The presence of the Pacific Oyster OSHV-1 resistance marker on chromosome 8 does not impact susceptibility to infection by Vibrio *aestuarianus*

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Abstract

The Pacific oyster, *Crassostrea gigas*, is an important species in global aquaculture, but its production is threatened by pathogenic infections such as Ostreid herpesvirus 1 (OsHV-1) and *Vibrio aestuarianus*. A genetic marker for OsHV-1 resistance has been identified on chromosome 8 of the Pacific oyster genome, and selective breeding has been performed to produce OsHV-1-resistant oysters. However, the potential impact of this marker on susceptibility to other pathogens remains unknown. In this study, we assess the effect of the presence and allelic dosage of the OsHV-1 resistance marker on susceptibility to *V. aestuarianus* infections in selectively bred oyster families from the Molluscan Broodstock Program of Oregon State University. Sixteen families were produced with various states of the OsHV-1 resistance marker, and a disease challenge with *V. aestuarianus* was performed on these families to track mortality in each individual. Challenged oysters were genotyped at the OsHV-1 resistance locus, and *Vibrio* susceptibility was compared among genotype groups within and between families. Additionally, double digest reduced representation sequencing (ddRADseq) was performed on challenged oysters, and a genome-wide association study was conducted for survivorship from the *Vibrio* exposure. Results showed that prior to the *Vibrio* exposure, the OsHV-1 resistance marker was not observed at the expected Mendelian inheritance ratios in families, with a significantly reduced presence of the homozygous alternate genotype in 75% of heterozygote family crosses. Among the 16 families, mortality rates differed significantly following exposure to *V. aestuarianus* (range: 85.4% to 47.9%), but there was no association found between the chromosome 8 marker and survival to Vibrio infection. No loci were found to be significantly associated to survivorship to *Vibrio* infection, suggesting Vibrio resistance in these families is not controlled by one or a few loci of large effect.

Keywords: genome-wide association study; OsHV-1; Ostreid herpesvirus 1; Pacific oyster; *Vibrio aestuarianus*; virus.

Introduction

The Pacific oyster *Crassostrea gigas* plays a prominent role in the aquaculture industry, accounting for more than 98% of all oyster production worldwide, with over five million metric tons produced annually (Azéma et al., 2017; Botta et al., 2020; Laurence et al., 2009; Okon et al., 2023). Native to the northwest Pacific Ocean, *C. gigas* has been introduced globally for aquaculture purposes due to its high growth rate and adaptability to varying environments (Zhai et al., 2021). One goal of the aquaculture industry is to supply aquatic-sourced protein to meet human dietary requirements in a fast-growing global population (Yang et al., 2022). However, the aquaculture industry faces substantial setbacks due to pathogenic infections and outbreaks caused by viruses and bacteria (Yang et al., 2022). Since 2008, there has been a global increase in oyster mass mortality events, which has substantially decreased *C. gigas* production in Europe and Australia (Barbosa-Solomieu et al., 2015; Fuhrmann et al., 2019). Given that oysters only possess innate immunity, resistance to pathogens cannot be enhanced long-term through vaccination but rather by immunostimulation, diet, and genetic selection (Divilov et al., 2021; Robinson & Green, 2020; Yang et al., 2022). Oysters are typically cultivated in open environments within straits and bays with complete contact with flow-through seawater, making it impossible to fully control diseases through the administration of disinfectants, antibiotics or similar treatments. Quarantining oysters in open environments is not feasible, and therefore, large-scale pathogen outbreaks are likely. Genetic improvement through selective breeding is considered to be the most practical approach to enhancing disease tolerance and resistance (Green et al., 2023; Zhai et al., 2021).

Ostreid herpesvirus 1 (OsHV-1) is a serious pathogen responsible for large-scale mortality events in Pacific oysters. In 2008, a highly virulent form of OsHV-1 termed OsHV-1 µvar was detected in France (Segarra et al., 2010). Outbreaks of OsHV-1 microvariants have subsequently occurred in Italy, the United Kingdom, Australia, New Zealand and the United States (de Lorgeril et al., 2018; Divilov et al., 2023b; Government of Canada, 2018). OsHV-1 impacts oyster immunity and allows opportunistic bacteria, such as *Vibrio* species, to infect the hosts (Siboni et al., 2024). OsHV-1 can infect all oyster life stages, especially larval and juvenile stages, resulting in up to 100% mortality (de Lorgeril et al., 2018). Therefore, substantial effort has been made to limit the spread of OsHV-1. In 2018, an OsHV-1 µvar was detected in San Diego Bay, California, USA, which poses a significant risk to oyster aquaculture on the Pacific west coast of North America (Thompson et al., 2024). Recent research indicates that this strain is different than the µvar detected in France with a 6 bp deletion in the microsatellite region instead of the 12 bp deletion in addition to other differences compared the OsHV-1 µvar genotype (Pelletier, 2023). Given the threat of OsHV-1, substantial effort has been made to implement strategies to mitigate OsHV-1 spread.

Extensive effort into selective breeding programs has improved the OsHV-1 survivability of Pacific oysters in the USA and Australia, as a potential mitigation strategy against OsHV-1 related mortality (Divilov et al., 2023b; Kube et al., 2018). A quantitative trait locus (QTL) has been identified that is associated with an upregulated interferon regulatory factor (IRF) antiviral gene (i.e., *irf2*) on chromosome 8, which is thought to increase resistance to OsHV-1 infections (Divilov et al., 2021). Oysters with the alternate allele of this locus nay be considered genetically primed to defend against viral infection. This marker has been used in marker-assisted selection by use of a rhAmp genotyping assay (Beltz et al., 2018; Divilov et al., 2023b). Selectively bred families that carry the OsHV-1 resistance marker have been produced by the Molluscan Broodstock Program (MBP) of Oregon State University. However, it is possible that selective breeding can lead to fitness trade-offs beyond growth (Divilov et al., 2024). For example, increased OsHV-1 resilience in offspring of oysters that were primed with an OsHV-1 viral mimic has been reported to co-occur with higher *Vibrio* infection levels in the offspring (Robinson & Green, 2020). This OsHV-1 resilience was also correlated with decreased larval size and altered microbiome composition. This raises the question as to whether virally-resistant oysters, as determined by the presence of resistant alleles, are more susceptible to bacterial pathogens, such as *Vibrio aestuarianus*.

Bacteria from the genus *Vibrio* are prevalent in the marine environment, and several pathogenic strains threaten vertebrates and invertebrates (Wendling et al., 2014). *Vibrio* species can utilize a variety of different niches, including forms that are free-living, mutualistic, opportunistic, or pathogenic. Some *Vibrio* spp. can result in vibriosis, a disease that causes mass oyster mortalities. Common species of *Vibrio* that are pathogenic to oysters include *V. splendidus*, *V. anguillarum*, *V. tubiashii* and *V. aestuarianus* (Wendling et al., 2014). In Europe, *V. aestuarianus* has caused recurrent mortalities since 2001, first in France and then in other European countries, including Italy, Ireland, and Spain (Mesnil et al., 2023). In recent years, the frequency of mortality events in France has risen from 30% in 2011 to 77% in 2013, where it has emerged as the primary pathogen identified during summer mortality events (Coyle et al., 2023). In British Columbia, Canada, *V. aestuarianus* is one of the most common pathogenic *Vibrio* species and is emerging as a serious threat to oyster aquaculture operations (Cowan et al., 2024). The effects of the presence of the OsHV-1 resistance allele on oyster survivability to *Vibrio* infection remains unknown, warranting further investigation. Loci have been found that are associated with increased survival to *Vibrio alginolyticus* in Pacific oysters (Yang et al., 2022). Using a genome-wide association study (GWAS) approach to explore allelic differences between resilient and susceptible oysters may also provide insight into the genes associated with higher survival during *V. aestuarianus* infection.

In this study, we evaluated the susceptibility of oysters selected for OsHV-1 resistance to *V. aestuarianus* infection by assessing the effects of the presence and allelic dosage of the resistance allele

for OsHV-1. Given the functional differences in the immunological responses to viruses and bacteria (Green et al., 2016), we hypothesize that there will be immunological trade-offs between resistance to viral and bacterial pathogens may be observed. Additionally, using the *Vibrio* exposed oysters from four of the MBP families and one family from the Vancouver Island University (VIU) Pacific oyster breeding program, we performed double digest RAD-sequencing (Peterson et al., 2012) to determine whether any loci are associated with *Vibrio* resistance. This work provides insight into how genetic predispositions towards resistance against one pathogen (OsHV-1) might affect the vulnerability of Pacific oysters to other pathogens, specifically *Vibrio* species.

Methods

Samples and crosses

Pacific oyster families with various genotypic states of the chromosome 8 (CHR8) resistant marker (i.e. position 9,719,736 on chromosome 8; Divilov et al., 2023) were generated by Oregon State University's Molluscan Broodstock Program (Oregon, USA). Spat from 16 of these families were provided to Vancouver Island University (VIU) to be used in experimental exposures to *V. aestuarianus*. Spat were 18 weeks old, greater than 4 mm long, and less than 6 mm wide. Animals were originally transported to Deep Bay Marine Field Station (VIU; British Columbia, Canada), where they were placed overnight into 12.5 ^{o}C flow-through seawater tanks, after which they were transported to the Center for Shellfish Research (CSR) at the campus of Vancouver Island University (VIU) in Nanaimo, BC. A sample of seawater and algae (*Chaetoceros muelleri*) was additionally collected from Deep Bay Marine Field Station and also transported to VIU.

Four of the 16 families were designated as 'mapping families' (i.e., F114-F117) based on the allelic state of the parents at the CHR8 marker (i.e., both parents were heterozygous). The other 12 families were designated as 'non-mapping families,' which had at least one homozygous reference parent (F101-F103, F105-F113; Table S1). None of the families were produced by crosses where both parents were homozygous for the alternate allele (i.e., the allele that confers resistance). Additionally, one family from the VIU oyster breeding program (Oyster Family Run 6, family 10; OFR6-10) was also included in the *Vibrio* exposure. OFR6-10 has not been selected for OsHV-1 resistance.

Disease challenge with Vibrio aestuarianus

A disease challenge was performed using *V. aestuarianus* to determine whether oysters with different genotypic states of the OsHV-1 resistance locus were differentially susceptible to infection by *V. aestuarianus*. Forty-eight spat from each of the 16 MBP families and the VIU family (total n=768) were exposed to *V. aestuarianus*. A mixture of samples from multiple families also was treated in the same way as the exposed oysters but without exposure to *Vibrio* and this was used as a non-exposed control group (n = 48 animals). For the exposure, spat were placed in 12 well plates, where each well contained 3 mL seawater and one spat. Spat in plates were acclimatized overnight in a temperature-controlled room at 16 ^{o}C.

A *Vibrio* suspension was prepared by inoculating *V. aestuarianus* (strain: BS032 2018) in two flasks containing TSB + 2% NaCl overnight in an orbital shaker (23 ^{o}C and 200 rpm). The cultures were then removed from the incubator, transferred into Falcon tubes, and centrifuged (5000 x g) at ambient temperature for 5 min. The supernatant was poured off, and cell pellets were resuspended in seawater. The optical density of the resuspended cells was measured at 600 nm using an Eppendorf BioSpectrometer on an Eppendorf µCuvette® G1.0. A standard curve was prepared to estimate the amount of *V. aestuarianus* in the preparation.

The exposure trial was initiated by adding 300 µL of the *V. aestuarianus* suspension (i.e., 7.35×10^{7} total cells) to each well on each experimental plate. Sterile seawater was added to the control plate. The temperature of the room was then increased to 23 ^{o}C for 5 days to simulate a marine heatwave event, and temperature was reduced to 16 ^{o}C on the sixth day of exposure (Green et al., 2019). After *Vibrio* inoculation, 100 µL of algae (*C. muelleri*) was added to each well of all plates, including the control plate. Morbidity was measured daily for six days, after which time the disease challenge was ended. Oysters were considered moribund or deceased when their shell valves were gaping and failed to close after the oysters were gently touched with a sterile toothpick. Oysters were placed into 25 mL Falcon tubes containing 100% ethanol, where whole individuals were pooled in the storage tube depending on their day of death and family. After six days, surviving oysters were preserved and stored in 100% ethanol (as described for dead oysters). Ethanol-preserved oysters were stored at room temperature until DNA extraction. Mortality data was analyzed using IBM SPSS Statistics v28.0.1.0 (142) (IBM Corp., Armonk, NY) and R (R Core Team, 2024). Mortality distributions were assessed using a Kaplan-Meier survival analysis. A log-rank test was conducted to determine the difference in survival distributions among families, and a pairwise log-rank test was performed to determine which families had different survival distributions.

Genomic DNA extraction

DNA extractions were performed on oysters from the mapping families (i.e., F114-F117) to extract total genomic DNA using a Monarch® Genomic DNA Purification Kit (#T3010L) according to manufacturer instructions. Briefly, oyster spat samples were extracted from their shells using sterilized forceps and rinsed with deionized water to remove residual ethanol. Spat tissue was then placed into a digestive solution containing lysis buffer and proteinase K (NEB). Samples were homogenized using a sterile pestle and digested at 56 ^{o}C for 2 hours, after which an RNase incubation was performed. Genomic DNA was purified through an extraction column as per manufacturer's guidelines and eluted in 35 µL molecular grade water held at 60 ^{o}C. Quantification of DNA was performed via absorbance and Qubit fluorimetry using an Eppendorf BioSpectrometer on an Eppendorf µCuvette® G1.0. Samples were checked for purity using the A260/A280 and A260/A230 ratios and quality by 1% agarose gel electrophoresis. Extracted genomic DNA was then stored at -20 ^{o}C.

OsHV-1 resistance allele dosage by rhAmp SNP genotyping assay

The rhAmp SNP assay (Design ID: CD.GT.ZKJS3200.1; Integrated DNA Technologies) targeting a G/A (ref/alt) SNP on position 9,719,736 on chromosome 8 (Divilov et al., 2023b) was used to genotype the OsHV-1 resistance marker in all exposed individuals in the mapping families (F114-F117). The assay was conducted on a CFX96 Touch Real-Time PCR Detection System (BIO-RAD) using the manufacturers' instructions for a 5 µL reaction volume in 96-well plates (Table S2). Samples were normalized to 10 ng in 2 µL molecular water (i.e., 5 ng/µl), and each sample was run in duplicate. Negative controls were also run on each plate, containing only molecular-grade water. The fluorophore dyes FAM and VIC were used for genotyping the reference and alternate alleles, respectively, and to call genotypes as homozygous reference, heterozygous, or homozygous alternate.

Results of the rhAmp assay were exported from the instrument in text format and processed in R v4.3.2 using a custom script (see *Data Availability*). As some likely false positives were observed, where one dye was detected at a much later Cq value than the other detected dye, a false positive heterozygote correction was applied. Specifically, Cq differences between FAM and VIC fluorophores were calculated per well, and any cases where the absolute value of the Cq difference between the two fluorophores was greater than four, the call was determined to be a false positive for the later Cq fluorophore, and the later fluorophore was set to zero, making the call a homozygous call for the earlier fluorophore (Figure S1 and Figure S2). Using the corrected data, genotypes were assigned depending on the retained fluorophore calls: FAM only = homozygous reference; VIC only = homozygous alternate; both FAM and VIC =

heterozygote; no fluorophore call = missing. Technical reproducibility was evaluated at the level of the genotype (Table S3), and the final call for technical replicates was based on the majority call, as inconsistent calls were re-run. Results of the genotyping were plotted and analyzed in R (see *Data Availability*).

Statistical analysis of chromosome 8 genotypes was carried out using IBM SPSS Statistics v28.0.1.0 (142) and R. The effect of the OsHV-1 resistance marker genotype on mortality was analyzed using a chi-square test of homogeneity (or Fisher's exact test if the chi-square assumptions could not be met). To use the chi-square test, samples had to meet the following criteria: one dependent variable measured at the dichotomous level (mortality: dead vs. alive or time until death), one independent variable with three or more categorical groups (genotype: *AA*, *Aa*, *aa*), and independence of observations. Finally, the sample had to meet a minimal sample size requirement. Minimal sample size with respect to expected frequencies was required to have an expected count greater than or equal to five. If samples did not meet this last criterion, a Fisher's exact test was performed instead of the chi-squared test. Genotype frequencies in each family, independent of mortality phenotype, were assessed using the same method to determine whether there were significant differences in baseline genotype frequencies across families.

Double-digest RAD sequencing (ddRADseq) sample preparation and read processing

Genomic DNA from the OSU mapping families (i.e., F114-F117) and their respective parents, and OFR6 Family 10 from the VIU breeding program were used for double-digest restriction site associated DNA sequencing (ddRADseq), which was carried out at the Institut de Biologie Intégrative et des Systems (IBIS) at Laval University, Quebec, Canada, as previously described (Sutherland et al., 2020) using *Nsi*I/ *Msp*I as cutting enzymes. Samples were pooled with 32 individuals per sequencing run and sequenced on an Ion Torrent Proton using a ThermoFisher Ion PI Chip Kit v3 chip, as per manufacturers' instructions. Base calling was performed using Torrent Suite 5.10.1 (ThermoFisher), and multiplexed FASTQ output files with barcodes still present were exported using the Torrent FileExporter plugin.

Sequence data processing and analysis were conducted using the repository *stacks_workflow* (Normandeau, 2013/2023) and Stacks v.2.3e (Rochette et al., 2019), accompanied by custom analysis scripts (see *Data Availability*). The quality of all FASTQ files was assessed before and after trimming using FastQC v0.11.8, and the results were consolidated using MultiQC (Andrews, 2010; Ewels et al., 2016). To prepare the data, universal adapters were eliminated and reads longer than 50 bp were retained using cutadapt v1.18 run in parallel (Martin, 2011; Tange, 2015). Quality trimming was performed on all reads with Phred >10 in sliding windows of size 15% of the read length, followed by truncation to 80 bp

and demultiplexing using the *process_radtags* function of Stacks (Catchen et al., 2011; Rochette et al., 2019) with two enzymes selected (i.e., *Nsi*I and *Msp*I) in parallel.

Demultiplexed and trimmed single-end reads per individual were aligned to a chromosome-level genome assembly for Pacific oyster (i.e., GCF_902806645.1_cgigas_uk_roslin_v1_genomic.fna) (Peñaloza et al., 2021) using bwa mem v0.7.12-r1039 (Li et al., 2009; Peñaloza et al., 2021). Samtools v1.9 was used to remove unmapped reads and secondary or low-quality alignments (Li et al., 2009). Remaining reads were used for genotyping with Stacks. The total number of reads per sample, total read alignments, and mapping percentage were calculated using samtools and custom scripts (Li et al., 2009).

Loci were identified with Stacks using the reference-based approach through the *gstacks* module with default settings. Genotype data was filtered using the *populations* module of Stacks, requiring that a locus is genotyped in at least 70% of the individuals in all six populations (i.e., F114-F117, MBP parents, and OFR6.10), and with a minimum global minor allele frequency (MAF) of 0.01. Samples with fewer than 1 M reads were identified and removed from the analysis, and samples were re-genotyped again (as described above) to evaluate the effect of removing low read count samples. Outlier samples, if detected, were identified by principal component analysis (PCA). Data were exported both as all variants (i.e., multiple per RAD-tag) and a single SNP per RAD-tag data using the *populations* module in VCF and plink formats. Plink data were transformed from .ped and .map to .raw formats using plink (Purcell, 2022; Purcell et al., 2007). Data was loaded into R using the *read.plink* function of adegenet (Jombart & Ahmed, 2011). The data was formatted and converted to genind format using the *df2genind* function from the adegenet package. Functions from the *simple_pop_stats* repository (see *Data availability*) were employed for analysis. The genotyping rate per sample was determined using the *percent_missing_by_ind* function from *simple_pop_stats*, retaining individuals with less than 30% missing data. Minor allele frequency (MAF) was re-calculated per locus, and loci with MAF < 0.01 were excluded. H_{OBS} and Hardy–Weinberg (HW) equilibrium filters were not applied due to anticipated family effects.

Genome-wide association study (GWAS)

A GWAS for survivorship from the *Vibrio* exposure was conducted using GEMMA v0.98.5 (Zhou & Stephens, 2012) using the single-SNP per locus VCF data set and the plink map file in R (see *Data Availability*). NCBI chromosome identifiers were converted to linkage groups using the Pacific oyster assembly *Cgigas_uk_roslin_v1*, and then linkage groups were converted to chromosomes following Peñaloza et al. (2021). Samples were separated into individual families and imputed using *LinkImputeR* (Money et al., 2017) and the *missMethods* R package (Rockel, 2022). The family identity was used as a

covariate in the model, and a GWAS was run using the phenotype mortality assessed as a dichotomous function (i.e., dead or alive at end of trial) or based on days-to-death, setting the survivors to one day past the end of the trial (i.e., day 7). A Bonferroni-corrected *p*-value of 0.05 was used as the genome-wide significance threshold. All families were analyzed together, with the family covariate applied. SNPs were coded 0, 1, and 2 to correspond to homozygote reference, heterozygote, and homozygote alternate genotypes, respectively. The R package *fastman* (Paria et al., 2022) was used to generate Manhattan plots from the GWAS analysis.

Results

Basal frequency of OsHV-1 resistance marker genotypes per family

Based on all oysters in the exposure trial (n = 48 per family), the CHR8 marker genotype frequencies per mapping family (i.e., F114-F117) differed slightly from the expected proportions based on Mendelian inheritance from the heterozygous parents (i.e., 1:2:1) for homozygous reference, heterozygous, and homozygous alternate. Heterozygotes were the most frequently observed genotype across all families (57%), followed by homozygous reference (25%), and the homozygous alternate genotypic state was the rarest (18%; Figure 1). When inspecting individual families (Figure 1), a skew was observed where family F115 had significantly more homozygous alternate genotypes (35%), and families F114 and F117 had significantly fewer homozygous alternate genotypes (11% and 7%, respectively; χ^2 test of homogeneity $p<0.05$). All other genotypes were not significantly different among the families (Table S4).

Mortality in Vibrio aestuarianus disease challenge

Deaths were tracked daily following the exposure to *V. aestuarianus* during the disease challenge experiment. No mortality was observed in the first two days post-exposure (DPE). Considering all families together, 37.8% of oysters died on three DPE, and by six DPE, 68.2% of all exposed oysters had died (n = 516) (Figure S3). The remaining 252 exposed oysters (32.8%) (not including controls) survived the disease challenge.

Considering cumulative mortality per family, the mortality rate during the disease challenge was on average (± s.d.) 67.2 ± 9.5% (Figure 2). Family F106 exhibited the highest mortality (85.4%), and family F116 exhibited the lowest mortality (47.9%). Rates of mortality also varied among families ($p<0.001$; Figure 3), and pairwise differences were observed between family F106 and families F103, F113, F114, F115, and F116 ($p < 0.003$; Table S5; Bonferonni correction significance threshold $p = 0.003$). Offspring of the family that had the highest and most rapid mortality (F106) would be expected to

all be heterozygous for the CHR8 marker, whereas F103 and F113 (i.e., low mortality families) would all be expected as homozygous for the reference allele (Table S1). Offspring of F114, F115, and F116 would be comprised of all three CHR8 marker genotypic states (Table S1). Families grouped by cross-types did not reveal a consistent overall mortality trend (Figure 2). The control group exhibited 100% survival.

Effects of OsHV-1 CHR8 resistance allele on survival to Vibrio challenge

The effects of the presence and dosage of the CHR8 resistance allele on mortality for families F114-F117 were evaluated based on days to death (Figure 4) or survival (dead or alive; Figure 5). No significant associations were observed between offspring genotype and mortality for any of the families considering days to death, nor dead or alive ($p>0.05$). The analysis was conducted on each family individually to avoid potential confounding interfamily effects.

Bioinformatics and genotyping

On average, 92.8 M single-end reads were provided per chip from the six chips sequenced (n = 523.1 M reads total). Demultiplexing resulted in an average of 91.8% of reads retained. On average (± s.d.), 65.0 ± 2.2% of reads aligned against the chromosome-level reference genome (Peñaloza et al., 2021) and 340,291,536 alignments were used for genotyping with gstacks. In total, 277.6 M primary alignments (81.6%) were retained (10.9% of alignments were discarded due to insufficient MAPQ and 7.5% due to excessive soft clipping). On average, 1.79 M alignments were obtained per sample (range: 0.265-4.681 M records per sample). Samples with fewer than 1 M reads were removed, resulting in the removal of 17 of the 191 total samples (Table S6). Notably, 14 of the 17 removed samples were oysters that died during the trial.

After low-yield samples were removed, genotyping used 330.6 M alignments (269.7 M primary alignments) to build 393,153 RAD loci to an average (± s.d.) effective coverage of 56.9 ± 22.6x. Loci were filtered to keep those that were genotyped in at least 70% of the individuals in all families and with a minor allele frequency (MAF) > 0.01. This resulted in the retention of 10,202 RAD loci comprised of 889,291 genomic sites, of which 25,801 contained variants (percent polymorphic = 2.9%). Considering only a single SNP per RAD locus resulted in 8,252 variants retained. Individuals missing more than 30% of loci were removed, leaving 168 retained individuals (Figure S4). This included the retention of 32, 36, 35, 33, and 25 individuals from F114, F115, F116, F117, and OFR6.10, respectively, as well as seven of the eight parents of families F114-F117. Reapplying the MAF filter after individuals with low genotyping

rates were removed resulted in a total of 8,193 SNPs retained, of which 4,218 are MAF > 0.1. Visualizing the samples clustered by PCA based on genotypes shows close clustering of samples within the four MBP families, with the parents of these families interspersed among the offspring and the VIU family grouping together but apart from the MBP families on PC1 (Figure S5).

Genome-wide association study for Vibrio resistance

GWAS analyses using survival to the *Vibrio* infection as a dichotomous variable (dead or alive) or as days to death did not identify any associated loci associated with *Vibrio* resistance (Figure 6; Figure S6). Both analyses included 4,559 SNPs with MAF > 0.05 (n = 120 individuals). The percent variation explained (PVE) ± standard error by the GWAS when assessing survival as a dichotomous variable was 45.1 ± 18.0%, and when assessing survival as days-to-death was 39.7 ± 25.2%.

Discussion

Host genetic and epigenetic factors are known to influence disease interactions (Krickeberg et al., 2012; Magwire et al., 2012). In this study, we assessed if the presence of a resistance marker for a virus would affect the host's susceptibility to a pathogen from a different biological domain (*i.e.* bacterial). Our results suggest that the presence and allelic dosage of the OsHV-1 resistance marker (*i.e.*, the CHR8 marker) in *C. gigas* does not affect susceptibility to *Vibrio aestuarianus* infections. This provides some important insights for disease resistance selective breeding in this species. It may also suggest an absence of genetic correlation between OsHV-1 resistance and *V. aestuarianus*, at least regarding the OsHV-1 resistance mechanism associated with the CHR8 locus. Given the independence observed, identifying loci involved in resistance to *Vibrio aestuarianus* may result in the ability to select for dual-resistant oysters (i.e., those resistant to both OsHV-1 and *Vibrio aestuarianus*), an outcome that would bring significant benefits to the field.

Effects of the OsHV-1 resistance allele on survival to Vibrio aestuarianus infection

The presence of the OsHV-1 resistance allele had no effect on the survival to *V. aestuarianus* infection. This finding is supported by another study that reported the absence of genetic correlations between OsHV-1 and *V. aestuarianus* resistance (Azéma et al., 2017). Azéma et al. analyzed the genetic parameters for resistance to OsHV-1 and *V. aestuarianus* at three different life stages: spat (3 to 6 months

old); juvenile (11 to 15 months old); and adult (25 to 26 months old). They reported no observable genetic correlation between survival to viral infection and survival to bacterial infection. However, the life stage of the oyster significantly affected susceptibility to either pathogen; susceptibility to OsHV-1 decreases with age and growth, while susceptibility to *V. aestuarianus* increases with age and growth. The different impacts of age on susceptibility to OsHV-1 and *Vibrio* potentially reflect the different immune responses from the oyster host. Further evidence of the independence of the resistance mechanisms was shown by Green et al. (2016), finding different transcriptome responses to OsHV-1 and *V. aestuarianus* infections. Thus, the immune genes involved in defence to OsHV-1 and *Vibrio* infection appear to be distinct.

The absence of a correlation between OsHV-1 resistance marker genotype and *Vibrio* susceptibility suggests that the mechanisms of resistance to these pathogens are independent. The OsHV-1 resistance allele is thought to upregulate *interferon regulatory factor 2* (*irf2*), an antiviral gene that can provide oysters with constitutively upregulated antiviral immunity (Divilov et al., 2023b). With constitutively overexpressed *irf2*, the overexpression of antiviral genes could be expected even in the absence of the virus. Therefore, the lack of increased susceptibility to *Vibrio* is notable, as it suggests that the upregulated antiviral immunity does not negatively or positively impact the oyster's ability to resist bacterial infection. Genetic mechanisms behind *Vibrio* resistance are not well known; however, a recent study using 52 Pacific oyster families found a total of 18 SNPs in three QTL associated with resistance to *Vibrio alginolyticus* in *C. gigas*, and the authors suggest that *Vibrio* resistance is polygenic (Yang et al., 2022). The majority of these genes were related to pathogen recognition and immune defence response (Yang et al., 2022). Collectively, genes that induce the expression of immune-related genes provide potential targets for selective breeding for increased *Vibrio* resilience.

Selective breeding for one trait can lead to fitness trade-offs by negatively impacting a second trait and consequently impacting overall fitness. Our understanding of the compromises in selection for disease resistance is limited, particularly regarding the impacts on other traits or resistance to other pathogens. Trade-offs can arise when beneficial traits are linked to detrimental ones, affecting fitness outcomes (Gallardo-Hidalgo et al., 2021). For example, although selection for increased growth rate has also improved larval resilience to ocean acidification, fast-growing oysters later in life are more susceptible to marine heatwaves and *V. aestuarianus* infections; mortality was found to increase by 76% per additional gram of body weight gained (Khtikian, 2021; Nordio et al., 2021). On the other hand, selection for OsHV-1 resistance in the MBP breeding population has not been observed to impact growth (Divilov et al., 2024). Selection for disease resistance is complex, as resistance is expected to be influenced by multifactorial biological functions, including reproduction and immunity (Bai & Plastow, 2022; Wang et al., 2021).

We expected a trade-off because the immune system upregulation increases the energetic requirements of the host (Lochmiller & Deerenberg, 2000; Robinson & Green, 2020). This can reduce overall reproductive success, growth rates, and survival to events such as pathogen outbreaks (Lochmiller & Deerenberg, 2000; Robinson & Green, 2020). Additionally, in some cases, over-reactive immune responses to pathogens can be more damaging than a weak immune response (Green et al., 2016), as discussed in the concept of immune tolerance (Råberg et al., 2007). The expression of extremely potent antibacterial or antiviral effectors can result in a toxic cellular environment that can be detrimental to the organism (Green et al., 2016), and strong immune effectors and responses can inflict damage to the tissues of the host. In the case of infectious diseases, a fitness trade-off might occur if the heightened immune response leads to greater collateral damage (Råberg et al., 2007). Furthermore, other environmental stress factors can reduce immune competence due to energetic constraints (Sutherland et al., 2014). Here, we did not measure other factors that may represent energetic trade-offs for increased viral resistance (e.g., growth rate, response to other stressors), but these factors could be integrated into future studies to evaluate the impact of the OsHV-1 marker on *Vibrio* resistance. Notably, before the *Vibrio* exposure occurred, there were basal differences in genotype frequencies of the CHR8 marker, specifically in the proportion of the homozygous alternate genotype. There may therefore be other unknown factors causing distortions in the frequency of this marker, e.g., potentially the presence of multiple linked QTL on chromosome 8 (Divilov et al., 2023a). Other distorting factors could impact future breeding practices around the CHR8 marker.

Although we did not observe a trade-off, selection for disease resistance may also indirectly increase resistance to other stresses, such as marine heat waves (MHW). MHWs increase water temperature, resulting in high mortality rates (Cowan et al., 2024). Vibriosis and *V. aestuarianus* proliferation are further linked to seawater temperature, with increased temperature increasing host susceptibility by weakening the immune system in addition to increasing the transmission and proliferation of the pathogenic *Vibrio* (Cowan et al., 2024; Wendling et al., 2014). MHWs are a consequence of climate change, which is driven by increased anthropogenic emissions of CO_2. The global average MHW frequency and duration increased by 34% and 17%, respectively, resulting in a 54% increase in annual marine heatwave days globally (Oliver et al., 2018). Bolstering resilience to MHWs may occur by producing an oyster with increased resistance to viral and bacterial pathogens. This can mitigate the detrimental effects of these temperature spikes on oyster populations. This dual-pathogen resistance strategy could serve as a multifaceted approach to bolstering oyster health and ensuring the sustainability of oyster aquaculture in the face of various environmental stressors.

Family effects and using GWAS to detect loci associated with resistance to Vibrio aestuarianus

In addition to investigating the potential effects of the presence and dosage of the OsHV-1 resistance allele within each family, we also analyzed family effects on the survival to *Vibrio* infection. Family differences were observed in both the per family cumulative mortality and mortality rates. Although different families have known OsHV-1 genotypes, they also would have other genetic differences that could impact resistance to *V. aestuarianus*. Since the OsHV-1 resistance marker did not explain the variance observed across families in *Vibrio* susceptibility, other factors may be involved.

In the present GWAS analysis, we did not identify any loci significantly associated with mortality or mortality rate in response to *V. aestuarianus* infection. However, our results suggest there is a trend towards significance on chromosome 2 (Figure 6). It is possible that our sample size was too low to detect a more subtle genetic association, such as that which would occur if resistance was polygenic. Resistance to *Vibrio* infections has previously been reported to exhibit polygenic architecture (Yang et al., 2022), and therefore could require a larger sample size to identify a significant association than would oligogenic or monogenic resistance mechanisms. It is also possible that the differences in resistance across families could involve epigenomic differences impacting immune or microbiome responses to bacterial infection. A recent GWAS for survival to Pacific oyster mortality syndrome indicated that epigenetic factors play a larger role than genetic factors for this disease (Gawra et al., 2023) and this could also be the case for *V. aestuarianus* infection. Future studies may benefit from including a larger number of individuals to account for the potential polygenic nature of *Vibrio* the resistance mechanisms.

Conclusion

In this study, we found that genotypes of families from the MBP breeding population at the OsHV-1 resistance marker do not affect susceptibility to *V. aestuarianus* infections in either overall mortality (i.e., dead or alive) or rate of mortality (i.e., days-to-death). This suggests that the OsHV-1 resistance allele does not specifically increase the susceptibility of *C. gigas* to *Vibrio* infection and, therefore, opens the potential for selecting for resistance to both pathogens. We observed significant family differences in *Vibrio* susceptibility, even within the MBP breeding program, which has been selectively bred for at least 6 generations (de Melo et al., 2016), and is therefore expected to be more genetically homogenous than other outbred oysters. These observations emphasize the need for further investigation into the role of genetics in *Vibrio* susceptibility among Pacific oysters. Given that no negative correlations were observed between resistance to the two pathogen types, the next steps for breeding programs may involve working towards dual-resistant oysters that can withstand OsHV-1 and *V. aestuarianus* infections. This should

enhance the health and resilience of cultured *C. gigas* and improve oyster aquaculture as a sustainable marine resource.

Supplementary Materials

Supplementary information for this article is provided in the attached file:
OSU_CHRM8_Manuscript_SI_2024-10-07.docx

Data Availability

Raw sequencing data have been uploaded to SRA under BioProject PRJNA1145972 with BioSamples SAMN43080749 - SAMN43080938.

FigShare RADseq genotypes and supporting files: 10.6084/m9.figshare.26524321

FigShare rhAmp assay raw data plate files: 10.6084/m9.figshare.26515438

This analysis was supported by the following repositories:

Manuscript code and README: https://github.com/bensutherland/ms_cgig_chr8_vibrio

simple_pop_stats repository: https://github.com/bensutherland/simple_pop_stats

Additional material is included on figshare, https://doi.org/10.6084/m9.figshare.26515438.v1

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Conflicts of Interest

Conflicts of interest: The authors declare no conflicts of interest

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Conceptualization, L. B. Surry, B. J.G. Sutherland, and T. J. Green; Methodology, L. B. Surry, B. J.G. Sutherland, T. J. Green, and K. Divilov; Software, L. B. Surry, B. J.G. Sutherland, and K. Divilov; Validation, L. B. Surry, B. J.G. Sutherland, and T. J. Green; Formal Analysis, L. B. Surry and B. J.G. Sutherland; Investigation, L. B. Surry, S. L. Lunda, A. Loudon and T. J. Green.; Resources, T.J. Green, C. J. Langdon, K. Divilov, and S. Lunda; Data Curation, B J.G. Sutherland; Writing – Original Draft Preparation, L. B. Surry and B J.G. Sutherland.; Writing – Review & Editing, B J.G. Sutherland, T. J. Green, C. J. Langdon, K. Divilov, and A. Loudon.; Visualization, L. B. Surry and B. J.G. Sutherland; Supervision, T. J. Green and Ben J.G. Sutherland.; Project Administration, T. J. Green and C. J. Langdon; Funding Acquisition, C. J. Langdon and T. J. Green.

References

Andrews, S. (2010). *Babraham Bioinformatics—FastQC A Quality Control tool for High Throughput Sequence Data*. https://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Azéma, P., Lamy, J.-B., Boudry, P., Renault, T., Travers, M.-A., & Dégremont, L. (2017). Genetic parameters of resistance to Vibrio aestuarianus, and OsHV-1 infections in the Pacific oyster, Crassostrea gigas, at three different life stages. *Genetics, Selection, Evolution : GSE*, *49*, 23. https://doi.org/10.1186/s12711-017-0297-2

Bai, X., & Plastow, G. S. (2022). Breeding for disease resilience: Opportunities to manage polymicrobial challenge and improve commercial performance in the pig industry. *CABI Agriculture and Bioscience*, *3*(1), 6. https://doi.org/10.1186/s43170-022-00073-y

Barbosa-Solomieu, V., Renault, T., & Travers, M.-A. (2015). Mass mortality in bivalves and the intricate case of the Pacific oyster, Crassostrea gigas. *Journal of Invertebrate Pathology*, *131*, 2–10. https://doi.org/10.1016/j.jip.2015.07.011

Beltz, K., Tsang, D., Wang, J., Rose, S., Bao, Y., Wang, Y., Larkin, K., Rupp, S., Schrepfer, D., Datta, K., Gunderson, K., Sailor, C., Hansen, S., Dobosy, J., Lewis, L., Menezes, A., Walder, J., Behlke, M., & Chen, C. (2018). A High-Performing and Cost-Effective SNP Genotyping Method Using rhPCR and Universal Reporters. *Advances in Bioscience and Biotechnology*, *09*, 497–512. https://doi.org/10.4236/abb.2018.99034

Botta, R., Asche, F., Borsum, J. S., & Camp, E. V. (2020). A review of global oyster aquaculture production and consumption. *Marine Policy*, *117*, 103952. https://doi.org/10.1016/j.marpol.2020.103952

Catchen, J. M., Amores, A., Hohenlohe, P., Cresko, W., & Postlethwait, J. H. (2011). Stacks: Building and Genotyping Loci De Novo From Short-Read Sequences. *G3 Genes|Genomes|Genetics*, *1*(3), 171–182. https://doi.org/10.1534/g3.111.000240

Cowan, M. W., Pearce, C. M., Green, T. J., Finston, T., Meyer, G. R., McAmmond, B., Van Hamme, J. D., Bottos, E. M., Marshall, R., Evans, W., Sutherland, T. F., & de la Bastide, P. Y. (2024). Abundance of Vibrio aestuarianus, water temperature, and stocking density are associated with summer mortality of Pacific oysters in suspended culture. *Aquaculture International*. https://doi.org/10.1007/s10499-024-01415-5

Coyle, N. M., O'Toole, C., Thomas, J. C. L., Ryder, D., Feil, E. J., Geary, M., Bean, T. P., Joseph, A. W., Waine, A., Cheslett, D., & Verner-Jeffreys, D. W. (2023). Vibrio aestuarianus clade A and clade B isolates are associated with Pacific oyster (Magallana gigas) disease outbreaks across Ireland. *Microbial Genomics*, *9*(8), mgen001078. https://doi.org/10.1099/mgen.0.001078

de Lorgeril, J., Lucasson, A., Petton, B., Toulza, E., Montagnani, C., Clerissi, C., Vidal-Dupiol, J., Chaparro, C., Galinier, R., Escoubas, J.-M., Haffner, P., Dégremont, L., Charrière, G. M., Lafont, M., Delort, A., Vergnes, A., Chiarello, M., Faury, N., Rubio, T., … Mitta, G. (2018). Immune-suppression by OsHV-1 viral infection causes fatal bacteraemia in Pacific oysters. *Nature Communications*, *9*(1), Article 1. https://doi.org/10.1038/s41467-018-06659-3

de Melo, C. M. R., Durland, E., & Langdon, C. (2016). Improvements in desirable traits of the Pacific oyster, *Crassostrea gigas*, as a result of five generations of selection on the West Coast, USA. *Aquaculture*, *460*, 105–115. https://doi.org/10.1016/j.aquaculture.2016.04.017

Divilov, K., Merz, N., Schoolfield, B., Green, T. J., & Langdon, C. (2023a). Genome-wide allele frequency studies in Pacific oyster families identify candidate genes for tolerance to ostreid herpesvirus 1 (OsHV-1). *BMC Genomics*, *24*(1), 631. https://doi.org/10.1186/s12864-023-09744-0

Divilov, K., Merz, N., Schoolfield, B., Green, T. J., & Langdon, C. (2023b). 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. https://doi.org/10.1016/j.aquaculture.2023.739291

Divilov, K., Schoolfield, B., Mancilla Cortez, D., Wang, X., Fleener, G. B., Jin, L., Dumbauld, B. R., & Langdon, C. (2021). Genetic improvement of survival in Pacific oysters to the Tomales Bay strain of OsHV-1 over two cycles of selection. *Aquaculture*, *543*, 737020. https://doi.org/10.1016/j.aquaculture.2021.737020

Divilov, K., Schoolfield, B., Merz, N., & Langdon, C. (2024). No trade-off between growth and OsHV-1 tolerance in Pacific oysters (Crassostrea gigas) farmed on the West Coast, USA. *Animal Genetics*, *55*(1), 158–162. https://doi.org/10.1111/age.13375

Ewels, P., Magnusson, M., Lundin, S., & Käller, M. (2016). MultiQC: Summarize analysis results for multiple tools and samples in a single report. *Bioinformatics*, *32*(19), 3047–3048. https://doi.org/10.1093/bioinformatics/btw354

Fuhrmann, M., Castinel, A., Cheslett, D., Furones Nozal, D., & Whittington, R. j. (2019). *The impacts of ostreid herpesvirus 1 microvariants on Pacific oyster aquaculture in the Northern and Southern Hemispheres since 2008*. *38(2)*, 491. https://doi.org/10.20506/rst.38.2.3000

Gallardo-Hidalgo, J., Barría, A., Yoshida, G. M., & Yáñéz, J. M. (2021). Genetics of growth and survival under chronic heat stress and trade-offs with growth- and robustness-related traits in rainbow trout. *Aquaculture*, *531*, 735685. https://doi.org/10.1016/j.aquaculture.2020.735685

Gawra, J., Valdivieso, A., Roux, F., Laporte, M., de Lorgeril, J., Gueguen, Y., Saccas, M., Escoubas, J.-M., Montagnani, C., Destoumieux-Garzón, D., Lagarde, F., Leroy, M. A., Haffner, P., Petton, B., Cosseau, C., Morga, B., Dégremont, L., Mitta, G., Grunau, C., & Vidal-Dupiol, J. (2023). Epigenetic variations are more substantial than genetic variations in rapid adaptation of oyster to Pacific oyster mortality syndrome. *Science Advances*, *9*(36), eadh8990. https://doi.org/10.1126/sciadv.adh8990

Government of Canada, F. and O. C. (2018, December 4). *Herpes-Type Virus Disease of Oysters*. Fisheries and Oceans Canada. https://www.dfo-mpo.gc.ca/science/aah-saa/diseases-maladies/htvdoy-eng.html

Green, K. M., Spalding, A. K., Ward, M., Levine, A., Wolters, E. A., Hamilton, S. L., & Rice, L. (2023). Oregon shellfish farmers: Perceptions of stressors, adaptive strategies, and policy linkages. *Ocean & Coastal Management*, *234*, 106475. https://doi.org/10.1016/j.ocecoaman.2022.106475

Green, T. J., Siboni, N., King, W. L., Labbate, M., Seymour, J. R., & Raftos, D. (2019). Simulated Marine Heat Wave Alters Abundance and Structure of Vibrio Populations Associated with the Pacific Oyster Resulting in a Mass Mortality Event. *Microbial Ecology*, *77*(3), 736–747. https://doi.org/10.1007/s00248-018-1242-9

Green, T. J., Vergnes, A., Montagnani, C., & de Lorgeril, J. (2016). Distinct immune responses of juvenile and adult oysters (Crassostrea gigas) to viral and bacterial infections. *Veterinary Research*, *47*(1), 72. https://doi.org/10.1186/s13567-016-0356-7

Jombart, T., & Ahmed, I. (2011). adegenet 1.3-1: New tools for the analysis of genome-wide SNP data. *Bioinformatics*, *27*(21), 3070–3071. https://doi.org/10.1093/bioinformatics/btr521

Khtikian, N. (2021). *Investigating the role of Vibrio aestuarianus in summer mortality of farmed Crassostrea gigas in Baynes Sound, British Columbia*. http://hdl.handle.net/1828/13534

Krickeberg, K., Pham, V. T., & Pham, T. M. H. (2012). The Idea of Epidemiology. In K. Krickeberg, V. T. Pham, & T. M. H. Pham (Eds.), *Epidemiology: Key to Prevention* (pp. 1–10). Springer. https://doi.org/10.1007/978-1-4614-1205-2_1

Kube, P., Dove, M., Cunningham, M., Kirkland, P., Gu, X., Hick, P., O'Connor, W., & Elliott, N. (2018). *Genetic Selection for Resistance to Pacific Oyster Mortality Syndrome*. CSIRO Agriculture and Food, NSW Department of Primary Industries, Australian Seafood Industries, Seafood CRC, and FRDC.

Laurence, M., Rose-marie, L., & Philippe, G. (2009). *Alien Species Alert: Crassostrea gigas (Pacific oyster)*.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., & 1000 Genome Project Data Processing Subgroup. (2009). The Sequence Alignment/Map

format and SAMtools. *Bioinformatics*, *25*(16), 2078–2079. https://doi.org/10.1093/bioinformatics/btp352

Lochmiller, R. L., & Deerenberg, C. (2000). Trade-offs in evolutionary immunology: Just what is the cost of immunity? *Oikos*, *88*(1), 87–98. https://doi.org/10.1034/j.1600-0706.2000.880110.x

Magwire, M. M., Fabian, D. K., Schweyen, H., Cao, C., Longdon, B., Bayer, F., & Jiggins, F. M. (2012). Genome-Wide Association Studies Reveal a Simple Genetic Basis of Resistance to Naturally Coevolving Viruses in Drosophila melanogaster. *PLoS Genetics*, *8*(11), e1003057. https://doi.org/10.1371/journal.pgen.1003057

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, *17*(1), Article 1. https://doi.org/10.14806/ej.17.1.200

Mesnil, A., Jacquot, M., Garcia, C., Tourbiez, D., Canier, L., Bidois, A., Dégremont, L., Cheslett, D., Geary, M., Vetri, A., Roque, A., Furones, D., Garden, A., Orozova, P., Arzul, I., Sicard, M., Charrière, G. M., Destoumieux-Garzón, D., & Travers, M.-A. (2023). Emergence and clonal expansion of Vibrio aestuarianus lineages pathogenic for oysters in Europe. *Molecular Ecology*, *32*(11), 2869–2883. https://doi.org/10.1111/mec.16910

Money, D., Migicovsky, Z., Gardner, K., & Myles, S. (2017). LinkImputeR: User-guided genotype calling and imputation for non-model organisms. *BMC Genomics*, *18*(1), 523. https://doi.org/10.1186/s12864-017-3873-5

Nordio, D., Khtikian, N., Andrews, S., Bertotto, D., Leask, K., & Green, T. (2021). Adaption potential of Crassostrea gigas to ocean acidification and disease caused by Vibrio harveyi. *ICES Journal of Marine Science*, *78*(1), 360–367. https://doi.org/10.1093/icesjms/fsaa080

Normandeau, E. (2023). *STACKS Workflow2* [Python]. https://github.com/enormandeau/stacks_workflow (Original work published 2013)

Okon, E. M., Birikorang, H. N., Munir, M. B., Kari, Z. A., Téllez-Isaías, G., Khalifa, N. E., Abdelnour, S. A., Eissa, M. E. H., Al-Farga, A., Dighiesh, H. S., & Eissa, E.-S. H. (2023). A Global Analysis of

Climate Change and the Impacts on Oyster Diseases. *Sustainability*, *15*(17), Article 17. https://doi.org/10.3390/su151712775

Oliver, E. C. J., Donat, M. G., Burrows, M. T., Moore, P. J., Smale, D. A., Alexander, L. V., Benthuysen, J. A., Feng, M., Sen Gupta, A., Hobday, A. J., Holbrook, N. J., Perkins-Kirkpatrick, S. E., Scannell, H. A., Straub, S. C., & Wernberg, T. (2018). Longer and more frequent marine heatwaves over the past century. *Nature Communications*, *9*(1), 1324. https://doi.org/10.1038/s41467-018-03732-9

Paria, S. S., Rahman, S. R., & Adhikari, K. (2022). *fastman: A fast algorithm for visualizing GWAS results using Manhattan and Q-Q plots*. https://doi.org/10.1101/2022.04.19.488738

Pelletier, C. (2023). *Diversité et évolution de l'Ostreid Herpesvirus type 1* [Phdthesis, Université de La Rochelle]. https://theses.hal.science/tel-04549017

Peñaloza, C., Gutierrez, A. P., Eöry, L., Wang, S., Guo, X., Archibald, A. L., Bean, T. P., & Houston, R. D. (2021). A chromosome-level genome assembly for the Pacific oyster Crassostrea gigas. *GigaScience*, *10*(3), giab020. https://doi.org/10.1093/gigascience/giab020

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double Digest RADseq: An Inexpensive Method for De Novo SNP Discovery and Genotyping in Model and Non-Model Species. *PLoS ONE*, *7*(5), e37135. https://doi.org/10.1371/journal.pone.0037135

Purcell, S. (2022). *PLINK* (Version v.1.90b6.26) [Computer software]. https://zzz.bwh.harvard.edu/plink/

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., Maller, J., Sklar, P., de Bakker, P. I. W., Daly, M. J., & Sham, P. C. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics*, *81*(3), 559–575. https://doi.org/10.1086/519795

R Core Team. (2024). *R: A Language and Environment for Statistical Computing* [Computer software]. https://www.r-project.org/

Råberg, L., Sim, D., & Read, A. F. (2007). Disentangling Genetic Variation for Resistance and Tolerance to Infectious Diseases in Animals. *Science*, *318*(5851), 812–814. https://doi.org/10.1126/science.1148526

Robinson, A. N., & Green, T. J. (2020). Fitness costs associated with maternal immune priming in the oyster. *Fish & Shellfish Immunology*, *103*, 32–36. https://doi.org/10.1016/j.fsi.2020.04.047

Rochette, N. C., Rivera-Colón, A. G., & Catchen, J. M. (2019). Stacks 2: Analytical methods for paired-end sequencing improve RADseq-based population genomics. *Molecular Ecology*, *28*(21), 4737–4754. https://doi.org/10.1111/mec.15253

Rockel, T. (2022). *missMethods: Methods for Missing Data* (Version 0.4.0) [Computer software]. https://cran.r-project.org/web/packages/missMethods/index.html

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 Research*, *153*(1), 92–99. https://doi.org/10.1016/j.virusres.2010.07.011

Siboni, N., King, W. L., Williams, N. L. R., Scanes, E., Giardina, M., Green, T. J., Ostrowski, M., O'Connor, W., Dove, M., Labbate, M., & Seymour, J. R. (2024). Increased abundance of potentially pathogenic Vibrio and a marine heatwave co-occur with a Pacific Oyster summer mortality event. *Aquaculture*, *583*, 740618. https://doi.org/10.1016/j.aquaculture.2024.740618

Sutherland, B. J. G., Hanson, K. C., Jantzen, J. R., Koop, B. F., & Smith, C. T. (2014). Divergent immunity and energetic programs in the gills of migratory and resident ncorhynchus mykiss. *Molecular Ecology*, *23*(8), 1952–1964. https://doi.org/10.1111/mec.12713

Sutherland, B. J. G., Rycroft, C., Ferchaud, A.-L., Saunders, R., Li, L., Liu, S., Chan, A. M., Otto, S. P., Suttle, C. A., & Miller, K. M. (2020). Relative genomic impacts of translocation history, hatchery practices, and farm selection in Pacific oyster Crassostrea gigas throughout the Northern Hemisphere. *Evolutionary Applications*, *13*(6), 1380–1399. https://doi.org/10.1111/eva.12965

Tange, O. (2015). *GNU Parallel 20150322 ('Hellwig')* [Computer software]. Zenodo. https://doi.org/10.5281/zenodo.16303

Thompson, N. F., Agnew, M. V., Calla, B., & Burge, C. A. (2024). Assessing selection potential for Pacific oyster (*Crassostrea gigas*) to a North American OsHV-1 μvar: Comparing two experimental assay methods. *Aquaculture*, *590*, 741076. https://doi.org/10.1016/j.aquaculture.2024.741076

Wang, D., Loor, A., Bels, L. D., Stappen, G. V., den Broeck, W. V., & Nevejan, N. (2021). Dynamic Immune Response to Vibriosis in Pacific Oyster Crassostrea gigas Larvae during the Infection Process as Supported by Accurate Positioning of GFP-Tagged Vibrio Strains. *Microorganisms*, *9*(7), 1523. https://doi.org/10.3390/microorganisms9071523

Wendling, C. C., Batista, F. M., & Wegner, K. M. (2014). Persistence, Seasonal Dynamics and Pathogenic Potential of Vibrio Communities from Pacific Oyster Hemolymph. *PLOS ONE*, *9*(4), e94256. https://doi.org/10.1371/journal.pone.0094256

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. https://doi.org/10.1016/j.aquaculture.2021.737592

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. https://doi.org/10.1016/j.aquaculture.2021.736545

Zhou, X., & Stephens, M. (2012). Genome-wide efficient mixed-model analysis for association studies. *Nature Genetics*, *44*(7), 821–824. https://doi.org/10.1038/ng.2310

TABLES AND FIGURES

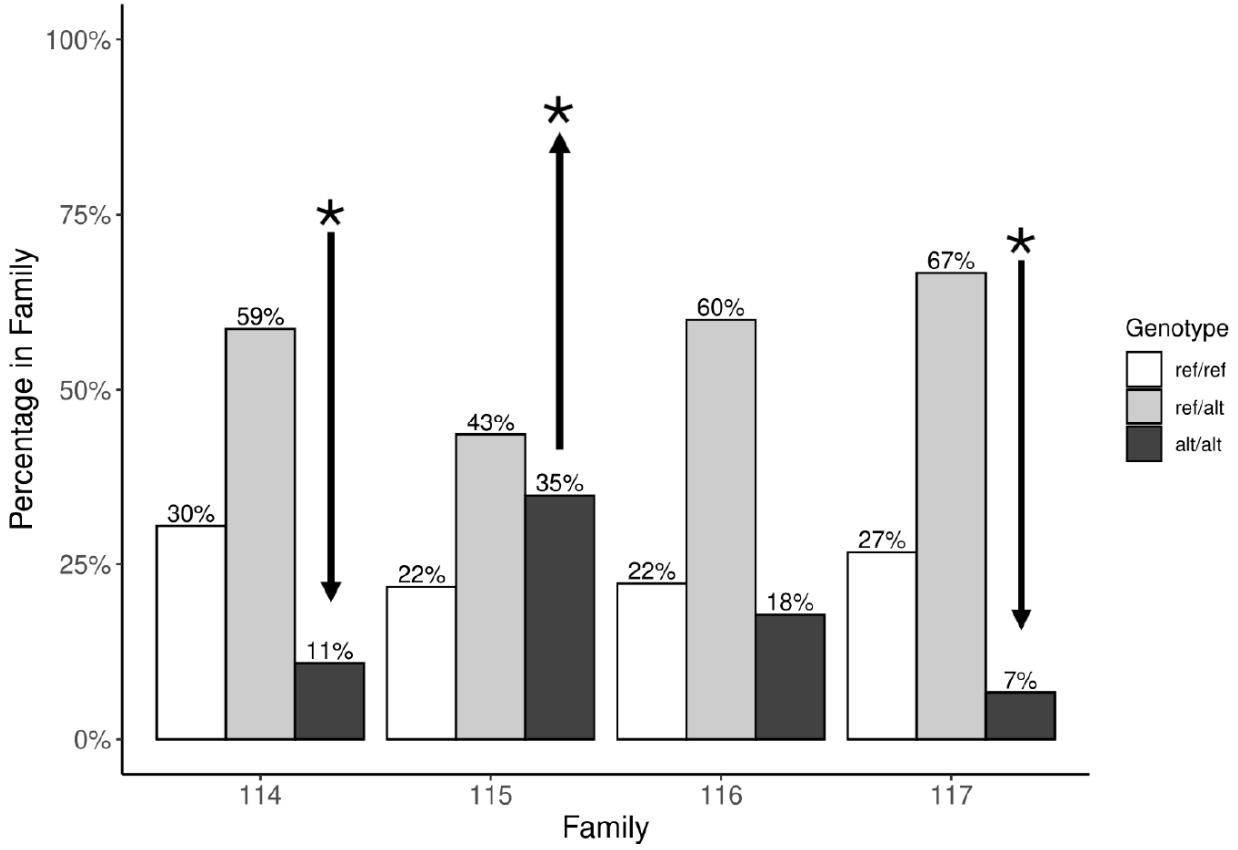

Figure 1: Frequency of basal genotypes (%) in OsHV-1 resistant oyster mapping families (F114-F117). Genotype is denoted by colour. Columns with arrows indicate a significant difference ($p < 0.05$) in genotype proportions between families. Significance was tested using a Fisher's exact test. Significant differences were observed in the number of homozygous alt's for F115 compared to F114 and F117 (χ^2=15.183, p-value = 0.019). No significant differences were observed in the number of heterozygotes or homozygous refs across all families.

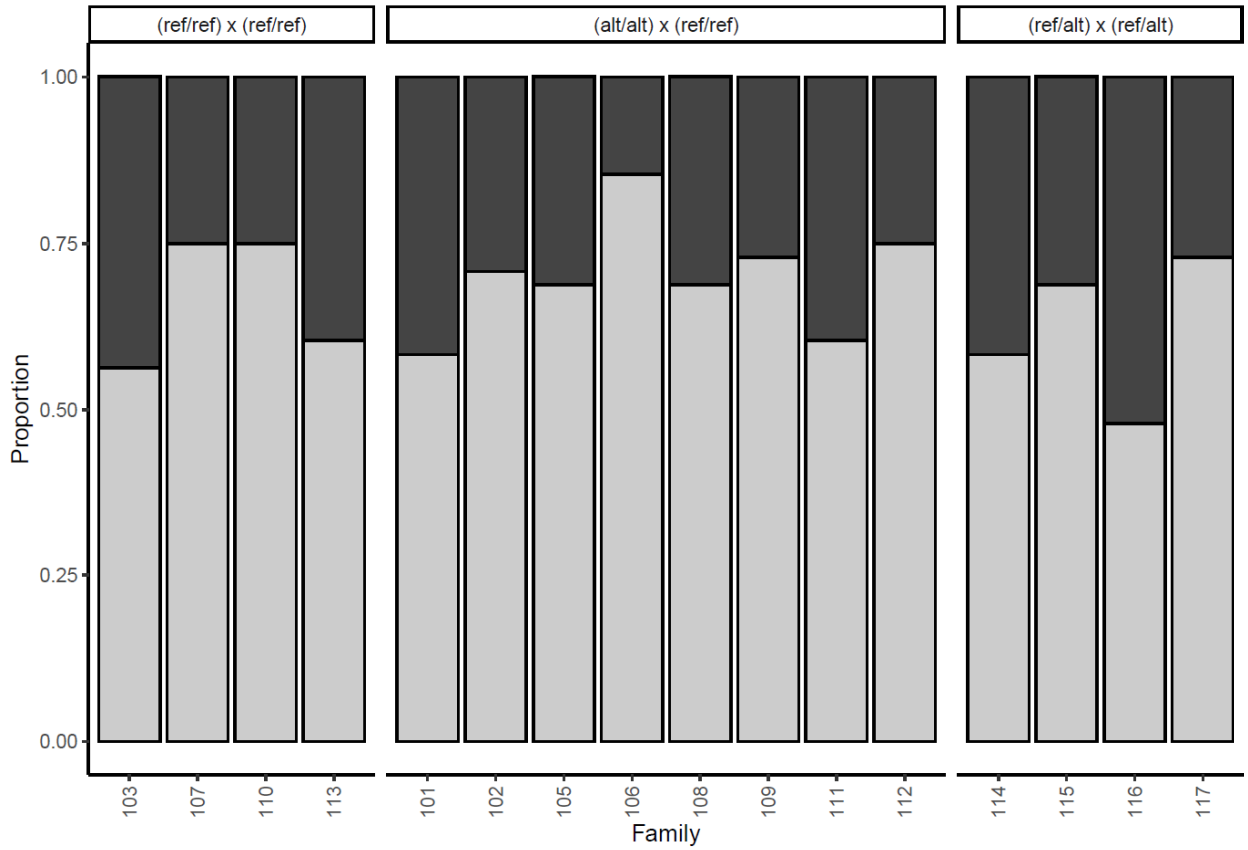

Figure 2: Proportion of oysters that died (light grey) or survived (dark grey) in each family to the *V. aestuarianus* disease challenge grouped by parental genotype. The control group exhibited 100% survival.

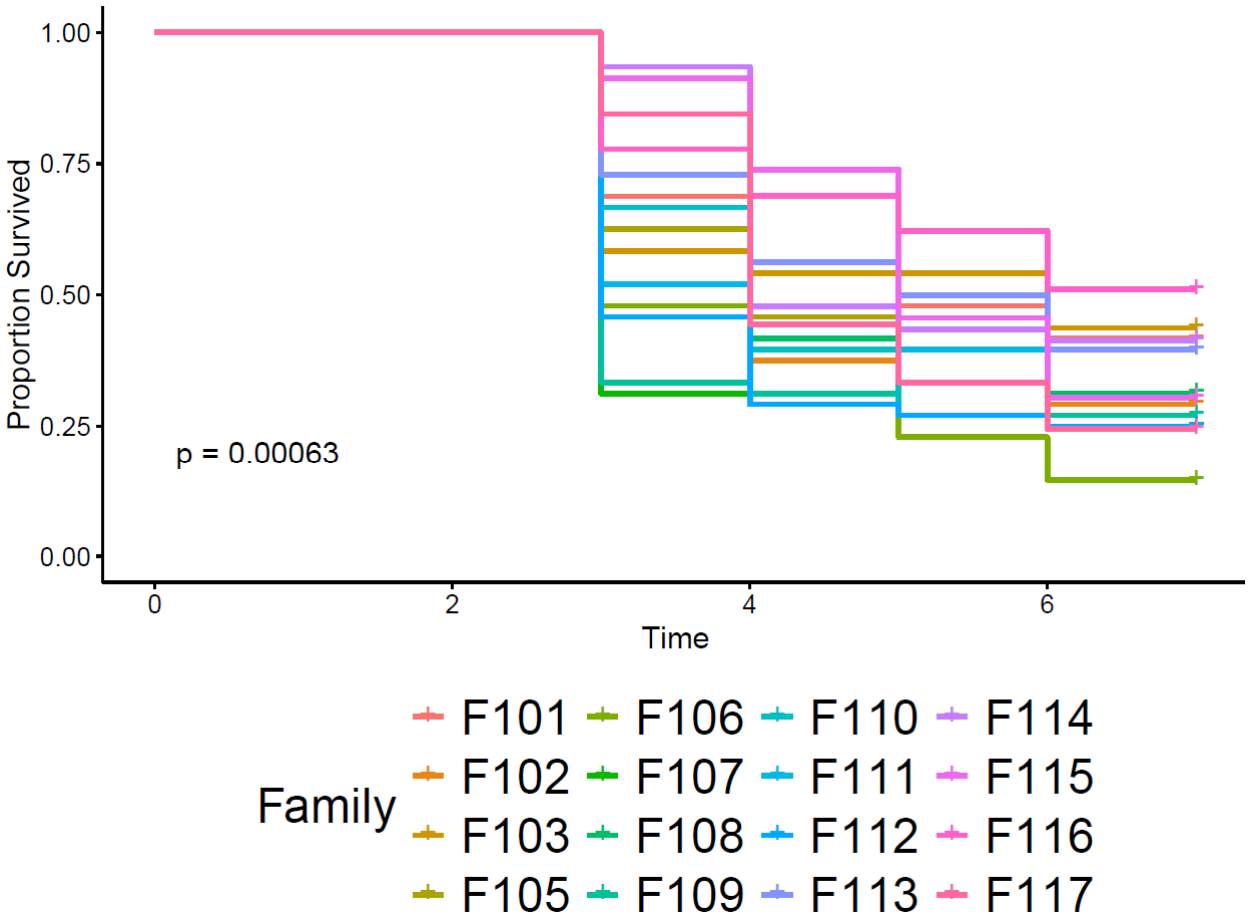

Figure 3: Kaplan-Meier survival curves for Pacific oyster families after exposure to *V. aestuarianus*. Family is denoted by colour. No mortality was observed on days 1 and 2. A log-rank test was conducted to determine if there were significant differences in the survival distributions for the family crosses. The survival distributions for different family crosses were statistically significant, =39.070, p<0.001. Pairwise log-rank comparisons were conducted to determine which intervention groups had different survival distributions. A Bonferroni correction was made with statistical significance accepted at the p < 0.003 level.

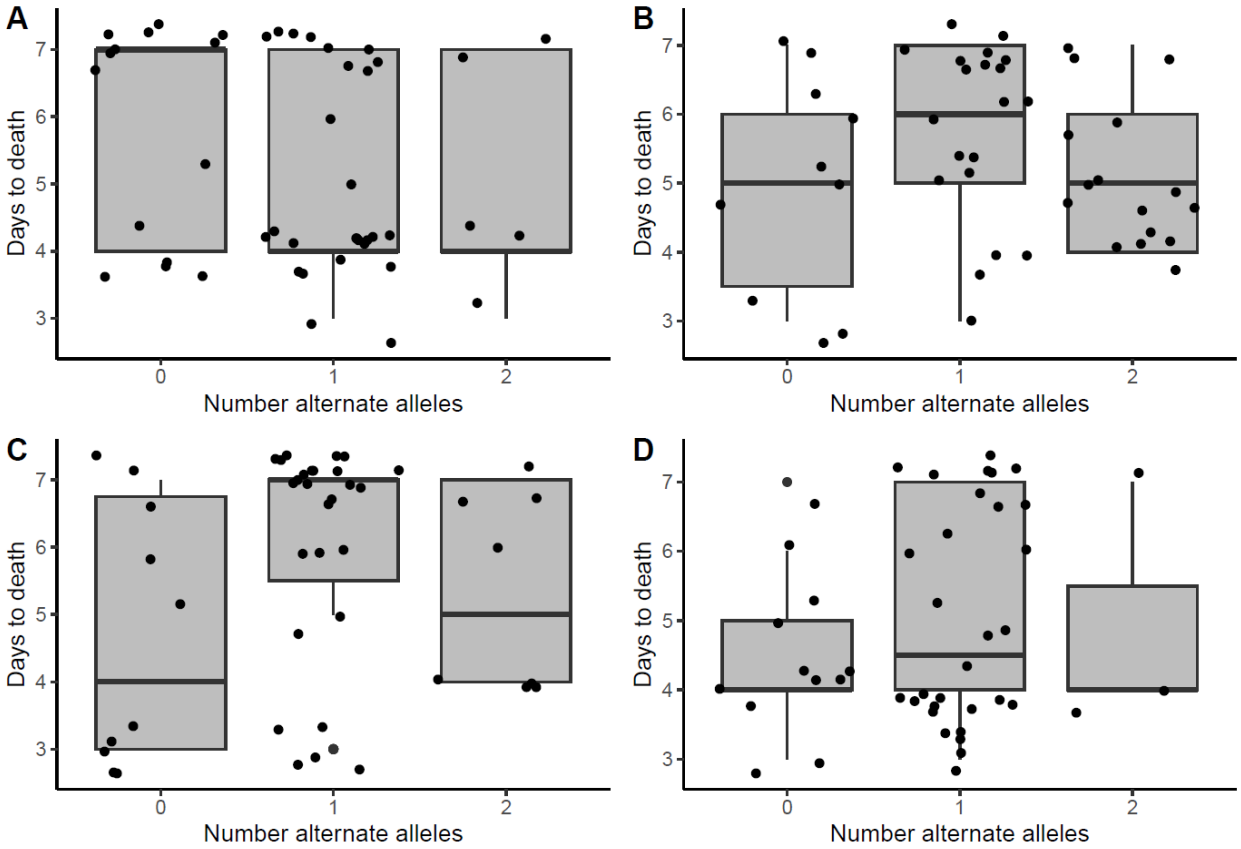

Figure 4: Effects of allelic dosage on mortality (quantified as day of death) to *V. aestuarianus* in oyster mapping families with the OsHV-1 resistance marker (A = F114, B = F115, C = F116, D = F117). Statistical analysis was conducted using a chi-squared test of homogeneity and a Fisher's exact test. No significant association was found between OsHV-1 resistance marker allelic dosage and mortality represented as day of death (p 0.05) (F114 =5.370, p>0.05; F115 =14.172, p>0.05; F116 =11.280, p>0.05; F117 =4.429, p>0.05).

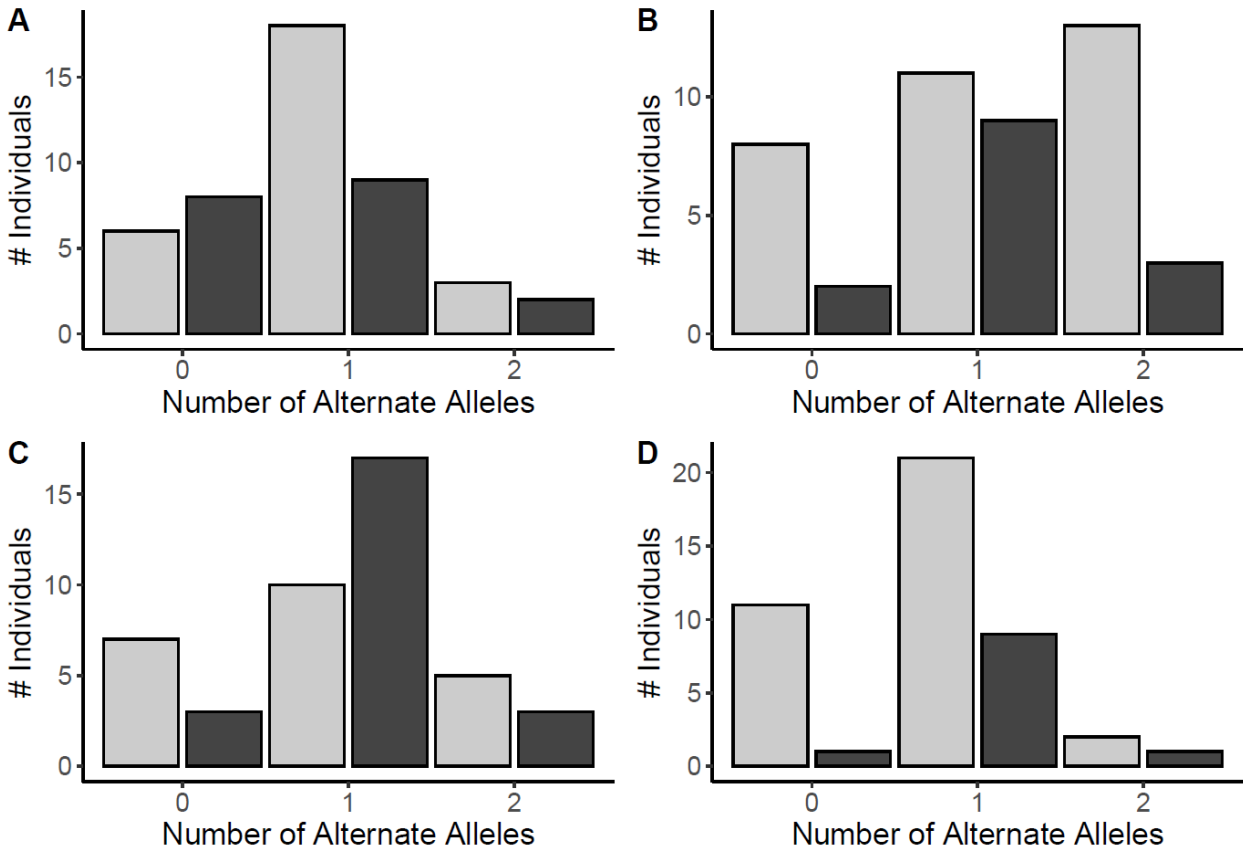

Figure 5: Effects of allelic dosage (i.e., number of alternate alleles) on mortality to Vibrio shown as the number of deaths (light grey) compared with the number of survivors (dark grey) for four families (panel A = F114; B = F115; C = F116; D = F117). Statistical analysis was conducted using a chi-squared test of homogeneity and a Fisher's exact test. No significant association was found between genotype and mortality represented as day of death (p-value 0.05 for all values).

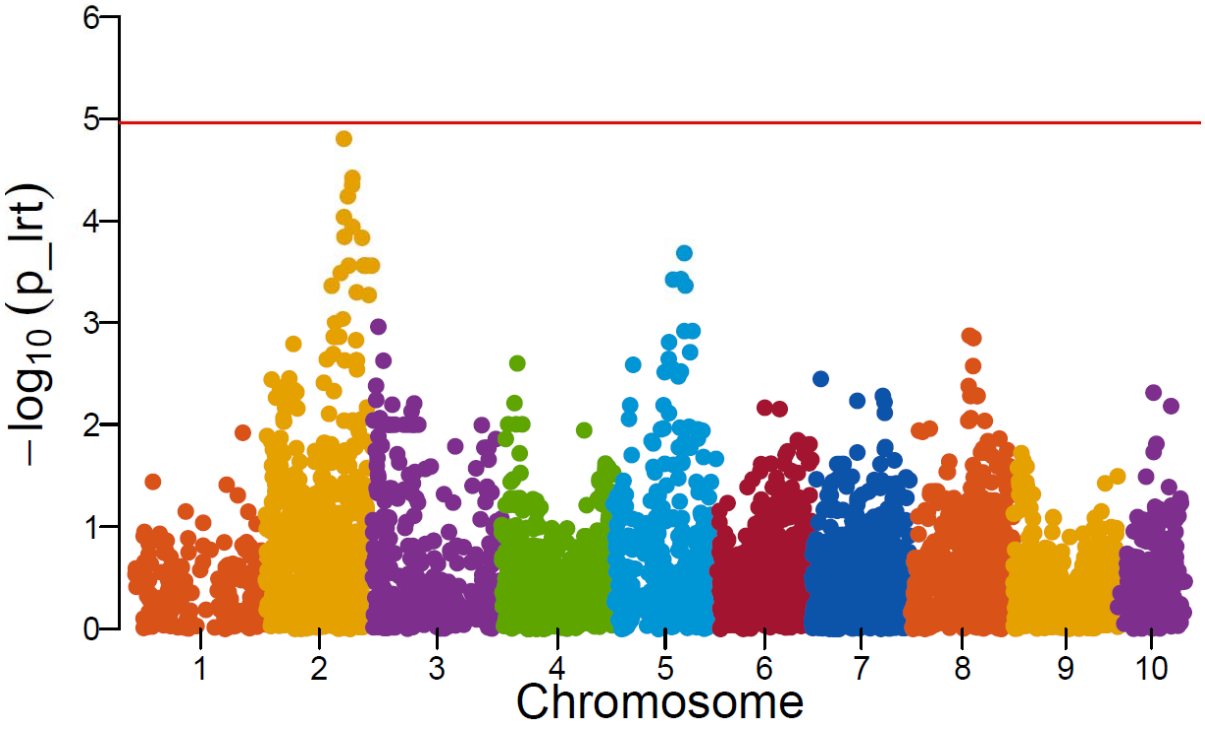

Figure 6: Manhattan plot showing the results of the genome-wide association study (GWAS) for survivorship from the *Vibrio* exposure in Pacific oysters (F114-F117) with the OsHV-1 resistance marker using the phenotype mortality assessed as dichotomous function (died or survived). The red horizontal line represents the genome-wide Bonferroni-corrected p-value significance threshold of 0.05. No significant association was observed.