Portuguese oyster, *C. angulate* (Vu et al., 2021) and harvest traits in channel catfish, *Ictalurus punctatus* (Garcia et al., 2018). The reason for this difference may be that genome-wide SNPs capture more genetic variation, which yields higher heritability (VanRaden, 2008). In fact, heritability can vary depending on the statistical methodology applied, genotyping method, and the type of markers utilized (Wray and Visscher, 2008; Visscher et al., 2008; Hu et al., 2020).

Using linear mixed model for GWAS, we identified 18 SNPs significantly associated with summer mortality resistance, and no major-effect QTLs for summer mortality resistance were found on these SNPs. Significant SNPs linked to summer mortality resistance were observed to be extensively distributed across 7 chromosomes, with no obvious clustering. In addition, the PVE of these SNPs was low, ranging from 8.25% to 10.14%. These results suggest that summer mortality resistance maybe a polygenic trait, regulated by complex regulatory networks of multiple interacting genes. From a practical breeding point of view, these SNPs could be used in GS to improve summer mortality resistance in *C. gigas*.

A total of nine candidate genes were identified in this study. Some of these genes are related to innate immune response, such as *TLR4* and *TBK1*. Innate immunity is the main defense mechanism against pathogen invasion in shellfish, as the adaptive immune system is not yet fully developed (Song et al., 2010). *TLR4* is a key protein for pathogen recognition during host defense and plays a crucial role in the activation of innate immunity (Schnare et al., 2001). It is worth noting that *TLR4* has been implicated in the immune response of *C. hongkongensis* against *Vibrio Parahaemolyticus* through the MyD88-dependent pathway (Chen et al., 2022; Yu et al., 2023). Once pathogens are recognized by PRRs, host cells initiate the recruitment of various adaptor proteins to activate

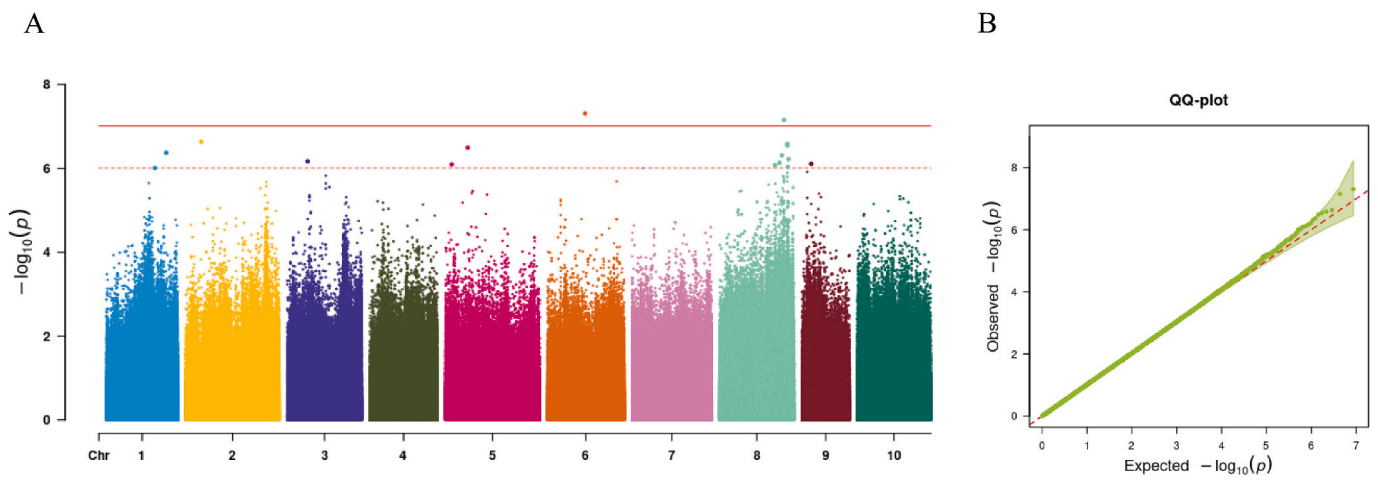


Fig. 3. Manhattan (A) and quantile-quantile (QQ) (B) plots in the genome-wide association study (GWAS) for summer mortality resistance in *C. gigas*. The solid red line represents the genome-wide significance threshold, and the dashed red line reaches the chromosome-wide significance threshold. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Summary of the 18 SNPs associated with summer mortality resistance of *C. gigas*.

Marker	-log10(*P* value)	Allele	Maf	Location	PVE%
1:37849088	6.94	G/T	0.061	Intergenic	8.25
1:46591678	6.37	A/T	0.290	Exonic	8.78
2:12227761	6.63	C/T	0.116	Intronic	9.16
3:16051384	6.17	T/G	0.086	Intronic	8.49
5:5133380	6.10	T/G	0.072	Exonic	8.38
5:17407160	6.50	A/T	0.156	Intergenic	8.96
6:29508000	7.31*	T/A	0.099	Intergenic	10.14
7:41147462	6.16	T/C	0.188	Intronic	8.48
8:39605558	6.14	C/T	0.091	Intronic	8.45
8:43460256	6.08	C/T	0.120	Intergenic	8.36
8:46826397	6.13	G/C	0.082	Intronic	8.43
8:48857575	6.31	G/A	0.258	Intronic	8.70
8:50391463	7.16*	C/G	0.197	Intergenic	9.92
8:52936220	6.59	T/C	0.186	Intronic	9.10
8:52936226	6.54	G/A	0.181	Intronic	9.03
8:53245946	6.04	A/T	0.163	Intergenic	8.29
8:53927457	6.22	T/A	0.082	Intronic	8.56
9:7293168	6.11	C/A	0.301	Intronic	8.40

Notes: * indicates genome-wide significant level, others are chromosome-wide significant level, Marker names were shown as chromosome/position, PVE%: phenotypic variances explained.

Table 2
Nine candidate genes associated with summer mortality resistance in *C. gigas*.

Gene symbol	Chr	Star (bp)	End (bp)	Gene annotation
TLR4	1	46,587,202	46,592,645	toll-like receptor 4
HEX	2	12,218,187	12,241,668	beta-hexosaminidase-like
SLC22A8	3	16,035,142	16,062,763	solute carrier family 22 member 8
PDE2A	7	41,110,551	41,157,061	cGMP-dependent 3′,5′-cyclic phosphodiesterase
HUWE1	8	39,549,711	39,612,297	E3 ubiquitin-protein ligase HUWE1
SLMAP	8	48,842,698	48,879,900	sarcolemmal membrane-associated protein
RAD52	8	52,928,401	52,940,340	DNA repair and recombination protein RAD52
TBK1	8	53,902,738	53,928,301	serine/threonine-protein kinase TBK1
RAPH1	9	7,228,488	7,305,133	ras-associated and pleckstrin homology domains-containing protein 1

Table 3
Estimation of variance components (σ_a^2: additive variance, σ_e^2: random residual variance) and heritability (h^2) for summer mortality resistance. Standard errors for variance components and heritability estimates are in parentheses.

Method	σ_a^2	σ_e^2	h^2
PBLUP	0.027(0.009)	0.219(0.011)	0.108(0.036)
GBLUP	0.041(0.026)	0.211(0.028)	0.161(0.102)
ssGLUP	0.034(0.011)	0.216(0.011)	0.134(0.043)

Table 4
Genomic prediction accuracy of summer mortality resistance in *C. gigas* under different models.

Method	Relationship matrix	Correlation	Accuracy
PBLUP	A	0.220 ± 0.010	0.549 ± 0.026
GBLUP	G	0.153 ± 0.030	0.381 ± 0.074
ssGBLUP	H	0.219 ± 0.011	0.544 ± 0.026
WssGBLUP	H	0.350 ± 0.007	0.869 ± 0.018

TBK1 (Takeuchi and Akira, 2009). Previous studies have shown that *TBK1* has a wide-ranging impact on the immune response of oysters to both bacteria and viruses (Tang et al., 2016; Li et al., 2022). *RAPH1* is associated with the Ras-ERK signaling cascade which is involved in proliferation, differentiation and apoptosis (Lewis et al., 1998; Matsunaga-Udagawa et al., 2010). In addition, *RAPH1* was found to be involved in the immune response in crustaceans (Ding et al., 2018). Oysters are exposed to many microorganisms in the field environment. Elevated sea water temperatures during summer months promote the growth of pathogens and suppress oysters' immune systems, making them more susceptible to pathogens (Vezzulli et al., 2010). These immune-related genes may be critical in fending off invading pathogens.

Pathogens stimulate phagocytosis and trigger a significant production of reactive oxygen species (ROS) within the host. However, this process can also result in oxidative damage to vital biological macromolecules like DNA and proteins (Cutler, 1991; Feuers et al., 1993; Dickson and Zhou, 2020). Notably, ROS production was reported to be an important factor in distinguishing susceptible and resistant oyster lines (Delaporte et al., 2007; Lambert et al., 2007). Oysters typically face heat stress during the summer, which is a significant cause of massive summer mortalities (Chávez-Villalba et al., 2007; Rodríguez-Jaramillo et al., 2022). Sustained high temperature disrupt the balance between the oxidative and antioxidant systems of oysters, leading to DNA damage. We identified a candidate gene RAD52 in the GWAS results, which

plays a key role in genome duplication and stability. In addition, it is involved in in repairing externally induced DNA damage, such as double-strand breaks (Essers, 2002). *RAD52* may help to mitigate the effects of DNA damage in oysters under high-pressure summer conditions, thereby improving summer survival. We have also identified an SLC22 family gene, *SLC22A8*, and the SLC22 family plays an important role in regulating homeostasis in the body by transporting small organic molecules. Notably, many of the metabolites transported by the SLC22 transporter play a role in the response to reactive oxygen species-induced oxidative stress (Engelhart et al., 2020). Higher antioxidant capacity may lead to lower levels of ROS, thereby increasing oyster summer mortality resistance. Among the candidate genes, we also identified *HUWE1*, which mediates proteasomal degradation of target proteins in organisms, suggesting that ubiquitin-dependent processes may play a key role in resistance to summer mortality. *HUWE1* has been reported to be closely associated with mammalian proliferation/differentiation, DNA repair and p53-dependent apoptosis (Thompson et al., 2014). In addition, *HUWE1* affects ROS and apoptotic signaling during WSSV infection in mud crabs (*Scylla paramamosain*) by promoting p53 ubiquitination (Gong et al., 2022). Thus, our results suggest that the regulatory mechanism of summer mortality resistance is relatively complex, and many important key genes are involved in the regulation of resistance mechanism (Fig. 4).

GS has been shown to be a useful approach to enhance resistance traits in aquaculture species, such as resistance to *Cryptocaryon irritans* and *Pseudomonas plecoglossicida* in large yellow croaker (Wang et al., 2022b; Bai et al., 2022) and amoebic gill disease resistance in Atlantic salmon (Verbyla et al., 2021). In this study, four methods (PBLUP, GBLUP, ssGBLUP and WssGBLUP) were used and their predictive accuracy for EBV or GEBV was compared by five-fold cross-validation with ten replications. Since ssGBLUP uses both genomic and pedigree information, several studies have shown that ssGBLUP outperforms GBLUP and PBLUP (Campos-Montes et al., 2023; Liu et al., 2023). However, ssGBLUP did not have an advantage over PBLUP in our study. The lower improvement in ssGBLUP may be due to the small number of genotyping individuals. It has been reported that ssGBLUP improves less compared to PBLUP when the number of genotyping reference population is <500 (Song et al., 2019).

Some studies have reported that WssGBLUP is superior to unweighted ssGBLUP (Vallejo et al., 2021; Song et al., 2022). In this study, prediction accuracy using WssGBLUP model was higher than other models, suggesting that WssGBLUP is an effective GS method for improving summer mortality resistance in this *C. gigas* population. Indeed, as reported in many studies, reference population size, genetic relatedness and population structure are important factors affecting the accuracy of genomic prediction (Song et al., 2023). Therefore, larger reference population sizes are still needed to ensure the accuracy of genomic prediction of summer mortality resistance in *C. giga*. In addition, heritability also affects predictive accuracy, with lower heritability being associated with lower prediction accuracy (Daetwyler et al., 2010). Therefore, when applying GS for selective breeding, various factors should be carefully considered beforehand, and then the most appropriate model should be selected based on specific traits.

5. Conclusion

In order to reveal the genetic basis of summer mortality resistance in *C. gigas*, GWAS was carried out based on whole-genome resequencing. In this study, a comprehensive analysis identified a total of 18 SNPs and nine candidate genes significantly associated with summer mortality resistance in *C. gigas*. Our results suggest that summer mortality resistance is driven by synergistic interactions among multiple genes associated with various biological processes. We found that summer mortality resistance in *C. gigas* has a low heritability, which was estimated using SNPs to be higher than the results of REML analysis of pedigree data. In addition, the WssGBLUP model was more suitable than the other three models (PBLUP, GBLUP and ssGBLUP) for predicting breeding values for summer mortality resistance in *C. gigas*. These findings not only help us to gain insight into the molecular regulatory mechanisms of summer mortality resistance, but also contribute to GS for resistance traits in *C. gigas*.

CRediT authorship contribution statement

Yong Chi: Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. **Hang Yang:** Software, Methodology. **Ben Yang:** Software, Methodology. **Chenyu Shi:** Software, Methodology. **Chengxun Xu:** Resources. **Shikai Liu:** Software, Methodology. **Qi Li:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

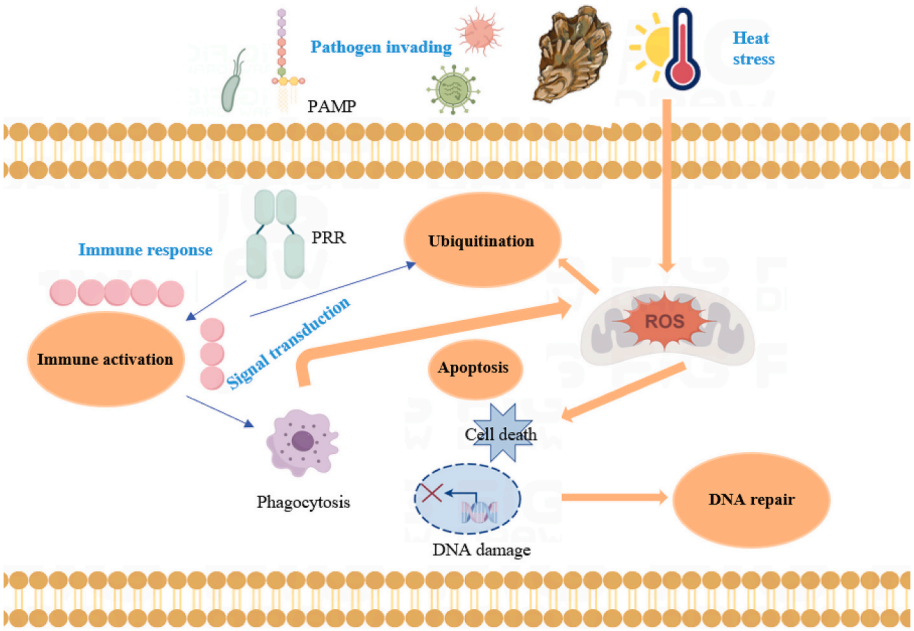


Fig. 4. Schematic diagram of putative molecular basis underlying summer mortality resistance in *C. gigas*.

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.

Data availability

The data presented in this study are available on request from the corresponding author. The raw sequence reads have been submitted to the SRA database of NCBI with the accession number PRJNA1099940

Acknowledgements

This work was supported by grants from the National Key Research and Development Program of China (2022YFD2400305), Shandong Province (2022LZGCQY010, 2021ZLGX03 and 2021TSGC1240), and Agriculture Research System of China Project (CARS-49).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2024.741023.

References

Aguilar, I., Misztal, I., Tsuruta, S., Wiggans, G.R., Lawlor, T.J., 2011. Multiple trait genomic evaluation of conception rate in Holsteins. J. Dairy Sci. 94, 2621–2624.
Alexander, D.H., Novembre, J., Lange, K., 2009. Fast model-based estimation of ancestry in unrelated individuals. Genome Res. 19, 1655–1664.
Alfaro, A.C., Nguyen, T.V., Merien, F., 2019. The complex interactions of *Ostreid herpesvirus* 1, Vibrio bacteria, environment and host factors in mass mortality outbreaks of *Crassostrea gigas*. Rev. Aquac. 11, 1148–1168.
Bai, Y., Wang, J., Zhao, J., Ke, Q., Qu, A., Deng, Y., Zeng, J., Gong, J., Chen, J., Pan, Y., Chi, H., Gong, H., Zhou, T., Xu, P., 2022. Genomic selection for visceral white nodules diseases resistance in large yellow croaker. Aquaculture 559.
Blay, C., Haffray, P., D'ambrosio, J., Prado, E., Dechamp, N., Nazabal, V., Bugeon, J., Enez, F., Causeur, D., Eklouh-Molinier, C., 2021. Genetic architecture and genomic selection of fatty acid composition predicted by Raman spectroscopy in rainbow trout. BMC Genomics 22, 1–19.
Burge, C.A., Griffin, F.J., Friedman, C.S., 2006. Mortality and herpesvirus infections of the Pacific oyster *Crassostrea gigas* in Tomales Bay, California, USA. Dis. Aquat. Org. 72, 31–43.
Campos-Montes, G.R., Garcia, B.F., Medrano-Mendoza, T., Caballero-Zamora, A., Montoya-Rodríguez, L., Quintana-Casares, J., Yáñez, J., 2023. Genetic and genomic evaluation for resistance to white spot syndrome virus in post-larvae of Pacific white shrimp (*Litopenaeus vannamei*). Aquaculture 575, 739745.
Chávez-Villalba, J., Villelas-Ávila, R., Cáceres-Martínez, C., 2007. Reproduction, condition and mortality of the Pacific oyster *Crassostrea gigas* (Thunberg) in Sonora, México. Aquac. Res. 38, 268–278.
Chen, S.F., Zhou, Y.Q., Chen, Y.R., Gu, J., 2018. Fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34, 884–890.
Chen, J.Y., Lin, J.J., Yu, F.F., Zhong, Z.M., Liang, Q.W., Pang, H.Y., Wu, S.Y., 2022. Transcriptome analysis reveals the function of TLR4-MyD88 pathway in immune response of *Crassostrea hongkongensis* against *Vibrio Parahemolyticus*. Aquacult. Rep. 25, 101253.
Chi, Y., Li, Q., Liu, S.K., Kong, L.F., 2021. Genetic parameters of growth and survival in the Pacific oyster *Crassostrea gigas*. Aquac. Res. 52, 282–290.
Chi, Y., Jiang, G., Liang, Y., Xu, C., Li, Q., 2022. Selective breeding for summer survival in Pacific oyster (*Crassostrea gigas*): genetic parameters and response to selection. Aquaculture 556, 738271.
Chi, Y., Yang, H., Shi, C., Yang, B., Bai, X., Li, Q., 2023. Comparative transcriptome and gene co-expression network analysis identifies key candidate genes associated with resistance to summer mortality in the Pacific oyster (*Crassostrea gigas*). Aquaculture 577, 739922.
Christensen, O.F., Lund, M.S., 2010. Genomic prediction when some animals are not genotyped. Genet. Sel. Evol. 42, 2.
Cotter, E., Malham, S.K., O'Keefe, S., Lynch, S.A., Latchford, J.W., King, J.W., Beaumont, A.R., Culloty, S.C., 2010. Summer mortality of the Pacific oyster, *Crassostrea gigas*, in the Irish Sea: the influence of growth, biochemistry and gametogenesis. Aquaculture 303, 8–21.
Cutler, R.G., 1991. Human longevity and aging: possible role of reactive oxygen species. Ann. N. Y. Acad. Sci. 621, 1859082.
Daetwyler, H.D., Pong-Wong, R., Villanueva, B., Woolliams, J.A., 2010. The impact of genetic architecture on genome-wide evaluation methods. Genetics 185, 1021–1031.
De Lorgeril, J., Petton, B., Lucasson, A., Perez, V., Stenger, P.L., Dégremont, L., Mitta, G., 2020. Differential basal expression of immune genes confers *Crassostrea gigas* resistance to Pacific oyster mortality syndrome. BMC Genomics 21, 63.
Dégremont, L., Ernande, B., Bedier, E., Boudry, P., 2007. Summer mortality of hatchery-produced Pacific oyster spat (*Crassostrea gigas*). I. Estimation of genetic parameters for survival and growth. Aquaculture 262, 41–53.
Dégremont, L., Garcia, C., Allen Jr., S.K., 2015. Genetic improvement for disease resistance in oysters: a review. J. Invertebr. Pathol. 131, 226–241.
Delaporte, M., Philippe, S., Lambert, C., Jegaden, M., Moal, J., Pouvreau, S., Dégremont, L., Boudry, P., Samain, J., 2007. Characterisation of physiological and immunological differences between Pacific oysters (*Crassotrea gigas*) genetically selected for high or low survival to summer mortalities and fed different rations under controlled conditions. J. Exp. Mar. Biol. Ecol. 353, 45–57.
Dickson, K.B., Zhou, J., 2020. Role of reactive oxygen species and iron in host defense against infection. Front. Biosci. 25, 1600–1616.
Ding, Z., Jin, M., Ren, Q., 2018. Transcriptome analysis of *macrobrachium rosenbergii* intestines under the white spot syndrome virus and poly (I:C) challenges. PLoS One 13, 1–12.
Divilov, K., Merz, N., Schoolfield, B., Green, T.J., Langdon, C., 2023. Marker-assisted selection in a Pacific oyster population for an antiviral QTL conferring increased survival to OsHV-1 mortality events in Tomales Bay. Aquaculture 567, 739291.
Engelhart, D.C., Azad, P., Ali, S., Granados, J.C., Haddad, G.G., Nigam, S.K., 2020. Drosophila SLC22 orthologs related to OATs, OCTs, and OCTNs regulate development and responsiveness to oxidative stress. Int. J. Mol. Sci. 21, 2002–2018.
Essers, J., 2002. Nuclear dynamics of RAD52 group homologous recombination proteins in response to DNA damage. EMBO J. 21, 2030–2037.
Falconer, D.S., Mackay, T.F.C., 1996. Introduction to Quantitative Genetics, 4th edn. Pearson Education Limited, Essex, England.
Feuers, R.J., Weindruch, R., Hart, R.W., 1993. Caloric restriction, aging, and antioxidant enzymes. Mutat. Res. 295, 191–200.
Fleury, E., Huvet, A., 2012. Microarray analysis highlights immune response of Pacific oysters as a determinant of resistance to summer mortality. Mar. Biotechnol. 14, 203–217.
Fleury, E., Moal, J., Boulo, V., Daniel, J.Y., Mazurais, D., Hénaut, A., Corporeau, C., Boudry, P., Favrel, P., Huvet, A., 2010. Microarray-based identification of gonad transcripts differentially expressed between lines of Pacific oyster selected to be resistant or susceptible to summer mortality. Mar. Biotechnol. 12, 326–339.
Garcia, A.L.S., Bosworth, B., Waldbieser, G., Misztal, I., Tsuruta, S., Lourenco, D.A.L., 2018. Development of genomic predictions for harvest and carcass weight in channel catfish. Genet. Sel. Evol. 50, 66.
Glude, J.B., 1975. A summary report of the Pacific coast oyster mortality investigations 1965–1972. In: Proceedings of the third U.S.–Japan meeting on aquaculture at Tokyo, Japan, p. 28. October 15–16, 1974.
Gong, Y., Kong, T., Aweya, J.J., Ma, H., Zhang, Y., Li, S., 2022. P53 ubiquitination comediated by HUWE1 and TRAF6 contributes to white spot syndrome virus infection in crustacean. J. Virol. 96, e0202921.
Gutierrez, Alejandro P., Bean, T.P., Hooper, C., Stenton, C.A., Sanders, M.B., Paley, R.K., Rastas, P., Bryrom, M., Matika, O., Houston, R.D., 2018. A genome-wide association study for host resistance to *Ostreid herpesvirus* in Pacific oysters (*Crassostrea gigas*). G3: Genes Genom. Genet. 8, 1273–1280.
Gutierrez, A.P., Symonds, J., King, N., Steiner, K., Bean, T.P., Houston, R.D., 2020. Potential of genomic selection for improvement of resistance to *ostreid herpesvirus* in Pacific oyster (*Crassostrea gigas*). Anim. Genet. 51, 249–257.
Hayes, B., Goddard, M., 2010. Genome-wide association and genomic selection in animal breeding. Genome 53, 876–883.
Hayes, B.J., Bowman, P.J., Chamberlain, A.C., Verbyla, K., Goddard, M.E., 2009. Accuracy of genomic breeding values in multi-breed dairy cattle populations. Genet. Sel. Evol. 41, 1–9.
Houston, R.D., Bean, T.P., Macqueen, D.J., Gundappa, M.K., Jin, Y.H., Jenkins, T.L., Selly, S.L.C., Martin, S.A., Stevens, J.R., Santos, E.M., 2020. Harnessing genomics to fast-track genetic improvement in aquaculture. Nat. Rev. Genet. 21, 389–409.
Hu, Y., Li, Y., Li, Z., Chen, C., Zang, J., Li, Y., Kong, X., 2020. Novel insights into the selective breeding for disease resistance to vibriosis by using natural outbreak survival data in Chinese tongue sole (*Cynoglossus semilaevis*). Aquaculture 529, 735670.
Koganezawa, A., 1974. Present status of studies on the mass mortality of cultured oysters in Japan and its prevention. In: Proceedings of the Third U.S.-Japan Meeting On Aquaculture. Tokyo, Japan, pp. 29–34. October 15–16, 1974.
Lambert, C., Soudant, P., Degremont, L., Delaporte, M., Moal, J., Boudry, P., Jean, F., Huvet, A., Samain, J.F., 2007. Hemocyte characteristics in families of oysters, *Crassostrea gigas*, selected for differential survival during summer and reared in three sites. Aquaculture 270, 276–288.
Legarra, A., Robert-Granié, C., Manfredi, E., Elsen, J.M., 2008. Performance of genomic selection in mice. Genetics 180, 611–618.
Legarra, A., Aguilar, I., Misztal, I., 2009. A relationship matrix including full pedigree and genomic information. J. Dairy Sci. 92, 4656–4663.
Lewis, T.S., Shapiro, P.S., Ahn, N.G., 1998. Signal transduction through MAP kinase cascades. Adv. Cancer Res. 74, 49–139.
Li, H., Durbin, R., 2009. Fast and accurate short read alignment with burrows-wheeler transform. Bioinformatics 25, 1754–1760.
Li, Q., Yu, H., Yu, R., 2006. Genetic variability assessed by microsatellites in cultured populations of the Pacific oyster (*Crassostrea gigas*) in China. Aquaculture 259, 95–102.
Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., Proc, G.P.D., 2009. The sequence alignment/map format and SAMtools. Bioinformatics 25, 2078–2079.
Li, F., Liu, W., Chen, J., Huang, B., Zheng, Y., Ma, J., Cai, S., Li, L., Liu, F., Wang, X., Wei, L., Liu, Y., Zhang, M., Han, Y., Zhang, X., Wang, X., 2022. CfIRF8-Like Interacts

With the TBK1/IKKε Family Protein And Regulates Host Antiviral Innate Immunity, 132, p. 108497.
Liu, P., Zhang, T., Lv, J., Ma, C., Yang, Z., Huang, X., Zhang, L., Bao, Z., Wang, S., 2021. An efficient integrated approach for nonlethal DNA sampling and genome-wide genotyping in bivalve molluscs. Aquaculture 536, 736489.
Liu, S., Li, L., Shi, R., Wang, W., Wu, F., Zhang, G., 2022. Genome-wide association study for desirable traits in the Pacific oyster *Crassostrea gigas* (Thunberg). Aquac. Res. 56, 4007–4015.
Liu, M., Dai, P., Kong, J., Meng, X., Sui, J., Luo, K., Chen, B., Fu, Q., Cao, B., Cao, J., Luan, S., 2023. Assessing accuracy of genomic breeding values of selection candidates under biosecurity restrictions by progeny testing in Chinese shrimp Fenneropenaeus chinensis. Aquaculture 566, 739181.
Matsunaga-Udagawa, R., Fujita, Y., Yoshiki, S., Terai, K., Kamioka, Y., Kiyokawa, E., Yugi, K., Aoki, K., Matsuda, M., 2010. The scaffold protein Shoc2/SUR-8 accelerates the interaction of Ras and Raf. J. Biol. Chem. 285, 7818–7826.
McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Daly, M., 2010. The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 20, 1297–130.
Meuwissen, T.H., Hayes, B.J., Goddard, M., 2001. Prediction of total genetic value using genome-wide dense marker maps. Genetics 157, 1819–1829.
Misztal, I., Tsuruta, S., Lourenco, D.A.L., Masuda, Y., Aguilar, I., Legarra, A., Vitezica, Z., 2018. Manual for BLUPF90 Family Programs. University of Georgia.
Peñaloza, C., Barria, A., Papadopoulou, A., Hooper, C., Preston, J., Green, M., Helmer, L., Kean-Hammerson, J., Nascimento-Schulze, J.C., Minardi, D., Gundappa, M.K., Macqueen, D.J., Hamilton, J., Houston, R.D., Bean, T.P., 2022. Genome-wide association and genomic prediction of growth traits in the European flat oyster (*Ostrea edulis*). Front. Genet. 13, 926638.
Peng, W., Yu, F., Wu, Y., Zhang, Y., Lu, C., Wang, Y., Huang, Z., Lu, Y., Chen, N., Luo, X., You, W., Ke, C., 2021. Identification of growth-related SNPs and genes in the genome of the Pacific abalone (*Haliotis discus hannai*) using GWAS. Aquaculture 541, 736820.
Price, A., Patterson, N., Plenge, R., Weinblatt, M., Shadick, N., Reich, D., 2006. Principal components analysis corrects for stratification in genome-wide association studies. Nat. Genet. 38, 904–909.
Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., De Bakker, P.I., Daly, M.J., 2007. PLINK: a tool set for whole-genome association and population -based linkage analysis. Am. J. Hum. Genet. 81, 559–575.
Rodríguez-Jaramillo, C., García-Corona, J.L., Zenteno-Savín, T., Palacios, E., 2022. The effects of experimental temperature increase on gametogenesis and heat stress parameters in oysters: comparison of a temperate-introduced species (*Crassostrea gigas*) and a native tropical species (*Crassostrea corteziensis*). Aquaculture 561, 738683.
Ruesink, J.L., Lenihan, H.S., Trimble, A.C., Heiman, K.W., Micheli, F., Byers, J.E., Kay, M. C., 2005. Introduction of non-native oysters: ecosystem effects and restoration implications. Annu. Rev. Ecol. Evol. Syst. 36, 643–689.
Schnare, M., Barton, G.M., Holt, A.C., 2001. Toll-like receptors control activation of adaptive immune responses. Nat. Immunol. 2, 947–950.
Segarra, A., Pépin, J.F., Arzul, I., Morga, B., Faury, N., Renault, T., 2010. Detection and description of a particular *Ostreid herpesvirus* 1 genotype associated with massive mortality outbreaks of Pacific oysters, *Crassostrea gigas*, in France in 2008. Virus Res. 153, 92–99.
Slatkin, M., 2008. Linkage disequilibrium - understanding the evolutionary past and mapping the medical future. Nat. Rev. Genet. 96, 477–485.
Solomieu, V.B., Renault, T., Travers, M.A., 2015. Mass mortality in bivalves and the intricate case of the Pacific oyster, *Crassostrea gigas*. J. Invertebr. Pathol. 131, 2–10.
Song, L., Wang, L., Qiu, L., Zhang, H., 2010. Bivalve immunity. Adv. Exp. Med. Biol. 708, 44–65.
Song, H., Zhang, J., Zhang, Q., Ding, X., 2019. Using different single-step strategies to improve the efficiency of genomic prediction on body measurement traits in pig. Front. Genet. 9, 730.
Song, H., Dong, T., Hu, M., Yan, X., Xu, S., Hu, H., 2022. First single-step genomic prediction and genome-wide association for body weight in Russian sturgeon (*Acipenser gueldenstaedtii*). Aquaculture 561, 738–713.
Song, H., Dong, T., Yan, X., Wang, W., Tian, Z., Sun, A., Dong, Y., Zhu, H., Hu, H., 2023. Genomic selection and its research progress in aquaculture breeding. Rev. Aquac. 15, 274–291.
Suquet, M., De Kermoysan, G., Araya, R.G., et. al., 2009. Anesthesia in pacific oyster, *Crassostrea gigas*. Aquat. Living Resour. 22, 29–34.
Takeuchi, O., Akira, S., 2009. Innate immunity to virus infection. Immunol. Rev. 227, 75–86.
Tang, X., Huang, B., Zhang, L., Li, L., Zhang, G., 2016. TANK-binding kinase-1 broadly affects oyster immune response to bacteria and viruses. Fish Shellfish Immunol. 56, 330–335.
Teslovich, T.M., Musunuru, K., Smith, A.V., Edmondson, A.C., Stylianou, I.M., Koseki, M., Pirruccello, J.P., Ripatti, S., Chasman, D.I., Willer, C.J., Johansen, C.T., Fouchier, S.W., Isaacs, A., Peloso, G.M., Barbalic, M., Ricketts, S.L., Bis, J.C., Aulchenko, Y.S., Thorleifsson, G., Kathiresan, S., 2010. Biological, clinical and population relevance of 95 loci for blood lipids. Nature 466, 707–713.
Thompson, J.W., Nagel, J., Hoving, S., Gerrits, B., Bauer, A., Thomas, J.R, Kirschner, M. W., Schirle, M., Luchansky, S.J., 2014. Quantitative Lys-ε-Gly-Gly (diGly) proteomics coupled with inducible RNAi reveals ubiquitin-mediated proteolysis of DNA damage-inducible transcript 4 (DDIT4) by the E3 ligase HUWE1. J. Biol. Chem. 289, 28942–28955.
Vallejo, R.L., Cheng, H., Fragomeni, B.O., Gao, G., Silva, R.M., Martin, K.E., Evenhuis, J. P., Wiens, G.D., Leeds, T.D., Palti, Y., 2021. The accuracy of genomic predictions for bacterial cold water disease resistance remains higher than the pedigree-based model one generation after model training in a commercial rainbow trout breeding population. Aquaculture 545, 737164.
VanRaden, P.M., 2008. Efficient methods to compute genomic predictions. J. Dairy Sci. 91, 4414–4423.
Verbyla, K.L., Kube, P.D., Evans, B.S., 2021. Commercial implementation of genomic selection in Tasmanian Atlantic salmon: scheme evolution and validation. Evol. Appl. 15, 631–644.
Vezzulli, L., Previati, M., Pruzzo, C., Marchese, A., Bourne, D.G., Cerrano, C., 2010. Vibrio infections triggering mass mortality events in a warming Mediterranean Sea. Environ. Microbiol. 12, 2007–2019.
Visscher, P.M., Hill, W.G., Wray, N.R., 2008. Heritability in the genomics era-concepts and misconceptions. Nat. Rev. Genet. 9, 255–266.
Vu, S.V., Knibb, W., Gondro, C., Subramanian, S., Nguyen, N.T.H., Alam, M., Dove, M., Gilmour, A.R., Vu, I.V., Bhyan, S., Tearle, R., Khuong, L.D., Le, T.S., O'Connor, W., 2021. Genomic prediction for whole weight, body shape, meat yield, and color traits in the Portuguese oyster *Crassostrea angulata*. Front. Genet. 12, 661276.
Wang, K., Li, M., Hakonarson, H., 2010. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 38, e164.
Wang, H., Misztal, I., Aguilar, I., Legarra, A., Muir, W.M., 2012. Genome-wide association mapping including phenotypes from relatives without genotypes. Genet. Res. 94, 73–83.
Wang, Z., Hu, H., Sun, T., Li, X., Lv, G., Bai, Z., Li, J., 2022a. Genomic selection for improvement of growth traits in triangle sail mussel (*Hyriopsis cumingii*). Aquaculture 561, 738692.
Wang, J., Zhao, J., Tong, B., Ke, Q., Bai, Y., Gong, J., Zeng, J., Deng, Y., Lan, B., Zhou, T., Xu, P., 2022b. Effects of artificial mating on genomic selection of resistance against *Cryptocaryon irritans* in large yellow croaker. Aquaculture 561, 738617.
Wray, N., Visscher, P., 2008. Estimating Trait Heritability. Nat. Educ. 1, 29.
Yang, B., Zhai, S., Li, X., Tian, J., Li, Q., Shan, H., Liu, S., 2021. Identification of *Vibrio alginolyticus* as a causative pathogen associated with mass summer mortality of the Pacific oyster (*Crassostrea gigas*) in China. Aquaculture 535, 736363.
Yang, B., Zhai, S., Zhang, F., Wang, H., Ren, L., Li, Y., Li, Q., Liu, S., 2022. Genome-wide association study toward efficient selection breeding of resistance to Vibrio alginolyticus in Pacific oyster, *Crassostrea gigas*. Aquaculture 548, 737592.
Yu, F., Chen, J., Lin, J., Zhong, Z., Lu, Y., Zeng, X., Lei, X., 2023. TLR4 involved in immune response against *Vibrio Parahaemolyticus* by MyD88-dependent pathway in *Crassostrea hongkongensis*. Fish Shellfish Immunol. 134, 108591.
Zhai, S., Yang, B., Zhang, F., Li, Q., Liu, S., 2021. Estimation of genetic parameters for resistance to *Vibrio alginolyticus* infection in the Pacific oyster (*Crassostrea gigas*). Aquaculture 538, 736545.
Zhang, C., Dong, S.-S., Xu, J.-Y., He, W.-M., Yang, T.-L., 2019. PopLDdecay: a fast and effective tool for linkage disequilibrium decay analysis based on variant call format files. Bioinformatics 35, 1786–1788.
Zhong, X.X., Wang, X.Z., Zhou, T., Jin, Y.L., Tan, S.X., Jiang, C., Geng, X., Li, N., Shi, H. T., Zeng, Q.F., Yang, Y.J., Yuan, Z.H., Bao, L.S., Liu, S.K., Tian, C.X., Peatman, E., Li, Q., Liu, Z.J., 2017. Genome-wide association study reveals multiple novel QTL associated with low oxygen tolerance in hybrid catfish. Mar. Biotechnol. 19, 379–390.
Zhou, X., Stephens, M., 2012. Genome-wide efficient mixed-model analysis for association studies. Nat. Genet. 44, 821–824.