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DNA methylation landscapes before and after Pacific Oyster Mortality Syndrome are different within and between resistant and susceptible *Magallana gigas*

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ABSTRACT

Pacific oysters face recurring outbreaks of Pacific Oyster Mortality Syndrome (POMS), a polymicrobial multifactorial disease. Although this interaction is increasingly understood, the role of epigenetics (*e.g.*, DNA methylation) appears to be of fundamental importance because of its ability to shape oyster resistance/susceptibility and respond to environmental triggers, including infections. In this context, we comprehensively characterized basal (no infection) and POMS-induced changes in the methylome of resistant and susceptible oysters, focusing on the gills and mantle. Our analysis identified differentially methylated regions (DMRs) that revealed distinct methylation patterns uniquely associated with the susceptible or resistant phenotypes in each tissue. Enrichment analysis of genes bearing DMRs highlighted that these epigenetic changes were specifically linked to immunity, signaling, metabolism, and transport. Notably, 31 genes with well-known immune functions were differentially methylated after POMS, with contrasting methylation patterns between the phenotypes. Based on the methylome differences between phenotypes, we identified a set of candidate epibiomarkers that could characterize whether an oyster is resistant or susceptible (1998 candidates) and whether a site has been exposed to POMS (164 candidates). Overall, the findings provide a deeper understanding of the molecular interactions between oysters and POMS infection, opening new questions about the broader implications of epigenetic mechanisms in host-pathogen dynamics and offering promising strategies for mitigating the impacts of this devastating disease. Beyond its biological aspects, this study provides insights into potential epigenetic biomarkers for POMS disease management and targets for enhancing oyster health and productivity.

KEYWORDS

OsHV-1 μ Var ; DNA methylation ; Immune response ; Epibiomarkers ; Oyster ; POMS

HIGHLIGHTS

- Oysters exhibit distinct pre-exposure DNA methylation patterns linked to POMS.
- POMS infection induces DNA methylation changes in resistant and susceptible oysters.
- Genes related to immunity and metabolism undergo methylation changes post-infection.
- DNA methylation as epimarkers may help manage POMS via selective breeding strategies.
- The study offers new insights for improving the resilience of the oyster industry.

1. Introduction

The Pacific oyster, *Magallana gigas* (Salvi and Mariottini, 2016, 2021), is one of the most widely exploited species in aquaculture. Notably, recurrent outbreaks of Pacific Oyster Mortality Syndrome (POMS) have jeopardized the sustainability of the oyster farming industry (EFSA Panel on Animal Health and Welfare (AHAW), 2010; Richard et al., 2021).

POMS is a polymicrobial disease that affects spats and juvenile oysters (Petton et al., 2021). The primary causative pathogen is the herpesvirus OsHV-1 μ Var, infecting cells and, ultimately, the hemocytes, leading to an immune-suppressed state (de Lorgeril et al., 2018). This state alters the control of the associated microbial community, leading to the proliferation of a consortium of opportunistic and pathogenic bacteria (Rubio et al., 2019; Oyanedel et al., 2023) and resulting in lethal bacteremia (Clerissi et al., 2023; de Lorgeril et al., 2018; Petton et al., 2021). In addition to the polymicrobial aspect, POMS is a multifactorial disease that involves a series of biotic and abiotic factors that influence the outcome of the POMS/oyster interaction (Petton et al., 2021). Genetic components are pivotal in oyster resistance and susceptibility (D'egremont et al., 2015; de Lorgeril et al., 2020). Additionally, factors such as temperature (Petton et al., 2013; Pernet et al., 2015; De Kantzow et al., 2016; Delisle et al., 2018, 2020), ageing (Hick et al., 2018; Peeler et al., 2012; Pernet et al., 2012), food availability (Pernet et al., 2019; Petton et al., 2023), and interactions with associated microbiota (Pathirana et al., 2019; Clerissi et al., 2020; Delisle et al., 2022; Fallet et al., 2022) can also influence the permissivity to POMS (*i. e.*, the development of the disease in a susceptible oyster).

The adaptive potential of the oyster population toward POMS was shown to rely on genomic variations (Azema et al., 2017; Gutierrez et al., 2017). However, a recent study revealed that non-genetic variations are also essential (Gawra et al., 2023). Concretely and independent of the DNA sequence, distinct methylation signatures in CG-rich regions (*i.e.*, CpGs), mostly harbored in immune genes, were found to be significantly associated with resistance to POMS in wild *M. gigas* populations (Gawra et al., 2023). Additionally, the interactions of oyster larvae (D- to veliger larval stages) with a rich microbial environment have been shown to result in epigenetic reconfigurations that contribute to immune shaping and increased resistance to POMS, even transgenerationally (Fallet et al., 2022). The impact of POMS infection on DNA methylation in oysters is, however, unexplored but is strongly hypothesized since pathogenic interactions are known to induce DNA methylation changes that can rely on the host response or the hijacking of the cellular machinery by the pathogen (Fischer, 2020; Netea et al., 2020). In the former, infections are known to induce immune priming, a phenomenon called trained immunity (Lanz-Mendoza and Contreras-Garduno, 2022). During the host response, epigenetic modifications regulate the innate immune response and induce long-lasting protection (Nieborak and Schneider, 2018). Although this phenomenon has mostly been demonstrated in plants and vertebrates, immune priming has been empirically demonstrated in oyster/POMS interactions (Lafont et al., 2017), and epigenetic changes in response to exposure to rich but non-pathogenic microbiota have also been demonstrated (Fallet et al., 2022). Concerning viral infections, the host epigenetic machinery is manipulated to optimize the multiplication and transmission of shedding viral particles (Balakrishnan and Milavetz, 2017). Herpesviridae has been shown to delay or impair the host immune response, induce metabolic shifts, and regulate the latent/lytic cycle (Pei and Robertson, 2020; Locatelli and Faure-Dupuy, 2023). Currently, no precise knowledge exists regarding OsHV-1 μ Var infection since no cellular system exists for *in vitro* infection assays, which is needed to characterize the lytic cycle. In other Herpesviridae, viral particles first attach to receptors on the host cell surface, which enables viral entry (Knipe et al., 2002). Viral proteins held by the envelope are then released, facilitating the transport of the capsid to the nucleus and the viral genome release (Everett, 2014). Viral replication begins with the sequenced expression of specific viral genes: (i) immediate-early genes (IE), (ii) early genes (E), and (iii) late genes (L) (Knipe et al., 2002; Zhu and Viejo-Borbolla, 2021; Damania et al., 2022). Genes of the IE and E categories are probably involved in manipulating the host epigenome as they prepare the cell to produce viral particles through the L genes.

Interest in epigenetic changes induced by the environmental *sensus lato* has also been steadily increasing in shellfish aquaculture. Research on *M. gigas* epigenetics with interest in aquaculture has been conducted to better understand the influence of epigenetics changes in sex determination (Jiang et al., 2016; Zhang et al., 2018; Sun et al., 2022, 2024) in response to thermal stress and thermotolerance (Fellous et al., 2015; C. Wang et al., 2024), intertidal effects (Wang et al., 2023), larvae development (Le Franc et al., 2021), and in the acquisition of resistance to POMS (Fallet et al., 2022; Gawra et al., 2023). In addition to their application in fundamental biology, these epigenetic changes (Law and Holland, 2019) can also be used as epibiomarkers for applied research (Bock, 2009; Chan and Baylin, 2010). Indeed, epibiomarkers hold the potential for various applications in aquaculture (Piferrer, 2023). Epibiomarkers can help farmers make decisions regarding breeding programs (Anastasiadi et al., 2018), disease control (Moralada-Prados et al., 2020), thermal history (Valdivieso et al., 2023a), environmental contamination (Rondon et al., 2017), and sustainable productivity enhancement (Valdivieso et al., 2023c). Protocols for developing epibiomarkers have already been established for several marine organisms (Anastasiadi and Beemelmans, 2023) and would assist in managing and controlling POMS. Among the putative solutions raised by epibiomarkers, the identification of resistance would improve oyster breeding programs through marker-assisted selection. Furthermore, the ability to identify potential new farming areas that are free of POMS outbreaks would enable safer growth in the oyster industry.

In this context, our study pursued two objectives based on two hypotheses. First, we hypothesized that resistant and susceptible oysters present different methylation patterns at some epiloci. Second, we hypothesized that POMS infection induces changes in oyster methylation. Therefore, we aimed to characterize the effect of POMS infection on the methylome of resistant and susceptible oysters and to identify epibiomarkers of past exposure to POMS and oyster resistance/susceptibility to assist in POMS management. To address these aims and test our hypothesis, we studied the methylome of resistant and susceptible oysters before and after POMS infection at the whole-genome scale in two tissues: the gills and mantle. These two aims were addressed using five oyster F₁ populations produced from wild progenitors collected from the main oyster production basins in France. Owing to the non-lethal sampling design, we accurately identified and quantified the methylation changes induced by POMS and further developed comparative approaches to identify candidate epibiomarkers of POMS exposure and the resistance/susceptible phenotype.

2. Material and methods

2.1. Oyster sampling and production

First-generation (F₁) oyster populations produced in the hatchery were used in this study (populations #1–5). Four of these F₁ populations were produced from progenitors collected in 2022 from four main oyster production basins in France: Thau Lagoon (#1), Arcachon Bay (#2), La Floride (#3), and Logonna Daoulas (#4) (Fig. S1). These natural populations experienced annual POMS events. The fifth F₁ population, SC18 (#5) (Fig. S1), remained unexposed to POMS events since 2007 because of biosecured maintenance under controlled conditions at the Institut Français de Recherche pour l'Exploitation de la Mer (Ifremer), La Tremblade facility (France). Each F₁ population was produced from the gametes of 20 females and 10 males, according to a previously described protocol (Azema et al., 2017). F₁ populations #1–5 were then grown in 150 L tanks for one year, fed seawater-enriched phytoplankton (*Skeletonema costatum*, *Isochrysis galbana*, and *Tetraselmis*

suecica; 550,000 cells/mL at 75 L/h), and maintained under biosecure conditions to avoid exposure to POMS. These F₁ populations were referred to as recipient oysters in the experimental infection procedure (see below).

To induce POMS infection, donor oysters from the F14 family (de Lorgeril et al., 2018) and the NSI population were used (Petton et al., 2013). F14 consists of a biparental oyster family displaying high susceptibility to POMS (expected susceptibility >90 %). The NSI population is a genetically diversified standardized oyster spat with an expected 50–60 % susceptibility to POMS. We used these two different donor oysters to maximize the production of OsHV-1 μ Var without compromising the genetic diversity of the viral populations produced. All oysters were produced and maintained in the Ifremer biosecure facilities in Argenton and Bouin (France) and never experienced POMS events. Before the POMS experiment, donor and recipient oysters were acclimatized in a dedicated chamber for two weeks and fed *ad libitum* with *Skeletonema costatum* (700,000 cells/mL). Every two days, the water temperature was gradually increased by 2 °C until it reached 21 °C, and water was renewed at the rate of 30 %/hour (BIO-UV ultraviolet- filtration).

2.2. OsHV-1 μ Var viral suspension for donor oysters

The viral suspension used for experimental infection was an equimolar mix of suspensions obtained from infected oysters collected from three different locations in France: Rade de Brest, La Tremblade, and Thau Lagoon. The viral suspension was prepared as previously described (Schikorski et al., 2011). Donor oysters were injected with 20 μ L of viral suspension (6.0 E⁷ genomic units) using a 26-gauge needle attached to a multi-dispensing hand pipette into the adductor muscle to facilitate spreading into the circulatory system.

2.3. POMS infection by cohabitation between donor and recipient oysters

After acclimatization, 20 recipient oysters from each of the five F₁ populations (#1–5, N_{size} = 100) were individually tagged and anesthetized (Suquet et al., 2009), and parts of their gills and mantle were excised (5–6 mm²). The extracted samples were labeled as “Pre-infection-T₀” and were the control group. Finally, 100 biopsied recipient oysters were monitored for a recovery period of 30 days (Fig. 1A).

To distinguish between POMS-susceptible and POMS-resistant among the recipient oysters, a cohabitation protocol with a randomized complete block design was used to mimic “natural” POMS infection (Schikorski et al., 2011; Gawra et al., 2023). The infection began with the inoculation of OsHV-1 μ Var viral suspension in 100 donor oysters (F14 = 60 and NSI = 40, Fig. 1B). After injection, five sets of 20 donor oysters (F14 = 12 and NSI = 8) were randomly distributed into five tanks (volume = 10 L; technical replicates). Each tank was equipped with an air-bubbling system to ensure oxygen saturation and water flow. The infected donor oysters were allowed to stand alone for 24 h. Five sets of 20 biopsied and tagged recipient oysters (four from each of the five F₁ populations #1–5) were introduced into each of the five tanks containing donors (Fig. 1B). At 24 h post-cohabitation (hpc), the donors were removed, and their mortality was assessed daily over seven days in an isolated tank. Simultaneously, 1 mL of seawater from each of the five tanks was sampled daily for quantifying the OsHV-1 μ Var viral load until the end of the experiment.

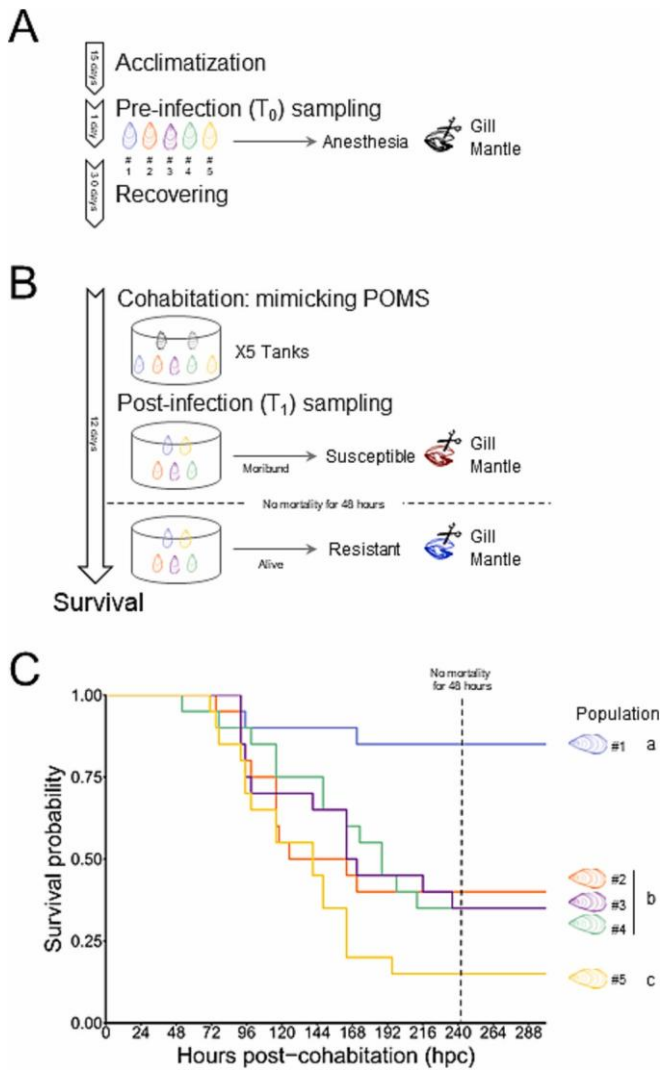


Fig. 1. Schematic representation of the experimental design and survival analysis. A) First-generation (F_1) offspring from five wild oyster populations (#1–5) were acclimatized in controlled conditions for two weeks and tagged. After anesthetizing, a piece of gill and mantle were excised and labeled as “Pre- infection- T_0 ” (control group). This sampling was followed by 30 days of recovery. B) Then, a cohabitation protocol between recipient and donor oysters previously infected by injection with 20 μ L OshV-1 μ Var (6.0 E^7 genomic units) was used to mimic an event of Pacific Oyster Mortality Syndrome (POMS). Recipient oysters were phenotyped for susceptibility (moribund, red) and resistance to POMS (those who survived POMS infection, blue). The second sampling of gills and mantle was carried out and labeled “Post-infection- T_1 ” (treatment group). C) Kaplan–Meier survival analysis for the five recipient oyster F_1 populations (#1–5) during POMS infection.

The POMS progression in the recipient oysters was monitored every 2 h to assess their status as either “susceptible” or “resistant,” as previously described (Gawra et al., 2023). Briefly, an oyster was considered susceptible if it could not close its valves within 30 s after emergence from the experimental tanks. Conversely, if the oysters closed their valves, they were deemed alive and returned to their tanks for subsequent phenotypic assessment. This monitoring process allowed the evaluation of the phenotype of each oyster over time in response to POMS exposure.

Susceptible oysters were immediately removed from the tanks, and the gills and mantle were sampled (5–6 mm^2). The experiment terminated when no mortality was recorded for 48 h in any of the five tanks. All surviving recipient oysters were categorized as resistant, and their gills and mantle were sampled (Fig. 1B). This second sampling, for both susceptible and resistant oysters, was labeled as “Post-infection- T_1 ” and was the treatment group. All dissected gills and mantle were promptly flash-frozen in liquid nitrogen and stored at -80°C . For further analysis, ten oysters were selected, with one resistant and one susceptible oyster for each F_1 population #1–5 among the five tanks.

Temperature and salinity were controlled twice daily and did not show a departure from the initial settings (20 $^\circ\text{C}$ and 34 g/L), and oxygen was maintained at saturation by air bubbling in the experimental tanks.

2.4. Survival analysis in donor and recipient oysters

Kaplan-Meier model was used with the ‘survfit’ and ‘ggsurvplot’ functions of ‘survival’ (Therneau and Lumley, 2015) (v3.2-11) and ‘survminer’ (Kassambara et al., 2017) (v0.4.9) packages, respectively. Then, the Cox proportional hazard model was performed using the ‘coxph’ function from the ‘survival,’ and results were plotted using the ‘ggforest’ function from the ‘survminer’ package. Survival was considered significant at $P \leq 0.05$.

2.5. DNA extraction from gills and mantle of susceptible and resistant oysters

The genomic DNA (gDNA) was extracted from gills and mantle using a NucleoSpin® Tissue kit (MACHEREY-NAGEL GmbH & Co. KG, Düren, Nordrhein-Westfalen, Germany) with 15 min RNase A digestion to remove co-purified RNA, and then stored at -20°C . The gDNA purity was assessed using a Nanodrop spectrophotometer (ND1000; Thermo Fisher Scientific, Waltham, MA, USA), and the concentration was verified using the Qubit 2.0 Fluorometric (Thermo Fisher Scientific) with the dsDNA HS assay kit (Q32851; Thermo Fisher Scientific). The presence of a consistent band of high-molecular-weight gDNA was evaluated using 1% agarose gel electrophoresis.

2.6. Quantification of OsHV-1 μ Var viral load

Quantification of the OsHV-1 μ Var viral load was performed using quantitative PCR (qPCR). The 20 μL qPCR reaction consisted of 5 μL of gDNA (5 ng μL^{-1}), 2 μL of each primer at the final concentration of 550 nM (Eurogentec), 1 μL of distilled water, and 10 μL of Brilliant III Ultra-Fast SYBR®Green PCR Master Mix (Agilent). The virus-specific primer pairs targeted a region of the OsHV-1 μ Var genome predicted to encode a DNA polymerase catalytic subunit (ORF100, AY509253): Forward-TTGATGATGTGGATAATCTGTG and Reverse-GTAAATACCATTGGTC TTGTCC (Webb et al., 2007; Pepin, 2013). The amplification reactions were carried out using the Mx3005P Real-Time thermocycler (Stratagene, San Diego, CA, USA) for 3 min at 95°C followed by 40 cycles of amplification at 95°C for 5 s and 60°C for 20 s. A melting temperature curve of the amplicon was generated to verify the specificity of the amplification, and absolute quantification of the virus was estimated by comparing the observed cycle threshold (Ct) values to a standard curve of the DP amplification product cloned into the pCR4-TOPO vector for the OsHV-1 μ Var.

2.7. Enzymatic methyl-seq library preparation and sequencing

NEBNext Enzymatic Methyl-seq (EM-seq™) library preparations and sequencing were carried out by IntegraGen (Evry, France). Briefly, 100 ng gDNA was end-repaired, A-tailed, and ligated to methylated universal adapters. The libraries were then purified and converted using the NEBNext Enzymatic Methyl-seq Conversion Module according to the manufacturer's recommendations. After PCR amplification and indexing, the samples were sequenced in paired-end (PE) reads with 150 base pairs (bp) lengths using an Illumina NovaSeq sequencer.

2.8. The bioinformatics pipeline for EM-seq reads

The raw PE read quality for each sample was analyzed using FastQC (v0.53) (Andrews, 2010), and adapters were trimmed using TrimGalore! (v0.6.7) (Krueger, 2015) with parameters: `-q 30 -paired -clip_R1 5 and -clip_R2 5 -Illumina`. Any remaining adapters were removed in a second trimming round using the default parameters. We utilized Bismark (v0.23.1) (Krueger and Andrews, 2011), employing the `'bismark_genome_preparation'` to perform bisulfite conversion *in silico* of the *M. gigas* genome (cgigas_uk_roslin_v1, Assembly: GCA902806645v1) (Penalzo et al., 2021). Then, the trimmed PE reads were aligned to the bisulfited converted genome using `'bismark'` with parameters: `-q -N 1 -score_min L,0,-0.4`. The duplicated aligned reads were removed using the `'deduplicate_bismark'` and the methylation calling was accomplished using `'bismark_methylation_extractor'` with parameters: `-no_overlap -cutoff 10` only in the CpG context. To assess the enzymatic conversion efficiency, each sample included unmethylated sequences of the bacteriophage lambda (48,502 bp from the cl857ind 1 Sam 7 strain) as a spike-in control. The efficiency was calculated for each sample by aligning the trimmed PE reads to the bacteriophage lambda genome (same procedure as above) and retained samples showing conversion efficiency $\leq 99.0\%$ for the DNA methylation level analysis.

2.9. DNA methylation level analysis

The `'MethylKit'` package (v1.24.0) (Akalin et al., 2012) was employed along with the `'bismark_cpg_report2mycpg.pl'` script (available at github.com/avilella/methylKit) to process the input data and identify common CpG sites among the selected samples. After normalization and filtering, principal component analysis (PCA) was conducted to assess sample clustering for each tissue and phenotype before and after POMS infection. For differential methylation analysis, we processed the output from methylation calling using the `'DSS'` package (v2.50.1) (Feng and Wu, 2019) to identify the differentially methylated region(s) (DMR) using a smoothing strategy on 500 bp windows. A DMR was considered significant when it contained at least four CpGs in a sequence of 50 bp and a minimum methylation level difference of 10%, with a False Discovery rate (FDR) ≤ 0.05 . Significant DMRs were automatically merged if they were within 50 bp of each other. In bivalves, as described for *M. gigas* and *Crassostrea virginica*, the methylated fraction primarily occurs within the gene body regions (Gavery and Roberts, 2014; Manner et al., 2021; Venkataraman et al., 2020, 2022). To identify DMRs within gene body regions, we obtained the gene coordinates (*i.e.*, chromosome, start and end positions) of all coding genes of *M. gigas* from the `'biomaRT'` package (v2.54.1) (Durinck et al., 2009) and we overlapped with the significant DMRs using the `'foverlaps'` function from the `'data.table'` package (v1.14.8) (Dowle et al., 2019) obtaining a list of genes displaying DMRs. Finally, the average methylation value was calculated when a gene had more than one DMR at its boundary.

2.10. Gene Ontology enrichment (GO-terms) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analysis of methylated genes affected by POMS

We performed Gene Ontology (GO-terms) enrichment analysis to extract the biological process (BP) GO-terms associated with the genes with DMRs affected by POMS infection. The gene list of *M. gigas* containing the complete set of annotated genes was used for functional annotation using binary analysis, and a score of 1 or 0 was assigned to genes with or without methylation, respectively, to identify enriched GO-terms (based on Fisher's exact test). We employed the GO_MWU package with adaptive clustering (github.com/zoon/GO_MWU) (Wright et al., 2015) with the following parameters: `largest = 0.1`, `smallest = 3`, and `cluster cut height = 0.25`. A BP GO-term was considered significant with an FDR correction ≤ 0.05 . To visualize the significant BP GO-terms, we used ReViGO (v1.8.1) (Supek et al., 2011) with the following parameters: `large = 0.9`, the Whole UniProt database as the background, and SimRel as the semantic similarity measures of the relationship of the GO-terms. The Database for Annotation Visualization and Integrated Discovery platform (v2023q4) was used to obtain the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (Huang et al., 2009; Sherman et al., 2022). A KEGG pathway was significant when it had a minimum of five genes and an EASE Score (modified Fisher exact test) ≤ 0.05 .

2.11. Statistical analysis and software

Statistical analyses were conducted using the R environment (v4.3.2) (Team, 2021) in RStudio (v2023.06.1). The heatmaps were constructed using the 'ComplexHeatmap' package (v2.14.0) (Gu, 2022; Gu et al., 2016), while plot visualizations were created using the 'ggplot2' package (v3.4.4) (Wickham, 2009). Data was processed using the 'dplyr' package (v1.1.4) (Wickham et al., 2020). To plot the map of the sites where the oyster progenitors were collected, we used 'ggspatia' (v1.1.9), 'geodata' (v0.5–9), 'terra' (v1.7–71), and 'raster' (v3.6–26) and to illustrate the exons of the genes we used the 'ggtranscript' (v0.99.9) packages.

3. Results

3.1. Experimental POMS infection and mortality

After the "Pre-infection-T₀" sampling, no mortality was recorded in the recipient oysters, indicating a low impact induced by the biopsy (Fig. 1A). After three weeks of recovery, we phenotyped the five F₁ populations for POMS resistance through cohabitation with infected donor oysters (Fig. 1B). The first mortality among the recipient oysters was observed at 52 hpc. At the end of the infection experiment, mortality rates among the five F₁ populations displayed significant differences (Log-rank test, *P*-value ≤ 0.001) ranging from 15 % of survival for the most susceptible (*i.e.*, SC18, #5) to 85 % for the most resistant (*i.e.*, Thau Lagoon, #1) (Fig. 1C; Tables S1 and S2). No significant differences were observed among the five replicates (Log-rank test, *P*-value = 0.96) (Fig. S2A and Table S3). The mortality of donor oysters (*i.e.*, F14 and NSI) began at 24 hpc, and the survival rate decreased to 70 % for NSI and 0 % for F14, as expected from previous experiments (Fig. S2B). Quantification of the OsHV-1 μVar viral load sampled from the water of the five tanks showed that viral shedding reached 520 ± 340 genome copies/μL at 24 hpc and peaked at 48 hpc with 5781 ± 2360 genome copies/μL (Fig. S2C). In gills and mantle sampled from "Post-infection-T₁", the OsHV-1 μVar viral load was significantly higher (Mann-Whitney, *W* = 23, *P*-value = 0.032 for both tissues) in the susceptible oysters compared to their resistant counterparts (Fig. S2D). These results confirmed that the experimental POMS infection was efficient, with active viral replication and shedding starting in donors and then in recipient oysters.

3.2. Global methylation levels in gills and mantle of susceptible and resistant oysters

For each phenotype (*i.e.*, 5 susceptible and 5 resistant oysters), we analyzed their DNA methylation profile in two tissues (gills and mantle) and for two time points: before (*i.e.*, "Pre-infection-T₀") and after (*i.e.*, "Post-infection-T₁") POMS infection, making a total of 40 samples. After demultiplexing from sequencing, the number of raw PE reads per sample was 111,915,407 ± 19,799,732 (mean ± SD), with a mapping efficiency of 43.15 ± 1.85 %. The removed duplicated reads represented 8.99 ± 0.71 %, and the enzymatic conversion efficiency was 99.90 ± 0.09 %. Detailed information for each sample is presented in Table S4.

At the genome level, we observed a consistent increase in the global methylation level in both tissues of the "Post-infection-T₁" samples (Fig. S3). When comparing phenotypes, DNA methylation levels were substantially higher in the gills (One-way ANOVA; *F* = 4.62, *P*-value = 0.005) and mantle (One-way ANOVA; *F* = 7.49, *P*-value = 0.002) of susceptible oysters than the resistant oysters (Fig. S3A and S3B). In the PCA analysis conducted with CpGs common to all gills (1,886,331 CpGs, Fig. S4A) and mantle (1,806,224 CpGs, Fig. S4B) samples, we observed that samples at both "Pre-infection-T₀" and "Post-infection-T₁" predominantly clustered according to the populations' origin.

These results showed that POMS infection increased the whole-genome methylation level, especially in susceptible individuals. However, the infection had only a subtle impact on the *M. gigas* cytosine methylation landscape.

3.3. Block 1: resistant and susceptible oysters exhibited distinct methylation patterns in both their gills and mantle before and after POMS infection

We then conducted differential methylation analysis comparing the "Post-infection-T₁" (treated) vs. "Pre-infection-T₀" (control) samples for each phenotype and tissue separately. This analysis aimed to identify the methylation changes associated with POMS infection and to understand how these changes differ between resistant and susceptible oysters (Fig. S5).

3.3.1. DNA methylation profile in susceptible oyster gills

In the gills of susceptible oysters, our analysis revealed 3069 DMRs (Tables S5 and S6) located in 2014 genes (Table S7). Among these genes, 1014 were hypermethylated and 1000 were hypomethylated in response to POMS (Fig. 2A and Table S7). The 1014 genes exhibiting hypermethylation were mostly associated with biological processes involved in immunity and stress responses (*e.g.*, DNA damage response, GO:0006281; response to virus, GO:0009615) and transport (*e.g.*, nuclear transport, GO:0046907; cellular localization, GO:0051641). With the 1000 hypomethylated genes, a reduced number of GO enrichments were obtained, which corresponded to more general biological processes (*e.g.*, RNA metabolic processes, GO:0016070) (Fig. 2A). KEGG pathway analysis revealed that hypermethylated genes were involved in base excision repair (crg03410) and nucleocytoplasmic transport (crg03013). In contrast, the hypomethylated genes were predominantly associated with metabolic processes, *e.g.*, biosynthesis of cofactors (crg01240), urine metabolism (crg00230), and ubiquinone biosynthesis (crg00130) (Fig. 2A).

3.3.2. DNA methylation profile in resistant oyster gills

In the gills of resistant oysters, our analysis revealed 2594 DMRs (Tables S5 and S8) located in 1702 genes (Table S9). Among these, 923 and 779 genes were hypermethylated and hypomethylated, respectively, in response to POMS (Fig. 2B and Table S9). Genes with hypermethylation were associated with biological processes involved in signaling (*e.g.*, negative regulation of the Wnt signaling pathway, GO:003178; positive regulation of GTPase activity, GO:0043547; protein phosphorylation, GO:0006468) and transport (*e.g.*, cellular transport, GO:0006810). Hypomethylated genes were associated with only transport (*e.g.*, nucleobase-containing compound transport, GO:0015931) (Fig. 2B). KEGG pathway analysis showed that hypermethylated genes were predominantly involved in mitophagy (crg04137). In contrast, hypomethylation was observed in genes involved in cofactor biosynthesis, such as Pantothenate CoA biosynthesis (crg00770) and the biosynthesis of cofactors (crg01240) (Fig. 2B).

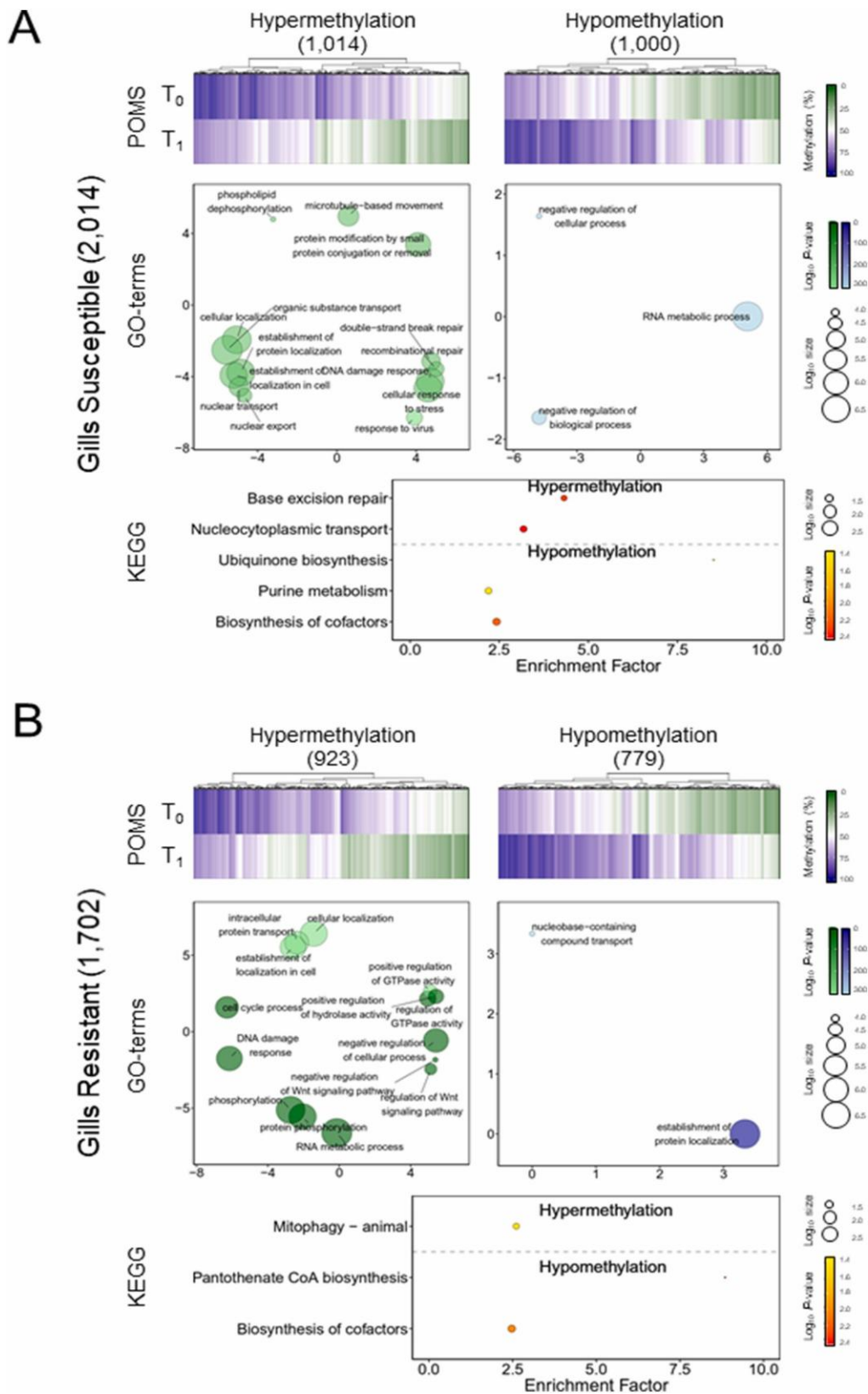


Fig. 2. DNA methylation analysis in gills comparing “Pre-infection-T₀” vs. “Post-infection-T₁” samples during Pacific Oyster Mortality Syndrome (POMS) infection. A) Analysis of 2014 genes with differentially methylated regions (DMRs) in gills of susceptible oysters and B) 1027 genes with DMRs in gills of resistant oysters, depicting their hypo- and hypermethylation changes after POMS infection, respectively. Gene Ontology terms (GO-terms) of the Biological Processes (BP) category and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed to elucidate the functional implications and molecular pathways associated with the differential genes with DMRs for each phenotype.

3.3.3. Similarities between susceptible and resistant oyster gills

Based on the above results, we performed a delta rank correlation analysis on GO-terms enriched in response to POMS infection in both phenotypes.

The results showed a significant, albeit moderate, positive correlation (Spearman's correlation; P -value = 0.0089; R^2 = 0.31; Fig. S6A). Among the 2014 and 1702 genes with DMRs in the gills associated with susceptible and resistant phenotypes, respectively, 527 genes overlapped (Fig. S6C). From these shared genes, GO-term enrichment for biological processes vital for the POMS response was identified, specifically in the stress response (*i.e.*, regulation of apoptotic process, GO:0042981; DNA damage response, GO:0006974; and double-strand break repair, GO:0006302) (Fig. S6E). Additionally, among these 527 shared genes, 277 displayed consistency in methylation change patterns after POMS infection, irrespective of the phenotype (*i.e.*, hypomethylation in 120 genes and hypermethylation in 157 genes, Fig. S6G).

However, even if these changes were similar and common in the susceptible and resistant strains, the methylation levels at T_0 and T_1 between phenotypes were strongly different, probably because of a different genetic background or progenitor history. For example, the rapamycin complex 2 subunit MAPKAP1 (G25230) gene, which plays a critical role in the TOR signaling pathway by regulating cell growth, proliferation, and survival, was hypomethylated in both phenotypes. Methylation values of G25230 gene ranged from 36.92 % in resistant oysters and 35.10 % in susceptible oysters at "Pre-infection- T_0 " to 5.04 % and 31.73 % at "Post-infection- T_1 ," respectively.

3.3.4. Methylation profile in susceptible oyster mantle

In the susceptible oyster mantle, 2836 DMRs were identified (Tables S5 and S10), encompassing 1866 genes, of which 690 were hypermethylated and 1176 were hypomethylated in response to POMS (Fig. 3A and Table S11). In the 690 hypermethylated genes, we identified the involvement of apoptosis (*e.g.*, regulation of programmed cell death, GO:0043067; programmed cell death GO:0012501) and cellular integrity maintenance (*e.g.*, riboflavin biosynthetic, GO:0009231) by GO-term enrichment analysis. The 1176 hypomethylated genes were involved in biological processes linked to intracellular transport (*e.g.*, calcium ion transport, GO:0048193; nuclear transport, GO:0051169), RNA and ncRNA metabolism (*e.g.* RNA metabolic process, GO:0016070; regulatory ncRNA processing, GO:0070918), and protein metabolic processes (*e.g.*, positive regulation of protein metabolic process, GO:0051247) (Fig. 3A). KEGG pathway analysis showed significant enrichment for metabolic pathways with riboflavin metabolism (crg00740) and β -alanine metabolism (crg00410) in hypermethylated genes. In the hypomethylated genes, pathways such as protein turnover and associated regulation of the proteasome (crg03050) and translation (nucleocytoplasmic transport, crg03013) were identified (Fig. 3A).

3.3.5. Methylation profile in resistant oyster mantle

In the mantle of resistant oysters, our analysis revealed 5469 DMRs between infected and non-infected samples (Tables S5 and S12). Among the DMRs located within the gene body regions, 1683 and 1294 exhibited hypermethylation and hypomethylation, respectively, in response to POMS (Fig. 3B and Table S13). The 1683 genes with hypermethylation were associated with biological processes of immunity (*e.g.*, response to virus, GO:0009615; response to stress GO:0006950), protein modification (protein modification, GO:0036211; protein modification by small protein removal GO:0070646), transport (*e.g.*, organic substance transport GO:0071702; cellular localization GO:0051641), and metabolism (*e.g.*, amide metabolic process, GO:0043603; glycolipid metabolic process, GO:0006664). The 1294 hypomethylated genes were associated with stress response (*e.g.* cellular stress response, GO:0033554; recombination repair, GO:0000725; double-strand break repair GO:0006302), GTPase activity (*e.g.*, regulation of GTPase activity GO:0043087; positive regulation of hydrolase activity, GO:0051345), and transport (*e.g.*, cellular localization, GO:0051641) (Fig. 3B). KEGG pathway analysis of the hypermethylated genes revealed the involvement of other glycan degradation (crg00511) and nucleocytoplasmic transport (crg03013). In contrast, hypomethylation was associated with the basal transcription factor (crg03022) and aminoacyl-tRNA biosynthesis (crg00970) (Fig. 3B).

3.3.6. Similarities between the susceptible and resistant oyster mantle

Based on the above results obtained for the mantle, delta rank correlation analysis of enriched GO-terms identified in the mantle of both phenotypes did not show a significant correlation (Spearman; P -value = 0.38; R^2 = 0.0089; Fig. S6B). When focusing on genes with DMRs, we found that 788 genes overlapped between phenotypes (Fig. S6D). Among the 788 common genes, several were implicated in key cellular processes, including immunity (*e.g.*, cellular response to virus, GO:0009615; response to virus, GO:0009615), intracellular transport (*e.g.*, nucleocytoplasmic transport, GO:0000063), and positive regulation of enzymatic activity (*e.g.*, positive regulation of catalytic activity, GO:0048554) (Fig. S6F). Analysis of DNA methylation patterns in the mantle of oysters revealed distinct responses between the susceptible and resistant phenotypes following POMS infection. These changes were primarily associated with processes related to intracellular transport, RNA metabolism, and protein turnover. In contrast, resistant oysters exhibited a broader and more pronounced response, with a larger number of DMRs encompassing genes involved in immunity, stress responses, protein modification, and metabolism. As observed in the gills, among the 788 common genes in the mantle, 384 displayed the same directional changes (213 hypermethylation and 171 hypomethylation), irrespective of the phenotype (Fig. S6H). For example, the interferon-induced protein 44 (G31185) gene displayed methylation levels of 47.52 % and 43.64 % in resistant and susceptible oysters, respectively, at T_0 and 27.14 % and 12.91 %, respectively, at T_1 .

3.4. Genes with DMRs associated with immune response

Our analysis of genes with DMRs in the gills and mantle revealed distinct overlaps and highlighted specific functional categories in the two tissues after POMS infection. Interestingly, some genes that displayed methylation changes due to infection were associated with immunity. From the list of genes found in gills, we identified 222 (Table S7) and 185 (Table S9) immune-related genes in susceptible and resistant oysters, respectively. In the mantle, there were 181 (Table S11) and 284, respectively (Table S13). When comparing the four lists, 31 genes were common to all tested conditions and displayed differential methylation patterns in response to POMS, regardless of the tissue or phenotype (Fig. S7 and Table S14). Among them, we identified the bcl-2 homologous antagonist/killer-like (*bak1*, G17360), the baculoviral IAP repeat-containing protein 7-like (*birc3*, G19919), few genes encoding for E3 ubiquitin-protein ligases (*trim3*, G18939; *trim36*, G18954; and *trim71*, G19802), the ubiquitin-like modifier-activating enzyme 1 (*uba1*, G29533), and the integrin alpha-2-like gene (*itga2*, G31928).

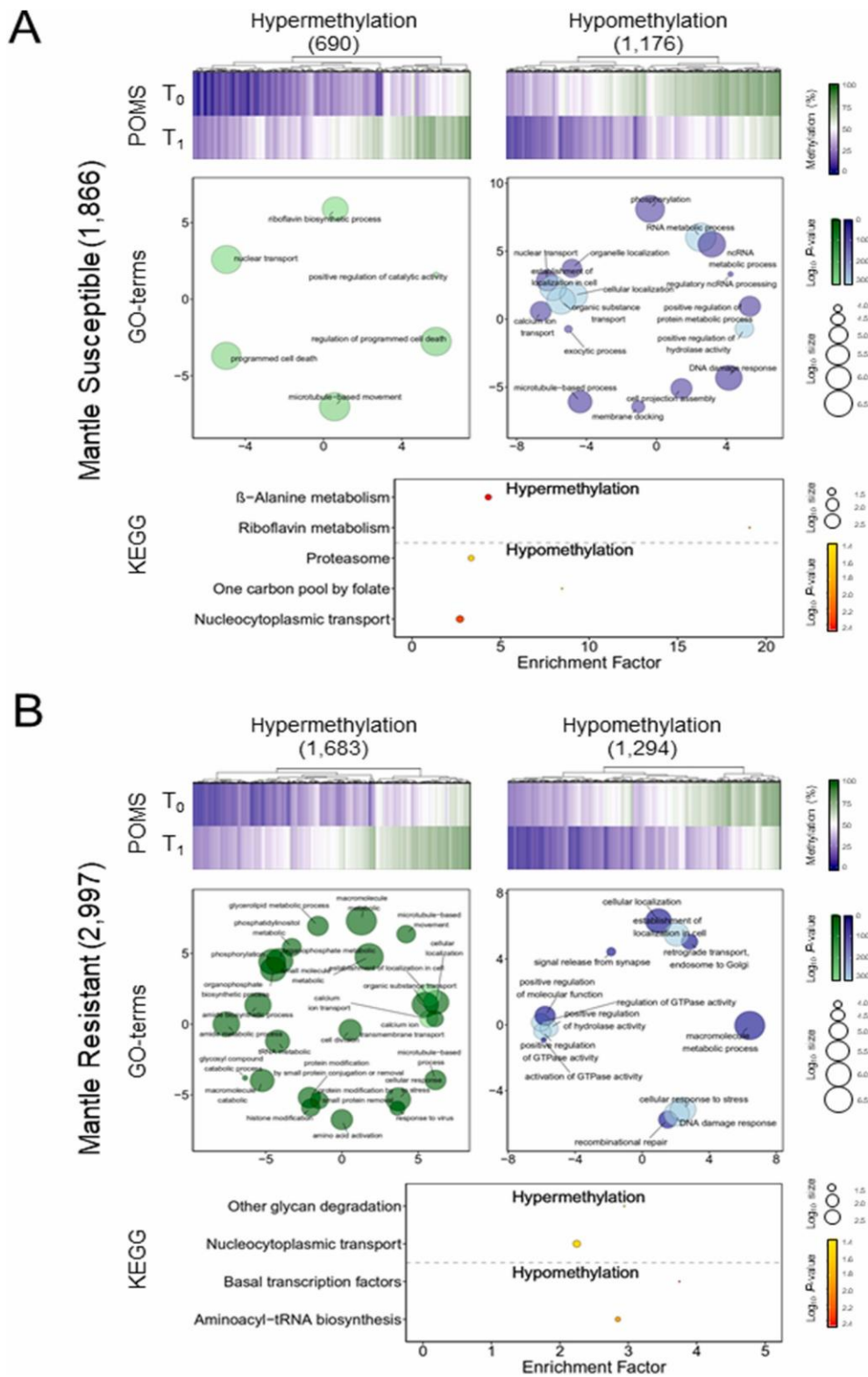


Fig. 3. DNA methylation analysis in mantle comparing “Pre-infection-T₀” vs. “Post-infection-T₁” samples during Pacific Oyster Mortality Syndrome (POMS) infection. A) Analysis of 1866 genes with differentially methylated regions (DMRs) in the mantle of susceptible oysters and B) 2997 genes with DMRs in the mantle of resistant oysters, depicting their hypo- and hypermethylation changes after the POMS infection, respectively. Additionally, the Gene Ontology enrichment (GO-terms) of the Biological Processes (BP) category and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed to elucidate the functional implications and molecular pathways associated with the differential genes with DMRs for each phenotype.

Overall, for both tissues, the methylation levels before and after the response to POMS revealed stark distinctions between the resistant and susceptible phenotypes at the specific and common gene levels. This dynamic response suggests a nuanced interplay between the genetic predisposition or history of progenitors and environmental triggers, where epigenetic modifications may serve as key mediators in orchestrating host defense mechanisms. Consequently, while genetic predisposition or progenitor history may shape the epigenetic profile of initial vulnerability, shared alterations in DNA methylation of the same genes may reflect the existence of a core response. This was also observed in 31 genes related to the immune response. This unified mechanism offers valuable insights into potential targets for enhancing oyster resilience to POMS infection.

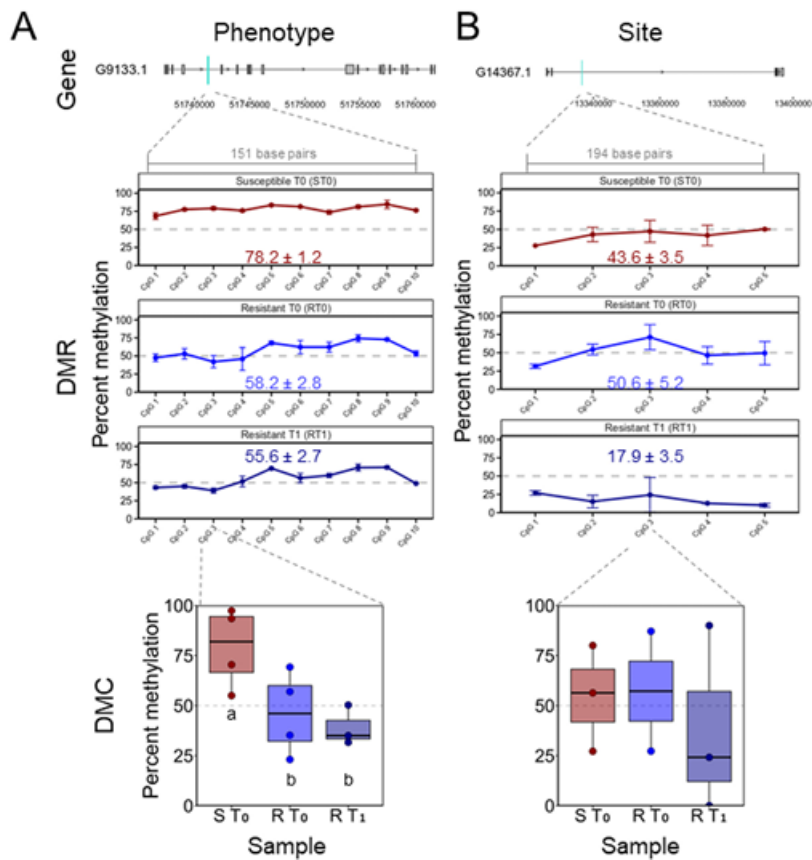


Fig. 4. Example of epibiome markers for phenotype and site selection in gills tissue. A) Example of a candidate epibiome marker for phenotype selection. Methylation levels of the CpGs located in the 151 base pairs (bp) long differentially methylated region (DMR) located in the gene body of the Hedgehog interacting protein-like gene (G9133) for the “Susceptible T0”, “Resistant T0”, and “Resistant T1” oysters. Among these CpGs, #CpG3 was differentially methylated cytosine (DMC) between phenotypes. B) Example of a candidate epibiome marker for site selection. Methylation levels of the CpGs located in the 194 bp long DMR located in the gene body of the THO complex subunit 3-like gene (G14367) for the “Susceptible-T0”, “Resistant-T0”, and “Resistant-T1”.

Table 1

List of epibiomarkers in gills and mantle tissue for phenotype selection with different levels of DNA methylation (from lower to higher stringency conditions).

Tissue	Δ ST ₀ vs. RT ₀	Δ ST ₀ vs. RT ₁ & RT ₀ vs. RT ₁	No. DMRs	No. genes with DMRs	Immune response
Gills	≤ 5	≥ 20	1204	1030	115
		≥ 30	420	392	45
		≥ 40	133	126	15
		≥ 50	33	32	4
		≥ 60	7	7	0
	≤ 2.5	≥ 20	816	717	79
		≥ 30	272	257	29
		≥ 40	78	76	8
		≥ 50	22	21	2
		≥ 60	5	5	0
	≤ 1	≥ 20	429	391	49
		≥ 30	126	120	14
		≥ 40	34	34	3
		≥ 50	7	7	1
		≥ 60	1	1	0
Mantle	≤ 5	≥ 20	794	718	85
		≥ 30	281	268	32
		≥ 40	77	74	9
		≥ 50	23	23	4
		≥ 60	9	9	1
	≤ 2.5	≥ 20	531	489	61
		≥ 30	199	191	28
		≥ 40	52	50	8
		≥ 50	16	16	4
		≥ 60	7	7	1
	≤ 1	≥ 20	292	278	35
		≥ 30	106	102	17
		≥ 40	28	28	7
		≥ 50	11	11	2
		≥ 60	5	5	0

Table 2

List of epibiomarkers in gills and mantle tissue for site selection with different levels of DNA methylation (from lower to higher stringency conditions).

Tissue	Δ ST ₀ vs. RT ₀	Δ ST ₀ vs. RT ₁ & RT ₀ vs. RT ₁	No. DMRs	No. genes with DMRs	Immune response
Gills	≤ 5	≥ 20	66	65	2
		≥ 30	22	22	1
		≥ 40	7	7	0
		≥ 50	1	1	0
		≥ 60	0	0	0
	≤ 2.5	≥ 20	35	35	1
		≥ 30	14	14	1
		≥ 40	2	2	0
		≥ 50	0	0	0
		≥ 60	0	0	0
	≤ 1	≥ 20	15	15	0
		≥ 30	4	4	0
		≥ 40	1	1	0
		≥ 50	0	0	0
		≥ 60	0	0	0
Mantle	≤ 5	≥ 20	98	94	9
		≥ 30	34	34	4
		≥ 40	17	17	2
		≥ 50	9	9	1
		≥ 60	3	3	0
	≤ 2.5	≥ 20	66	64	6
		≥ 30	25	25	2
		≥ 40	14	14	1
		≥ 50	9	9	1
		≥ 60	3	3	0
	≤ 1	≥ 20	41	39	6
		≥ 30	13	13	2
		≥ 40	7	7	1
		≥ 50	5	5	1
		≥ 60	1	1	0

3.5. Block 2: DNA methylation as epibiomarkers for POMS disease management

In this study, we uncovered distinct DNA methylation profiles within the gills and mantle of resistant and susceptible oysters before and after POMS infection. Beyond the biological interest in understanding the epigenetic patterns associated with each phenotype, we aimed to identify potential CpG sites and DMRs that could serve as predictive biomarkers for POMS infection (exposed vs. non-exposed sites) and outcomes (resistant vs. susceptible). POMS is a highly virulent syndrome that occurs in the wild and decimates of all susceptible individuals. In the case of a non-exposed site, the oyster populations will be composed of a mix of resistant and susceptible individuals displaying the “Pre- infection-T₀” methylation profile. On the contrary, already exposed sites will host only those resistant individuals with a resistant “Post-infection T₁” methylation profile. Based on this rationale, we focused on identifying the most promising candidate epibiomarkers.

3.5.1. Epibiomarkers for phenotype selection

To identify the epibiomarker(s) of resistance, we made two comparisons, where the reference group was the Susceptible-T₀ group (Fig. S8). We established the following criteria that candidate epibiomarkers must meet: 1) an absolute methylation difference ≥ 20 % between |Resistant-T₀ vs. Susceptible-T₀|, 2) an absolute methylation difference ≥ 20 % between |Resistant-T₁ vs. Susceptible-T₀|, and finally 3) an absolute methylation difference ≤ 5 % between |Resistant-T₀ vs. Resistant-T₁| (Fig. S8). Thus, we identified 1204 DMRs in the gills, of which 1030 were located within the gene body region (Table 1 and Table S15), and 794 DMRs in the mantle, of which 718 were located within the gene body region (Tables 1 and S16). Among these candidate epibiomarkers, one example was selected for description, as shown in Fig. 4A. Specifically, this DMR spanned over 151 bp, with 10 CpGs within the Hedgehog interacting protein-like gene body region (G9133) (Fig. 4A). When comparing the mean methylation difference in the DMR between the RT₀ vs. ST₀ and RT₁ vs. ST₀ groups, we observed 20.0 % and 22.6 % hypomethylation, respectively, with only a 2.6 % methylation difference between the RT₀ vs. RT₁ groups. At the single CpG level, the third CpG exhibited significant (One-way ANOVA, $F = 5.02$; P -value = 0.039) differential methylation levels between susceptible (ST₀, $n = 4$ samples) and resistant (RT₀, $n = 4$, and RT₁, $n = 3$) oysters (Fig. 4A). The results of this approach showed that several CpG candidate epibiomarkers located in gene body regions could be used as the foundation for developing a panel of putative informative DMRs or CpGs suitable for the phenotyping of oyster resistance in all epidemiological contexts.

3.5.2. Epibiomarkers for site selection

To identify epibiomarkers for diagnosing the presence/absence of POMS at an unknown site, we made two comparisons, where the reference group was the Resistant-T₁ group (Fig. S9). We established the following criteria that candidate epibiomarkers must meet: 1) an absolute methylation difference ≤ 5 % between |Susceptible-T₀ vs. Resistant-T₀|, 2) an absolute methylation difference ≥ 20 % between |Susceptible-T₀ vs. Resistant-T₁|, and finally 3) an absolute methylation difference ≥ 20 % between |Resistant-T₀ vs. Resistant-T₁|. Thus, we identified 68 candidate epibiomarkers in the gills, 66 of which were located within gene body regions (Tables 2 and S17). In the mantle, 128 DMRs were identified, 98 of which were located within the gene body region (Tables 2 and S18). Among these candidate epibiomarkers, one from the gills is depicted in Fig. 4B. This DMR spanned over 194 bp and encompassed five CpG sites in the body region of the THO complex subunit 3-like gene (G14367) (Fig. 4B). When comparing the methylation differences between non-infected and infected samples, the mean methylation was 2.4 (RT₀) and 2.8 (ST₀) folds higher than that of RT₁, respectively. Among the five CpGs within the DMR, CpG #3 was a putative epibiomarker for site selection.

4. Discussion

4.1. Epigenetic changes during POMS reveal the dynamic interplay of host-pathogen interactions

In the present study, we showed that epigenetic differences in oysters are associated with their resistant and susceptible phenotypes before POMS exposure, regardless of their geographical origin. Additionally, the exposure of these oysters to POMS induced DNA methylation changes in both phenotypes. These epigenetic differences between phenotypes and those induced by the disease open new questions about the fundamentals of the interaction between oysters and POMS and putative applications in POMS management.

Host-pathogen interactions are dynamic and involve constant co-evolutionary processes, where each partner constantly tries to circumvent molecular innovation, enhancing host resistance or pathogen infectivity. Among these mechanisms, the host epigenome can be a target of choice, given its ability to rapidly shape new molecular configurations with potentially improved fitness for both the host (Netea et al., 2020) and the pathogen (Fischer, 2020). Therefore, the DNA methylation changes identified in our study in response to POMS infection can affect the host response or the hijack of the cellular machinery by OshV1- μ Var.

In hosts, non-lethal biotic interactions that induce an immune response drive immune priming, leading to long-lasting protection against later pathogen encounters (Lanz-Mendoza and Contreras-Garduno, 2022). This “trained immunity” mechanism is a phenomenon of immune memory affecting the innate immune system *sensu lato* and metabolic shift (Lanz-Mendoza and Contreras-Garduno, 2022). Metabolic intermediates can serve as substrates and cofactors for chromatin modifiers, and the activities of related enzymes that fluctuate during infection can regulate innate immune responses *via* epigenetic mechanisms (Nieborak and Schneider, 2018). While numerous empirical studies have shown clear evidence of this phenomenon in mollusks, the mechanisms governing it remain misunderstood but may rely, at least partly, on metabolic shifts and epigenetic changes (Zhao et al., 2023), as in plants and vertebrates (Netea et al., 2020). In the case of oysters, immune priming has already been demonstrated (Lafont et al., 2017) and is associated with the specific regulation of genes involved in the innate immune response and metabolism (Lafont et al., 2020). Two studies have linked specific DNA methylation changes or patterns to oyster resistance/susceptibility to POMS. In a mesocosm context, one study showed that exposure to a microbial-rich environment during the larval stage induced an intergenerational increase in resistance against POMS (Fallet et al., 2022), whereas another showed a significant association between CpG methylation levels and oyster resistance/susceptibility to POMS in wild populations (Gawra et al., 2023). These findings indicate that trained immunity in the interaction between oysters and POMS may be influenced, in part, by alterations in DNA methylation.

Several studies have indicated that DNA methylation is an important epigenetic mechanism that controls inducible gene expression in *M. gigas* (Gavery and Roberts, 2010; Riviere et al., 2013). In our study, several genes belonging to biological functions linked to innate immunity and metabolism displayed such changes in response to POMS infection in both susceptible and resistant oysters (Figs. 2 and 3; Tables S7, S9, S11, and S13). This result can be interpreted as the first step toward inducing trained immunity. The affected genes displayed divergent functions between susceptible and resistant oysters from an approximately equal number of identified DMRs induced by POMS infection. Mechanistically, these DNA methylation changes targeting specific genes and functions are driven by the transcriptional activation/repression of these functions (de Lorgeril et al., 2018; Lafont et al., 2020; Fallet et al., 2022) during the POMS challenge. Indeed, as hypothesized in *Acropora millepora*, gene body methylation levels and transcription could influence each other; a higher transcription rate induces hypermethylation to a certain threshold, and a high methylation level decreases gene expression in a negative feedback loop (Dixon et al., 2018). The consistent methylation changes observed in specific shared genes in response to POMS infection (Fig. S6C and D) suggested that these genes may play a crucial role in the host response to POMS. This observation highlights the importance of epigenetic mechanisms, in which certain gene regions undergo similar methylation alterations in both resistant and susceptible oysters, reflecting a common response. However, the initial differences (T_0) in the methylation levels of these genes could explain, at least in part, the phenotypic differences between resistant and susceptible individuals. Thus, these regions appear to be under environmental influence due to OshV1- μ Var pressure, indicating potential epigenetic selection on those genes.

At the tissue level, we focused on two tissues closely interacting with the environment: the gills involved in respiratory function and food capture (Galbraith et al., 2009), and the mantle involved in shell biomineralization (Ivanina et al., 2017). Our results identified a set of 31 genes that showed substantial methylation changes after POMS infection, irrespective of the oyster phenotype and tissue (Fig. S7). These genes are involved in immune responses. Among them, the *bak1-like* gene (G9050), interferon alpha-inducible protein 27 (G9050), and macrophage mannose receptor 1 gene (G28068) have well-conserved immune functions in *M. gigas* (Lafont et al., 2020; Namwong et al., 2023; Qiao et al., 2022). While these similarities were observed, we also found tissue-specific methylation changes in immune genes such as viral A-type inclusion protein (G11123), Caspase P20 domain-containing protein (G16802), and programmed cell death protein 5 (G24071) in the gills, and C1q domain-containing protein (G18430), C-type lectin domain-containing protein (G2525), and macrophage-expressed 1 protein (G21614) in the mantle. These specific changes can be linked to the different cellular composition and the different roles played by these two tissues, but also because the gills are full of hemolymph and hemocytes. These are the oyster’s immune cells (Fisher, 1986; Morga et al., 2017), the cells that are the target of OshV1- μ Var (de Lorgeril et al., 2018). Therefore, the similarities and differences between these two tissues are likely the result of their role in the immune process linked to epithelia and the differences they harbor in terms of their immune cell content.

Pathogens are also known to induce epimutations in the host epigenome. This is especially true in diseases involving viruses in which the host cellular machinery is hijacked to optimize the multiplication and transmission of shedding viral particles (Balakrishnan and Milavetz, 2017). Among the viruses identified for epigenetic manipulation, several belong to the Herpesviridae family. OshV-1 μ Var, the primary agent of POMS, also belongs to this virus family (Pei et al., 2020). In general, virus-induced epigenetic reprogramming is mainly associated with the upregulation of host DNA methyltransferase (DNMTs), inducing hypermethylation of targeted genes and/or genome-wide hypermethylation (Locatelli and Faure-Dupuy, 2023). Our study mirrors this trend (especially in susceptible oysters), with methylation levels consistently increasing by ~ 0.54 % in the gills and ~ 0.48 % in the mantle post-POMS infection (Fig. S4). The Epstein-Barr virus (EBV) has been extensively studied in this context. It has been shown that the major EBV oncoprotein, the latent membrane protein 1 (LMP1), is a pleiotropic factor that reprograms, balances, and perturbs a wide spectrum of cellular mechanisms, including epigenetics (Wang and Ning, 2021). Tsai et al. showed that LMP1 downregulates the expression of critical host genes using the cellular DNA methylation machinery (Tsai et al., 2002); more specifically, the LMP1 transcriptionally upregulates the DNA methyltransferase 1 (DNMT1) thanks to its COOH-terminal activation region-2 YYD domain (Tsai et al., 2006). We searched for the presence of the YYD domain in the OshV-1 μ Var proteins and found that 6 of the 128 ORFs encode proteins containing this domain, and only one (ORF088) is predicted to encode a membrane protein (Burioli et al., 2017). Further investigations are required to determine the potential role of the protein encoded by this ORF in hijacking the oyster methylome and the epigenetic landscape of DNMT genes in *M. gigas*.

During Herpesviridae interactions with vertebrate hosts, manipulations to hijack cellular machinery have three major objectives: to delay or impair the host immune response, to induce a metabolic shift (both retrieved in biological processes displaying DNA methylation changes, as observed in Figs. 2 and 3; Tables S7, S9, S11, and S13), and to regulate the latent/lytic cycle (Pei and Robertson, 2020; Locatelli and Faure-Dupuy, 2023). Concerning the former, several viruses of the Herpesviridae family were shown to immuno-modulate, through epigenetic changes, the different functions of the main antiviral pathways, such as the pathogen recognition, complement activation, interferons (IFN), JAK-STAT, and TGF- β signaling (Locatelli and Faure-Dupuy, 2023). Interestingly, we and others have previously shown that all these pathways are transcriptionally activated in response to POMS and that differences between resistant and susceptible oyster families are mostly characterized by a slight delay in the activation of these antiviral pathways in susceptible oysters infected by the OshV-1 μ Var (de Lorgeril et al., 2018, 2020; Lepretre et al., 2021).

4.2. Development of putative epibiomarkers for improving Pacific oyster aquaculture

With the constant increase in food demand worldwide, aquaculture is constantly expanding due to increased production and the establishment of new farms in uncultured areas (Gentry et al., 2017). One of the main limitations to this increase in the oyster industry is that it is affected by diseases that strongly affect cultured stocks and brings uncertainties for geographical expansion (Pernet et al., 2016). In this context, providing epibiomarkers for epigenomic selection and site selection are two essential aspects that could improve current zootechnical practices for the sustainable growth of the Pacific oyster industry, given the outcomes of climate change (Reid et al., 2019). For this purpose, we identified a set of candidate epibiomarkers that could be used to characterize whether an oyster is resistant or susceptible (1998 candidate epibiomarkers) and whether a site has been exposed to POMS (164 candidate epibiomarkers).

Genetic selection for resistance to POMS has been shown to be an effective approach for enhancing oyster farming because it displays high heritability (Azema et al., 2017; Gutierrez et al., 2018, 2020). However, despite the identification of several quantitative trait locus (QTLs) (Sauvage et al., 2010; Gutierrez et al., 2018, 2020; Gawra et al., 2023), unique determinants of oyster resistance have not yet been identified, probably because of the polygenic nature of this trait (de Lorgeril et al., 2020). Recent studies from our group have shown that epigenetic variations play a major role in oyster resistance to POMS (Fallet et al., 2022; Gawra et al., 2023). Additionally, we have identified that the resistance trait to POMS is indeed polygenic but controlled at the genetic and epigenetic levels, with the latter explaining the phenotypic variation better than the former (Gawra et al., 2023). In another study, resistance was environmentally induced, resulting in the appearance of new epialleles in the epigenome (Fallet et al., 2022). Meiotic inheritance in the absence of environmental inducers of some of these epialleles also demonstrates the suitability of such epimutations for epigenomic selection (Fallet et al., 2022). The difficulty in identifying genetic markers associated with resistance owing to its polygenic nature and the significant influence of epigenetic variation on oyster resistance to POMS presents a novel opportunity for POMS management. Therefore, epibiomarker-assisted selection offers a promising avenue for optimization. These epibiomarkers could serve as a potent toolkit for further refinement of POMS management strategies.

Another avenue for sustainable development of the oyster industry relies on selecting disease-free sites to set up new farms. Currently, the characterization of the POMS exposure history of a natural site is costly and time-consuming. Indeed, it is still impossible to detect directly the initial agent causing the POMS (*i.e.*, OsHV-1 μ Var) in seawater (Richard et al., 2021). The unique applicable method is based on deploying dedicated pathogen-free oyster cohorts on the site of interest, their weekly monitoring during 4–5 months of the POMS season, and the detection of OsHV-1 μ Var in moribund individuals by qPCR. Therefore, the induction of specific epimutations in response to POMS events is a powerful alternative solution for diagnosing the presence or absence of POMS in a putative oyster farming site hosting a wild oyster population—a solution using the set of epibiomarker candidates identified in this study.

To identify candidate epibiomarkers for phenotyping or site selection, we performed whole-genome sequencing using a limited number of samples. Our genome-wide approach enabled the identification of candidate epibiomarkers without relying on prior assumptions regarding specific loci, thereby offering exploratory power across the entire genome. However, this method has some limitations. First, the relatively small sample size may limit the statistical power, reducing the sensitivity for detecting subtle epigenetic differences and potentially increasing false positives or negatives. Additionally, we used a conservative threshold to identify DMRs in gene body regions, which, while helpful for identifying changes in the landscape of methylation, may have inadvertently excluded biologically relevant loci in intergenic regions potentially linked to POMS. Our study is further limited by the use of five families as the sample population. Although this design minimizes external variability under controlled conditions, it does not fully represent the biological and genetic diversity of natural populations. Consequently, findings based on these limited families may have reduced their applicability across diverse oyster populations, potentially affecting the generalizability of the identified epibiomarkers. Environmental factors also influence epigenetic regulation, and controlled family-based studies may not replicate the complex conditions experienced by wild oysters.

To address these limitations, a second validation phase was designed and will be applied in future studies to reinforce the findings presented here. This phase involves targeted, locus-specific methods, specifically multiplex bisulfite sequencing (MBS) (Anastasiadi et al., 2018; Moraleda-Prados et al., 2020; Valdivieso et al., 2023a, 2023b), along with a significant increase in sample size (>300). By focusing on the targeted loci, we will apply more stringent statistical criteria, such as multiple testing corrections or a higher confidence threshold (*e.g.*, P -value < 0.001) (Anastasiadi and Beemelmanns, 2023). Machine learning algorithms assist in selecting the most robust features, enabling the development of a restricted panel of highly predictive epibiomarkers. To ensure broad applicability, we will study independent test populations from wild oyster beds along the Mediterranean and Atlantic coasts of France, covering both POMS-affected and unaffected areas. Seasonal sample collection, reflecting variations in temperature and water conditions, and the inclusion of oysters of various sizes (representing different ages) will help capture a comprehensive range of genetic and environmental variations, enhancing the relevance of these epibiomarkers in real-world conditions. This sampling strategy ensures that epibiomarkers are tested across diverse genetic and environmental backgrounds, thereby improving the generalizability and robustness of the candidate loci.

The final step in these developments will be the implementation of these epibiomarkers as practical tools for the sustainable management of the oyster industry. From a methodological perspective, this implementation will have to deal with the balance between the number of epibiomarkers needed to characterize the status of an individual and the number of individuals needed to address the aims of the industry. A small number of epibiomarkers (*e.g.*, N epibiomarkers < 5 and approximately 10 individuals) can be quantified by direct characterization methods, such as targeted bisulfite PCR. For a higher number of epibiomarkers (*e.g.*, $5 < N$ epibiomarkers < 20 and hundred individuals), multiplexing methods such as MBS are preferred (Anastasiadi et al., 2018; Moraleda-Prados et al., 2020; Valdivieso et al., 2023a, 2023b). Ultimately, for high-throughput applications (*e.g.*, N epibiomarker > 20, more than hundreds of individuals), the development of a DNA methylation array, such as that used for diagnostics in humans, would offer a cost-effective solution. For example, for producing a batch of oyster spats in a hatchery through the characterization of 10 epibiomarkers, we ended with 5000 measures. Indeed, such production classically requires ~20 males and 80 females; producing resistant spats from the top 20 % of epigenetically resistant adults would, therefore, require the characterization of 500 individuals based on 10 epibiomarkers. This fast and simplistic evaluation highlights that implementing epibiomarkers in the oyster industry will require good structuration and the involvement of dedicated institutions such as the SYSAAF in France. All these steps will assess the generalizability and robustness of the predictive model and open the use of epibiomarkers in non-model marine species of aquaculture interest, such as *Sepia esculenta* (Y. Wang et al., 2024).

5. Conclusion

This study provides new information about the epigenetic response of oysters to Pacific Oyster Mortality Syndrome (POMS), highlighting dynamic host-pathogen interactions. We showed that oysters exhibited distinct DNA methylation patterns associated with resistance or susceptibility to POMS before pathogen exposure. We also demonstrated that POMS infection induces further methylation changes in both phenotypes. These findings highlight the putative role of DNA methylation in imprinting the host response and potentially enhancing immune priming and trained immunity. This study revealed that specific genes related to

innate immunity and metabolism undergo methylation changes post-POMS infection, indicating their critical role in host response. This study also raises the possibility that epigenetic modifications can be leveraged for POMS management, offering new avenues for enhancing the resilience of the oyster industry through targeted breeding and site selection. Overall, our findings provide a deeper understanding of the molecular interactions between oysters and POMS, opening new questions about the broader implications of epigenetic mechanisms in host-pathogen dynamics and offering promising strategies for mitigating the impacts of this devastating disease.

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CRedit authorship contribution statement

Alejandro Valdivieso: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Benjamin Morga:** Writing – review & editing, Methodology. **Lionel Degremont:** Writing – review & editing, Methodology. **Mickael Mege:** Writing – review & editing, Methodology. **Gaëlle Courty:** Investigation. **Yann Dorant:** Writing – review & editing. **Jean-Michel Escoubas:** Writing – review & editing. **Janan Gawra:** Writing – review & editing. **Julien de Lorgeril:** Writing – review & editing. **Guillaume Mitta:** Writing – review & editing, Supervision, Resources. **Celine Cosseau:** Writing – review & editing. **Jeremie Vidal-Dupiol:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT to manage the data tables properly, improve the code for plotting figures, and improve the English language for clarity. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the contents of the publication.

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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.

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Data availability

Raw reads are available at the European Nucleotide Archive (ENA) under the project accession number PRJEB81880.

References

- Akalin, A., Kormaksson, M., Li, S., Garrett-Bakelman, F.E., Figueroa, M.E., Melnick, A., Mason, C.E., 2012. MethyKit: a comprehensive R package for the analysis of genome-wide DNA methylation profiles. *Genome Biol.* 13 (10), R87. <https://doi.org/10.1186/gb-2012-13-10-r87>.
- Anastasiadi, D., Beemelmanns, A., 2023. Development of epigenetic biomarkers in aquatic organisms. In: Piferrer, F., Wang, H. (Eds.), *Epigenetics in Aquaculture*, 1st ed. Wiley, pp. 413–438. <https://doi.org/10.1002/9781119821946.ch18>.
- Anastasiadi, D., Vandeputte, M., Sanchez-Baiz[´] an, N., Allal, F., Piferrer, F., 2018. Dynamic epimarks in sex-related genes predict gonad phenotype in the European sea bass, a fish with mixed genetic and environmental sex determination. *Epigenetics* 13 (9), 988–1011. <https://doi.org/10.1080/15592294.2018.1529504>.
- Andrews, S., 2010. *FastQC: A Quality Control Tool for High Throughput Sequence Data* [Computer Software]. Babraham Bioinformatics, Babraham Institute, Cambridge, United Kingdom.
- Azema, P., Lamy, J.-B., Boudry, P., Renault, T., Travers, M.-A., Degremont, L., 2017. Genetic parameters of resistance to *Vibrio aestuarianus*, and OsHV-1 infections in the Pacific oyster, *Crassostrea gigas*, at three different life stages. *Genet. Sel. Evol.* 49 (1), 23. <https://doi.org/10.1186/s12711-017-0297-2>.
- Balakrishnan, L., Milavetz, B., 2017. Epigenetic regulation of viral biological processes. *Viruses* 9 (11), 346. <https://doi.org/10.3390/v9110346>.
- Bock, C., 2009. Epigenetic biomarker development. *Epigenomics* 1 (1), 99–110. <https://doi.org/10.2217/epi.09.6>.
- Burioli, E.A.V., Prearo, M., Houssin, M., 2017. Complete genome sequence of Ostreid herpesvirus type 1 μ Var isolated during mortality events in the Pacific oyster *Crassostrea gigas* in France and Ireland. *Virology* 509, 239–251. <https://doi.org/10.1016/j.virol.2017.06.027>.
- Chan, T.A., Baylin, S.B., 2010. Epigenetic biomarkers. In: Mellinghoff, I.K., Sawyers, C.L. (Eds.), *Therapeutic Kinase Inhibitors*, Vol. 355. Springer Berlin Heidelberg, pp. 189–216. https://doi.org/10.1007/82_2011_165.
- Clerissi, C., de Lorgeril, J., Petton, B., Lucasson, A., Escoubas, J.-M., Gueguen, Y., Degremont, L., Mitta, G., Toulza, E., 2020. Microbiota composition and evenness predict survival rate of oysters confronted to Pacific oyster mortality syndrome. *Front. Microbiol.* 11, 311. <https://doi.org/10.3389/fmicb.2020.00311>.
- Clerissi, C., Luo, X., Lucasson, A., Mortaza, S., De Lorgeril, J., Toulza, E., Petton, B., Escoubas, J.-M., Degremont, L., Gueguen, Y., Destoumieux-Garzón, D., Jacq, A., Mitta, G., 2023. A core of functional complementary bacteria infects oysters in Pacific Oyster Mortality Syndrome. *Animal Microbiome* 5 (1), 26. <https://doi.org/10.1186/s42523-023-00246-8>.
- Damania, B., Kenney, S.C., Raab-Traub, N., 2022. Epstein-Barr virus: biology and clinical disease. *Cell* 185 (20), 3652–3670.
- De Kantzow, M., Hick, P., Becker, J., Whittington, R., 2016. Effect of water temperature on mortality of Pacific oysters *Crassostrea gigas* associated with microvariant ostreid herpesvirus 1 (OsHV-1 μ Var). *Aquac. Environ. Interact.* 8, 419–428. <https://doi.org/10.3354/aei00186>.
- de Lorgeril, J., Lucasson, A., Petton, B., Toulza, E., Montagnani, C., Clerissi, C., Vidal-upiol, J., Chaparro, C., Galinier, R., Escoubas, J.-M., Haffner, P., Degremont, L., Charriere, 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. *Nat. Commun.* 9 (1), 4215. <https://doi.org/10.1038/s41467-018-06659-3>.
- de Lorgeril, J., Petton, B., Lucasson, A., Perez, V., Stenger, P.-L., Degremont, L., Montagnani, C., Escoubas, J.-M., Haffner, P., Allienne, J.-F., Leroy, M., Lagarde, F., Vidal-Dupiol, J., Gueguen, Y., Mitta, G., 2020. Differential basal expression of immune genes confers *Crassostrea gigas* resistance to Pacific oyster mortality syndrome. *BMC Genomics* 21 (1), 63. <https://doi.org/10.1186/s12864-020-6471-x>.
- Degremont, L., Garcia, C., Allen, S.K., 2015. Genetic improvement for disease resistance in oysters: a review. *J. Invertebr. Pathol.* 131, 226–241. <https://doi.org/10.1016/j.jip.2015.05.010>.
- Delisle, L., Petton, B., Burguin, J.F., Morga, B., Corporeau, C., Pernet, F., 2018. Temperature modulate disease susceptibility of the Pacific oyster *Crassostrea gigas* and virulence of the Ostreid herpesvirus type 1. *Fish Shellfish Immunol.* 80, 71–79. <https://doi.org/10.1016/j.fsi.2018.05.056>.
- Delisle, L., Pualetto, M., Vidal-Dupiol, J., Petton, B., Bargelloni, L., Montagnani, C., Pernet, F., Corporeau, C., Fleury, E., 2020. High temperature induces transcriptomic changes in *Crassostrea gigas* that hinders progress of Ostreid herpesvirus (OsHV-1) and promotes survival. *J. Exp. Biol.* 226233. <https://doi.org/10.1242/jeb.226233>.
- Delisle, L., Laroche, O., Hilton, Z., Burguin, J.-F., Rolton, A., Berry, J., Pochon, X., Boudry, P., Vignier, J., 2022. Understanding the dynamic of POMS infection and the role of microbiota composition in the survival of Pacific oysters, *Crassostrea gigas*. *Microbiology Spectrum* 10 (6). <https://doi.org/10.1128/spectrum.01959-22> (e01959-22).
- Dixon, G., Liao, Y., Bay, L.K., Matz, M.V., 2018. Role of gene body methylation in acclimatization and adaptation in a basal metazoan. *Proc. Natl. Acad. Sci.* 115 (52), 13342–13346. <https://doi.org/10.1073/pnas.1813749115>.
- Dowle, M., Srinivasan, A., Gorecki, J., Chirico, M., Stetsenko, P., Short, T., Lianoglou, S., Antonyan, E., Bensch, M., Parsonage, H., 2019. Package ‘data.Table’. Extension of ‘data.Frame’ 596.
- Durinck, S., Spellman, P.T., Birney, E., Huber, W., 2009. Mapping identifiers for the integration of genomic datasets with the R/Bioconductor package biomaRt. *Nat. Protoc.* 4 (8), 1184–1191. <https://doi.org/10.1038/nprot.2009.97>.
- EFSA Panel on Animal Health and Welfare (AHAW), 2010. Scientific opinion on the increased mortality events in Pacific oysters, *Crassostrea gigas*. *EFSA J.* 8 (11), 1894.
- Everett, R.D., 2014. HSV-1 biology and life cycle. In: Diefenbach, R.J., Fraefel, C. (Eds.), *Herpes Simplex Virus*, Vol. 1144. Springer New York, pp. 1–17. https://doi.org/10.1007/978-1-4939-0428-0_1.
- Fallet, M., Montagnani, C., Petton, B., Dantan, L., De Lorgeril, J., Comarmond, S., Chaparro, C., Toulza, E., Boitard, S., Escoubas, J.-M., Vergnes, A., Le Grand, J., Bulla, I., Gueguen, Y., Vidal-Dupiol, J., Grunau, C., Mitta, G., Cosseau, C., 2022. Early life microbial exposures shape the *Crassostrea gigas* immune system for lifelong and intergenerational disease protection. *Microbiome* 10 (1), 85. <https://doi.org/10.1186/s40168-022-01280-5>.
- Fellous, A., Favrel, P., Riviere, G., 2015. Temperature influences histone methylation and mRNA expression of the Jmj-C histone-demethylase orthologues during the early development of the oyster *Crassostrea gigas*. *Mar. Genomics* 19, 23–30. <https://doi.org/10.1016/j.margen.2014.09.002>.
- Feng, H., Wu, H., 2019. Differential methylation analysis for bisulfite sequencing using DSS. *Quantitative Biology* 7 (4), 327–334. <https://doi.org/10.1007/s40484-019-0183-8>.
- Fischer, N., 2020. Infection-induced epigenetic changes and their impact on the pathogenesis of diseases. *Semin. Immunopathol.* 42 (2), 127–130. <https://doi.org/10.1007/s00281-020-00793-1>.
- Fisher, W., 1986. Structure and functions of oyster hemocytes. In: *Immunity in Invertebrates: Cells, Molecules, and Defense Reactions*. Springer, pp. 25–35.
- Galbraith, H.S., Frazier, S.E., Allison, B., Vaughn, C.C., 2009. Comparison of gill surface morphology across a guild of suspension-feeding unionid bivalves. *J. Molluscan Stud.* 75 (2), 103–107. <https://doi.org/10.1093/mollus/eyn045>.
- Gavery, M.R., Roberts, S.B., 2010. DNA methylation patterns provide insight into epigenetic regulation in the Pacific oyster (*Crassostrea gigas*). *BMC Genomics* 11 (1), 483. <https://doi.org/10.1186/1471-2164-11-483>.
- Gavery, M.R., Roberts, S.B., 2014. A context dependent role for DNA methylation in bivalves. *Brief. Funct. Genomics* 13 (3), 217–222. <https://doi.org/10.1093/bfpg/elt054>.
- 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., Degremont, 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. *Sci. Adv.* 9 (36), eadh8990. <https://doi.org/10.1126/sciadv.adh8990>.
- Gentry, R.R., Froehlich, H.E., Grimm, D., Kareiva, P., Parke, M., Rust, M., Gaines, S.D., Halpern, B.S., 2017. Mapping the global potential for marine aquaculture. *Nature Ecology & Evolution* 1 (9), 1317–1324. <https://doi.org/10.1038/s41559-017-0257-9>.
- Gu, Z., 2022. Complex heatmap visualization. *iMeta* 1 (3). <https://doi.org/10.1002/imt2.43>.
- Gu, Z., Eils, R., Schlesner, M., 2016. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics* 32 (18), 2847–2849. <https://doi.org/10.1093/bioinformatics/btw313>.
- Gutierrez, A.P., Turner, F., Gharbi, K., Talbot, R., Lowe, N.R., Penaloza, C., McCullough, M., Prodhon, P.A., Bean, T.P., Houston, R.D., 2017. Development of a medium density combined-species SNP array for Pacific and European oysters (*Crassostrea gigas* and *Ostrea edulis*). *G3 Genes|Genomes|Genetics* 7 (7), 2209–2218. <https://doi.org/10.1534/g3.117.041780>.
- Gutierrez, A.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|Genomes|Genetics* 8 (4), 1273–1280. <https://doi.org/10.1534/g3.118.200113>.
- 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 (2), 249–257. <https://doi.org/10.1111/age.12909>.
- Hick, P.M., Evans, O., Rubio, A., Dhand, N.K., Whittington, R.J., 2018. Both age and size influence susceptibility of Pacific oysters (*Crassostrea gigas*) to disease caused by Ostreid herpesvirus-1 (OsHV-1) in replicated field and laboratory experiments. *Aquaculture* 489, 110–120. <https://doi.org/10.1016/j.aquaculture.2018.02.013>.
- Huang, D.W., Sherman, B.T., Lempicki, R.A., 2009. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat. Protoc.* 4 (1), 44–57. <https://doi.org/10.1038/nprot.2008.211>.
- Ivanina, A.V., Falfushynska, H.I., Beniash, E., Piontkivska, H., Sokolova, I.M., 2017. Biomineralization-related specialization of hemocytes and mantle tissues of the Pacific oysters *Crassostrea gigas*. *J. Exp. Biol.* 160861. <https://doi.org/10.1242/jeb.160861>.
- Jiang, Q., Li, Q., Yu, H., Kong, L., 2016. Inheritance and variation of genomic DNA methylation in diploid and triploid Pacific oyster (*Crassostrea gigas*). *Mar. Biotechnol.* 18 (1), 124–132. <https://doi.org/10.1007/s10126-015-9674-4>.
- Kassambara, A., Kosinski, M., Biecek, P., Fabian, S., 2017. Package ‘survminer’. In: *Drawing Survival Curves Using ‘Ggplot2’ (R Package Version 03.1)*.

- Knipe, D.M., Howley, P.M., Griffin, D.E., Lamb, R.A., Martin, M.A., Roizman, B., Straus, S.E., Rubin, D.H., 2002. Fundamental virology, 4th edition; and fields virology, 4th edition, volumes I and II: fundamental virology, 4th edition; fields virology, 4th edition, volumes I and II. Clin. Infect. Dis. 34 (7), 1029–1030. <https://doi.org/10.1086/339330>.
- Krueger, F., 2015. Trim galore. A wrapper tool around Cutadapt and FastQC to consistently apply quality and adapter trimming to FastQ files, 516 (517).
- Krueger, F., Andrews, S.R., 2011. Bismark: a flexible aligner and methylation caller for Bisulfite-seq applications. Bioinformatics 27 (11), 1571–1572. <https://doi.org/10.1093/bioinformatics/btr167>.
- Lafont, M., Petton, B., Vergnes, A., Pauletto, M., Segarra, A., Gourbal, B., Montagnani, C., 2017. Long-lasting antiviral innate immune priming in the Lophotrochozoan Pacific oyster, *Crassostrea gigas*. Sci. Rep. 7 (1), 13143. <https://doi.org/10.1038/s41598-017-13564-0>.
- Lafont, M., Vergnes, A., Vidal-Dupiol, J., De Lorgeril, J., Gueguen, Y., Haffner, P., Petton, B., Chaparro, C., Barrachina, C., Destoumieux-Garzon, D., Mitta, G., Gourbal, B., Montagnani, C., 2020. A sustained immune response supports long-term antiviral immune priming in the Pacific oyster, *Crassostrea gigas*. mBio 11 (2). <https://doi.org/10.1128/mBio.02777-19> (e02777-19).
- Lanz-Mendoza, H., Contreras-Garduno, J., 2022. Innate immune memory in invertebrates: concept and potential mechanisms. Dev. Comp. Immunol. 127, 104285. <https://doi.org/10.1016/j.dci.2021.104285>.
- Law, P.-P., Holland, M.L., 2019. DNA methylation at the crossroads of gene and environment interactions. Essays Biochem. 63 (6), 717–726. <https://doi.org/10.1042/EBC20190031>.
- Le Franc, L., Bernay, B., Petton, B., Since, M., Favrel, P., Riviere, G., 2021. A functional m⁵A-RNA methylation pathway in the oyster *Crassostrea gigas* assumes epitranscriptomic regulation of lophotrochozoan development. FEBS J. 288 (5), 1696–1711. <https://doi.org/10.1111/febs.15500>.
- Leprière, M., Faury, N., Segarra, A., Claverol, S., Degremont, L., Palos-Ladeiro, M., Armengaud, J., Renault, T., Morga, B., 2021. Comparative proteomics of ostreid herpesvirus 1 and Pacific oyster interactions with two families exhibiting contrasted susceptibility to viral infection. Front. Immunol. 11, 621994. <https://doi.org/10.3389/fimmu.2020.621994>.
- Locatelli, M., Faure-Dupuy, S., 2023. Virus hijacking of host epigenetic machinery to impair immune response. J. Virol. 97 (9). <https://doi.org/10.1128/jvi.00658-23> (e00658-23).
- Manner, L., Schell, T., Provataris, P., Haase, M., Greve, C., 2021. Inference of DNA methylation patterns in molluscs. Philos. Trans. R. Soc. B 376 (1825), 20200166. <https://doi.org/10.1098/rstb.2020.0166>.
- Moraleda-Prados, J., Caballero-Huertás, M., Valdivieso, A., Joly, S., Ji, J., Roher, N., Ribas, L., 2020. Epigenetic differences in the innate response after immune stimulation during zebrafish sex differentiation. Dev. Comp. Immunol. 103848. <https://doi.org/10.1016/j.dci.2020.103848>.
- Morga, B., Faury, N., Guesdon, S., Chollet, B., Renault, T., 2017. Haemocytes from *Crassostrea gigas* and OsHV-1: a promising in vitro system to study host/virus interactions. J. Invertebr. Pathol. 150, 45–53. <https://doi.org/10.1016/j.jip.2017.09.007>.
- Namwong, P., Wang, S., Kong, Q., Mou, H., Ma, L., Srisapoome, P., 2023. Transcriptome analysis and pattern recognition receptors (PRRs) identification in different tissues of adult Pacific oysters infected with *Vibrio parahaemolyticus*. Aquaculture 562, 738824. <https://doi.org/10.1016/j.aquaculture.2022.738824>.
- Netea, M.G., Domínguez-Andrés, J., Barreiro, L.B., Chavakis, T., Divangahi, M., Fuchs, E., Joosten, L.A.B., Van Der Meer, J.W.M., Mhlanga, M.M., Mulder, W.J.M., Riksen, N. P., Schlitzer, A., Schultze, J.L., Stabel Benn, C., Sun, J.C., Xavier, R.J., Latz, E., 2020. Defining trained immunity and its role in health and disease. Nat. Rev. Immunol. 20 (6), 375–388. <https://doi.org/10.1038/s41577-020-0285-6>.
- Nieborak, A., Schneider, R., 2018. Metabolic intermediates – cellular messengers talking to chromatin modifiers. Molecular Metabolism 14, 39–52. <https://doi.org/10.1016/j.molmet.2018.01.007>.
- Oyanedel, D., Lagorce, A., Bruto, M., Haffner, P., Morot, A., Labreuche, Y., Dorant, Y., De La Forest Divonne, S., Delavat, F., Ingumbert, N., Montagnani, C., Morga, B., Toulza, E., Chaparro, C., Escoubas, J.-M., Gueguen, Y., Vidal-Dupiol, J., De Lorgeril, J., Petton, B., Destoumieux-Garzon, D., 2023. Cooperation and cheating orchestrate *Vibrio* assemblages and polymicrobial synergy in oysters infected with OsHV-1 virus. Proc. Natl. Acad. Sci. 120 (40), e2305195120. <https://doi.org/10.1073/pnas.2305195120>.
- Pathirana, E., Fuhrmann, M., Whittington, R., Hick, P., 2019. Influence of environment on the pathogenesis of Ostreid herpesvirus-1 (OsHV-1) infections in Pacific oysters (*Crassostrea gigas*) through differential microbiome responses. Heliyon 5 (7), e02101. <https://doi.org/10.1016/j.heliyon.2019.e02101>.
- Peeler, E.J., Allan Reese, R., Cheslett, D.L., Geoghegan, F., Power, A., Thrush, M.A., 2012. Investigation of mortality in Pacific oysters associated with Ostreid herpesvirus-1 μ Var in the Republic of Ireland in 2009. Prev. Vet. Med. 105 (1–2), 136–143. <https://doi.org/10.1016/j.prevetmed.2012.02.001>.
- Pei, Y., Robertson, E.S., 2020. The crosstalk of epigenetics and metabolism in herpesvirus infection. Viruses 12 (12), 1377. <https://doi.org/10.3390/v12121377>.
- Pei, Y., Wong, J.H., Robertson, E.S., 2020. Herpesvirus epigenetic reprogramming and oncogenesis. Annual Review of Virology 7 (1), 309–331. <https://doi.org/10.1146/annurev-virology-020420-014025>.
- Penalzo, C., Gutierrez, A.P., Eory, 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>.
- Pepin, J.F., 2013. European Union Reference Laboratory for Molluscs Diseases, IFREMER. <https://www.eurl-mollusc.eu/content/download/57054/795216/file/>.
- Pernet, F., Barret, J., Le Gall, P., Corporeau, C., D'egremont, L., Lagarde, F., P'epin, J., Keck, N., 2012. Mass mortalities of Pacific oysters *Crassostrea gigas* reflect infectious diseases and vary with farming practices in the Mediterranean Thau lagoon, France. Aquaculture Environment Interactions 2 (3), 215–237. <https://doi.org/10.3354/aei00041>.
- Pernet, F., Tamayo, D., Petton, B., 2015. Influence of low temperatures on the survival of the Pacific oyster (*Crassostrea gigas*) infected with ostreid herpes virus type 1. Aquaculture 445, 57–62. <https://doi.org/10.1016/j.aquaculture.2015.04.010>.
- Pernet, F., Lupo, C., Bacher, C., Whittington, R.J., 2016. Infectious diseases in oyster aquaculture require a new integrated approach. Philosophical Transactions of the Royal Society B: Biological Sciences 371 (1689), 20150213. <https://doi.org/10.1098/rstb.2015.0213>.
- Pernet, F., Tamayo, D., Fuhrmann, M., Petton, B., 2019. Deciphering the effect of food availability, growth and host condition on disease susceptibility in a marine invertebrate. J. Exp. Biol. jeb.210534. <https://doi.org/10.1242/jeb.210534>.
- Petton, B., Pernet, F., Robert, R., Boudry, P., 2013. Temperature influence on pathogen transmission and subsequent mortalities in juvenile Pacific oysters *Crassostrea gigas*. Aquac. Environ. Interact. 3 (3), 257–273. <https://doi.org/10.3354/aei00070>.
- Petton, B., Destoumieux-Garzon, D., Pernet, F., Toulza, E., De Lorgeril, J., Degremont, L., Mitta, G., 2021. The Pacific oyster mortality syndrome, a polymicrobial and multifactorial disease: state of knowledge and future directions. Front. Immunol. 12, 630343. <https://doi.org/10.3389/fimmu.2021.630343>.
- Petton, B., Alunno-Bruscia, M., Mitta, G., Pernet, F., 2023. Increased growth metabolism promotes viral infection in a susceptible oyster population. Aquac. Environ. Interact. 15, 19–33. <https://doi.org/10.3354/aei00450>.
- Pifferrer, F., 2023. Epigenetics in aquaculture: Knowledge gaps, challenges, and future prospects. In: Pifferrer, F., Wang, H. (Eds.), Epigenetics in Aquaculture, 1st ed. Wiley, pp. 451–463. <https://doi.org/10.1002/9781119821946.ch20>.
- Qiao, X., Li, Y., Jin, Y., Wang, S., Hou, L., Wang, L., Song, L., 2022. The involvement of an interferon-induced protein 44-like (CgIFI44L) in the antiviral immune response of *Crassostrea gigas*. Fish Shellfish Immunol. 129, 96–105. <https://doi.org/10.1016/j.fsi.2022.08.064>.
- Reid, G., Gurney-Smith, H., Marcogliese, D., Knowler, D., Benfey, T., Garber, A., Forster, I., Chopin, T., Brewer-Dalton, K., Moccia, R., Flaherty, M., Smith, C., De Silva, S., 2019. Climate change and aquaculture: considering biological response and resources. Aquac. Environ. Interact. 11, 569–602. <https://doi.org/10.3354/aei00332>.
- Richard, M., Rolland, J.L., Gueguen, Y., De Lorgeril, J., Pouzadoux, J., Mostajir, B., Bec, B., Mas, S., Parin, D., Le Gall, P., Mortreux, S., Fiandrino, A., Lagarde, F., Messiaen, G., Fortune, M., Roque d'Orbcastel, E., 2021. In situ characterisation of pathogen dynamics during a Pacific oyster mortality syndrome episode. Mar. Environ. Res. 165, 105251. <https://doi.org/10.1016/j.marenvres.2020.105251>.
- Riviere, G., Wu, G.-C., Fellous, A., Goux, D., Sourdaire, P., Favrel, P., 2013. DNA methylation is crucial for the early development in the oyster *C. gigas*. Mar. Biotechnol. 15 (6), 739–753. <https://doi.org/10.1007/s10126-013-9523-2>.
- Rondon, R., Grunau, C., Fallet, M., Charlemagne, N., Sussarellu, R., Chaparro, C., Montagnani, C., Mitta, G., Bach'ere, E., Akcha, F., Cosseau, C., 2017. Effects of a parental exposure to diuron on Pacific oyster spat methylome. Environmental Epigenetics 3 (1). <https://doi.org/10.1093/eep/dvx004>.
- Rubio, T., Oyanedel, D., Labreuche, Y., Toulza, E., Luo, X., Bruto, M., Chaparro, C., Torres, M., De Lorgeril, J., Haffner, P., Vidal-Dupiol, J., Lagorce, A., Petton, B., Mitta, G., Jacq, A., Le Roux, F., Charriere, G.M., Destoumieux-Garzon, D., 2019. Species-specific mechanisms of cytotoxicity toward immune cells determine the successful outcome of *Vibrio* infections. Proc. Natl. Acad. Sci. 116 (28), 14238–14247. <https://doi.org/10.1073/pnas.1905747116>.
- Salvi, D., Mariottini, P., 2016. Molecular taxonomy in 2D: a novel ITS2 rRNA sequence-structure approach guides the description of the oysters' subfamily Saccostreinae and the genus *Magallana* (Bivalvia: Ostreidae). Zool. J. Linnean Soc. <https://doi.org/10.1111/zoj.12455>.
- Salvi, D., Mariottini, P., 2021. Revision shock in Pacific oysters taxonomy: the genus *Magallana* (formerly *Crassostrea* in part) is well-founded and necessary. Zool. J. Linnean Soc. 192 (1), 43–58. <https://doi.org/10.1093/zoolinnean/zlaa112>.
- Sauvage, C., Boudry, P., de Koning, D.-J., Haley, C.S., Heurtebise, S., Lap'egue, S., 2010. QTL for resistance to summer mortality and OsHV-1 load in the Pacific oyster (*Crassostrea gigas*). Anim. Genet. <https://doi.org/10.1111/j.1365-2052.2009.02018.x>.
- Schikorski, D., Renault, T., Saulnier, D., Faury, N., Moreau, P., Pépin, J.-F., 2011. Experimental infection of Pacific oyster *Crassostrea gigas* spat by ostreid herpesvirus 1: demonstration of oyster spat susceptibility. Vet. Res. 42 (1), 27. <https://doi.org/10.1186/1297-9716-42-27>.
- Sherman, B.T., Hao, M., Qiu, J., Jiao, X., Baseler, M.W., Lane, H.C., Imamichi, T., Chang, W., 2022. DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). Nucleic Acids Res. 50 (W1), W216–W221. <https://doi.org/10.1093/nar/gkac194>.

- Sun, D., Li, Q., Yu, H., 2022. DNA methylation differences between male and female gonads of the oyster reveal the role of epigenetics in sex determination. *Gene* 146260. <https://doi.org/10.1016/j.gene.2022.146260>.
- Sun, D., Yu, H., Kong, L., Liu, S., Xu, C., Li, Q., 2024. The role of DNA methylation reprogramming during sex determination and sex reversal in the Pacific oyster *Crassostrea gigas*. *Int. J. Biol. Macromol.* 259, 128964. <https://doi.org/10.1016/j.ijbiomac.2023.128964>.
- Supek, F., Bosnjak, M., Skunca, N., Smuc, T., 2011. REVIGO summarizes and visualizes long lists of gene ontology terms. *PLoS One* 6 (7), e21800. <https://doi.org/10.1371/journal.pone.0021800>.
- Suquet, M., de Kermoisan, G., Araya, R.G., Queau, I., Lebrun, L., Le Souchu, P., Mingant, C., 2009. Anesthesia in Pacific oyster, *Crassostrea gigas*. *Aquat. Living Resour.* 22 (1), 29–34. <https://doi.org/10.1051/alr/2009006>.
- Team, R.C., 2021. R: a language and environment for statistical computing. <http://www.R-project.org/>.
- Therneau, T.M., Lumley, T., 2015. Package ‘survival’. *R Top Doc* 128 (10), 28–33.
- Tsai, C.-N., Tsai, C.-L., Tse, K.-P., Chang, H.-Y., Chang, Y.-S., 2002. The Epstein–Barr virus oncogene product, latent membrane protein 1, induces the downregulation of E-cadherin gene expression via activation of DNA methyltransferases. *Proc. Natl. Acad. Sci.* 99 (15), 10084–10089. <https://doi.org/10.1073/pnas.152059399>.
- Tsai, C.-L., Li, H.-P., Lu, Y.-J., Hsueh, C., Liang, Y., Chen, C.-L., Tsao, S.W., Tse, K.-P., Yu, J.-S., Chang, Y.-S., 2006. Activation of DNA methyltransferase 1 by EBV LMP1 involves c-Jun NH2-terminal kinase signaling. *Cancer Res.* 66 (24), 11668–11676. <https://doi.org/10.1158/0008-5472.CAN-06-2194>.
- Valdivieso, A., Anastasiadi, D., Ribas, L., Piferrer, F., 2023a. Development of epigenetic biomarkers for the identification of sex and thermal stress in fish using DNA methylation analysis and machine learning procedures. *Mol. Ecol. Resour.* 23 (2), 453–470. <https://doi.org/10.1111/1755-0998.13725>.
- Valdivieso, A., Caballero-Huertas, M., Moraleda-Prados, J., Piferrer, F., Ribas, L., 2023b. Exploring the effects of rearing densities on epigenetic modifications in the zebrafish gonads. *Int. J. Mol. Sci.* 24 (21), 16002. <https://doi.org/10.3390/ijms242116002>.
- Valdivieso, A., Sanchez-Baizán, N., Mitrizakis, N., Papandroulakis, N., Piferrer, F., 2023c. Development of epigenetic biomarkers with diagnostic and prognostic value to assess the lasting effects of early temperature changes in farmed fish. *Aquaculture* 563, 738918. <https://doi.org/10.1016/j.aquaculture.2022.738918>.
- Venkataraman, Y.R., Downey-Wall, A.M., Ries, J., Westfield, I., White, S.J., Roberts, S.B., Lotterhos, K.E., 2020. General DNA methylation patterns and environmentally-induced differential methylation in the eastern oyster (*Crassostrea virginica*). *Front. Mar. Sci.* 7, 225. <https://doi.org/10.3389/fmars.2020.00225>.
- Venkataraman, Y.R., White, S.J., Roberts, S.B., 2022. Differential DNA methylation in Pacific oyster reproductive tissue in response to ocean acidification. *BMC Genomics* 23 (1), 556. <https://doi.org/10.1186/s12864-022-08781-5>.
- Wang, L., Ning, S., 2021. New look of EBV LMP1 signaling landscape. *Cancers* 13 (21), 5451. <https://doi.org/10.3390/cancers13215451>.
- Wang, X., Cong, R., Li, A., Wang, W., Zhang, G., Li, L., 2023. Transgenerational effects of intertidal environment on physiological phenotypes and DNA methylation in Pacific oysters. *Sci. Total Environ.* 871, 162112. <https://doi.org/10.1016/j.scitotenv.2023.162112>.
- Wang, C., Jiang, Z., Du, M., Li, Q., Cong, R., Wang, W., Zhang, G., Li, L., 2024a. Comparative chromatin dynamics reveal differential thermal tolerance mechanisms between two congeneric oyster species. *Aquaculture* 579, 740177. <https://doi.org/10.1016/j.aquaculture.2023.740177>.
- Wang, Y., Liu, X., Wang, W., Sun, G., Feng, Y., Xu, X., Li, B., Luo, Q., Li, Y., Yang, J., Cui, C., Li, Z., 2024b. The investigation on stress mechanisms of *Sepia esculenta* larvae in the context of global warming and ocean acidification. *Aquaculture Reports* 36, 102120. <https://doi.org/10.1016/j.aqrep.2024.102120>.
- Webb, S.C., Fidler, A., Renault, T., 2007. Primers for PCR-based detection of ostreid herpes virus-1 (OsHV-1): application in a survey of New Zealand molluscs. *Aquaculture* 272 (1–4), 126–139. <https://doi.org/10.1016/j.aquaculture.2007.07.224>.
- Wickham, H., 2009. *Elegant graphics for data analysis*. *Media* 35 (211), 10–1007.
- Wickham, H., François, R., Henry, L., Müller, K., 2020. Dplyr: a grammar of data manipulation (version 0.8.5) [computer software]. <https://CRAN.R-project.org/package=dplyr>.
- Wright, R.M., Aglyamova, G.V., Meyer, E., Matz, M.V., 2015. Gene expression associated with white syndromes in a reef building coral, *Acropora hyacinthus*. *BMC Genomics* 16 (1), 371. <https://doi.org/10.1186/s12864-015-1540-2>.
- Zhang, X., Li, Q., Kong, L., Yu, H., 2018. DNA methylation frequency and epigenetic variability of the Pacific oyster *Crassostrea gigas* in relation to the gametogenesis. *Fish. Sci.* 84 (5), 789–797. <https://doi.org/10.1007/s12562-018-1214-5>.
- Zhao, M., Lin, Z., Zheng, Z., Yao, D., Yang, S., Zhao, Y., Chen, X., Awaya, J.J., Zhang, Y., 2023. The mechanisms and factors that induce trained immunity in arthropods and mollusks. *Front. Immunol.* 14, 1241934. <https://doi.org/10.3389/fimmu.2023.1241934>.
- Zhu, S., Viejo-Borbolla, A., 2021. Pathogenesis and virulence of herpes simplex virus. *Virulence* 12 (1), 2670–2702. <https://doi.org/10.1080/21505594.2021.1982373>.