# Strong genotype-by-environment interaction across contrasted sites for summer mortality syndrome in the Pacific oyster *Crassostrea gigas*

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**Abstract**

Genotype-by-environment (GxE) interaction in aquaculture is usually estimated for continuous traits and based on data from a limited number of 2–3 rearing sites. Here we report the results of a GxE study for resistance to Pacific oyster mortality syndrome (POMS), a multi-factorial disease that severely impacts Pacific oyster production worldwide. The syndrome is largely associated with Ostreid Herpes Virus 1 (OsHV-1). Resistance to OsHV-1 in *Crassostrea gigas* has been shown to be heritable, meaning that selective breeding is a suitable strategy for reducing mortalities. However, limited information was available about GxE interaction or the possible need to consider it in selective breeding. Survival of two cohorts (C1 and C2), consisting of a total of 104 full-sib families, was evaluated during the summer of 2013 in 7 sites along the French Atlantic, Channel and Mediterranean coasts. Mean survivals in autumn 2013 were 12.6% ± 10.9 and 4.6% ± 6.4 for C1 and C2, respectively. Genetic parameters were computed by MCMC, which is suitable for binary data like survival. Heritability estimates ranged from 0.16 to 0.42 depending on site and cohort, with a mean of 0.24 [0.20; 0.27] when including all data. GxE interactions were estimated by the genetic correlations between pairs of sites. Genetic correlations were high ($\rho$ > 0.80) for C1 between most tidal Atlantic and Channel sites, and intermediate between tidal sites and a Mediterranean lagoon site, while they were lower and more variable for C2 (0.21–0.77). Expected genetic gains were maximal when production site was the same as selection site. They were closed to this expected maximum when the selection site was different from the production site along the Atlantic or Channel coast. Limited GxE interaction along the French Atlantic coast is favourable to wide dissemination of genetically improved oysters along this coast. Limited potential improvement was shown in the Mediterranean site if selection was carried out elsewhere, confirming the specificity of this environment. Consequently, a specific strategy such as dedicated breeding should be used to achieve genetic progress for this site.

Keywords: *Crassostrea gigas*, POMS, OsHV-1, heritability, genotype-by-environment interaction, expected response to selection

## 1. Introduction

Many quantitative genetics studies in aquaculture have examined genotype-by-environment (GxE) interactions (for review see Sae-Lim et al., 2016). For any one trait, GxE interaction is defined as the difference in the magnitude of the genetic variances between two environments or a re-ranking of genotypes across environments (Falconer and Mackay, 1996; Lynch and Walsh, 1998). Re-ranking means that the best genotypes in a given environment are not the best in another. As notably shown in dairy cattle and pigs, significant changes in the ranking of genotypes among rearing environments may be problematic if neglected because this can limit the efficiency of selection and return on investment when the breeding environment differs too much from production ones (Mulder and Bijma, 2005). Knowledge on the potential impact of GxE interactions is therefore crucial for designing a breeding programme and forecasting its efficiency. One possible but costly strategy would be to implement one specific breeding programme per environment or per production method. Ultimately, the benefit-cost ratio mostly favours such environment-specific breeding programmes (James, 1961). Due to the high cost of genetic evaluation, studies reporting GxE interactions in aquaculture are mainly focused on evaluating performances (e.g. growth, survival, carcass or fillet yields, meat composition, sex-ratio, external morphology) in a few (n = 2-3) environments differing in water temperature, salinity, diet, vaccination status, rearing system or density (Sae-Lim et al., 2016). Generalisation of such results across environments, years, seasons or rearing practices is poorly documented. In shellfish, available studies are rather scarce and limited to growth-related traits or survival in edible oysters *Crassostrea gigas* or *Crassostrea virginica* (Allen et al., 2021; Chi et al., 2023; de Melo et al., 2018; Proestou et al., 2016; Swan et al., 2007; Vu et al., 2021), clams (Chen et al., 2022; Rawson and Hilbish, 1991; Scott and Koehn, 1990), abalones (Gan et al., 2023), sea mussels (Díaz-Puente et al., 2020; Shields et al., 2008), pearl oyster *Pinctada maxima* (Kvingedal et al., 2010) and scallops *Argopecten nucleus* or *Argopecten ventricosus* (Barros et al., 2018; Cruz et al., 1998). Other traits, such as shell morphology or colour, pearl quality or feed efficiency have also been investigated in many environments in the species freshwater mussel *Hyriopsis cumingii*, pearl oyster *Pinctada maxima* and abalone *Haliotis discus*, respectively (Hu et al., 2021; Jerry et al., 2012; Sun et al., 2022; Yu et al., 2023). Despite their importance for aquaculture efficiency and profitability, evaluation of GxE interaction and genetic resistance to a specific pathogen are less often studied in shellfish than in plants or other animal species. This is probably due to the cost and difficulties of setting up an effective challenge protocol or the complexity of statistical analysis for these categorical traits.

Oyster farming around the world is carried out in open environments (i.e. intertidal or nearshore subtidal zones), which are therefore directly subjected to naturally variable seasonal or yearly conditions. Thanks to the very high fecundity of oysters and their ease of transport over long distances, seed produced in a hatchery from a given selected broodstock can be marketed to growers

across greatly differing rearing conditions. Since 2008, the Pacific oyster industry worldwide has been strongly impacted by increases in mortality. These can reach more than 70% at the spat stage in most French production sites and remain a major concern for the industry today (Mazaleyrat et al., 2022). Pacific Oyster Mortality Syndrome (POMS) was also reported elsewhere in Europe during the 2010s (Daehne et al., 2009; Lynch et al., 2012; Malham et al., 2009; Roque et al., 2012), in Australia and New Zealand (Cameron and Crane, 2011), and in Mexico (Chávez-Villalba et al., 2010). These mortalities were associated with a particular genotype of ostreid herpes virus 1, called micro-var OsHV-1, which can be considered as a causal agent of mortalities in *C. gigas* spat since 2008 (de Lorgeril et al., 2018b; Renault et al., 2012; Segarra et al., 2010). Nonetheless, many other environmental or rearing factors are also involved in the onset and intensity of mortalities, including microbiota (de Lorgeril et al., 2018a; Delisle et al., 2022) and genetics (Dégremont, 2011; Petton et al., 2021).

Heritability estimates of POMS survival estimated in various parts of the world are moderate to high, ranging from 0.12 to 0.65 (Azéma et al., 2017b; Camara et al., 2017; de Melo et al., 2018; Dégremont et al., 2015b; Divilov et al., 2021; Gutierrez et al., 2020). Genetic determinism of POMS survival was confirmed by 39.6% survival gain after four generations of mass selection in the field (Dégremont et al., 2015b), and 42.0% or 21.7% survival gain after two generations of family selection (Chi et al., 2022; Divilov et al., 2021).

Selective breeding therefore appears to be a realistic and efficient solution to deal with mortalities affecting the Pacific oyster industry, but little work has yet been done on GxE interactions. To our knowledge, the first study reporting significant GxE interactions for survival of Pacific oyster juveniles used 44 families reared in three French sites in 2001 (Dégremont et al., 2005). Heritability estimates ranged from 0.27 to 0.68 and between-environment genetic correlations were very high but had low accuracy (Dégremont et al., 2007). Comparable results were found on the US west coast where survival was recorded at two tidal exposure levels in each of two sites (Evans and Langdon, 2006), and between coastal and estuarine sites (de Melo et al., 2018). Nevertheless, a deeper study of GxE interaction across sites located in larger farming environments and involving a greater number of production areas has not been reported, although it appears key for the establishment of an effective breeding programme and to justify industry investment. Beyond the potential presence of significant GxE effect, specifying the threshold at which this interaction can be considered negligible compared with the cost of site-specific breeding is essential information for designing breeding programmes.

This study investigated genotype-by-environment interaction for resistance to POMS. Genetic parameters were evaluated using a relatively large number of oyster families in seven rearing sites located in the main production areas on the French Atlantic, Channel (intertidal sites) and Mediterranean (coastal lagoon site with continuous submersion) coasts. Expected genetic progress for

survival was also estimated based on genetic parameters. Our objective was to provide information to help breeders implement more efficient selection strategies.

## 2. Material and methods

### *2.1. Production of cohorts*

The first cohort (C1) was produced in September 2012. Adults were collected in eight wild beds along the French coasts in 2012 (Figure 1). They were maintained in conditioning tanks at the Ifremer facilities in Bouin (Vendée, France). Seawater temperature was raised by 1°C a week to reach 19°C during the conditioning period. The partly factorial mating design proposed by Berg and Henryon (1998) was used, with 20 sires and 19 dams. Each male was crossed with 4–5 females to create genetic links among the families bred and among broodstock origins, and to avoid the loss of genetic information in case of the loss of a large number of families. Artificial fertilization was performed as presented in Brizard et al. (2004). Briefly, sperm was collected by scarification of gonads and stored at 4°C after dilution in 5 mL of STOR-GIGAS conservation extender (IMV-Technologies, France). Oocytes of each female were collected by scarification of the gonad, rinsed with seawater on a 100 μm screen, retained on a 20 μm screen and diluted in seawater to obtain 1000 mL of egg solution. Then, 1 mL of sperm solution was diluted in 150 mL of seawater for 15 minutes to active the spermatozoids. Fertilization was carried out using 1 million eggs mixed with 30mL of activated sperm solution. One hour after fertilization, the zygotes were transferred to larval tubes. Larvae were screened at day 1 (D1, $\phi$ 40μm) to keep 250,000 larvae per larval tube, then at D6 ($\phi$ 60μm), at D12 ($\phi$ 120μm) and at D20 ($\phi$ 240μm). A total of 67 full-sib families was produced. These were transferred to a commercial nursery (Aquanurs, Bouin, France) in November 2012 and were grown on in sieves until their deployment in the field.

A total of 54 full-sib families was produced in February 2013 for the second cohort (C2) using 12 sires and 21 dams collected in Brest roadstead. The conditioning period of the adults and fertilization protocol were similar to C1. These oysters were grown on at the Ifremer nursery (Bouin, France) from March 2013 until field deployment.

### *2.2. Monitoring survival in the field*

All families were tested on the shore during summer 2013 in seven sites representing the main French oyster production areas (Figure 1): Thau lagoon on the Mediterranean Sea (TL ; 43°24’ N, 3°36’ E); Arcachon Bay (AB; 44°41’ N, 1°12’ W), Marennes-Oléron area (MA; 45°50’ N, 1°11’ W), Noirmoutier (NO; 46°57’ N, 2°08’ W), South Brittany (SB; 47°35’ N, 2°57’ W) and North Brittany (NB; 48°65’ N, 3°89’ W) on the Atlantic; and Normandy (NY; 49°23’ N, 1°06’ W) on the English Channel. Thau lagoon (7000

ha) is the largest lagoon on the Mediterranean Sea, with an average depth of 4.5 m. Oysters there are reared in lantern nets that remain immersed 24 h a day. Temperature varies from 3°C to 29°C and salinity from 27 to 40 ppt, with peaks between July and autumn. The 6 other sites are intertidal and oysters are grown in mesh bags on racks.

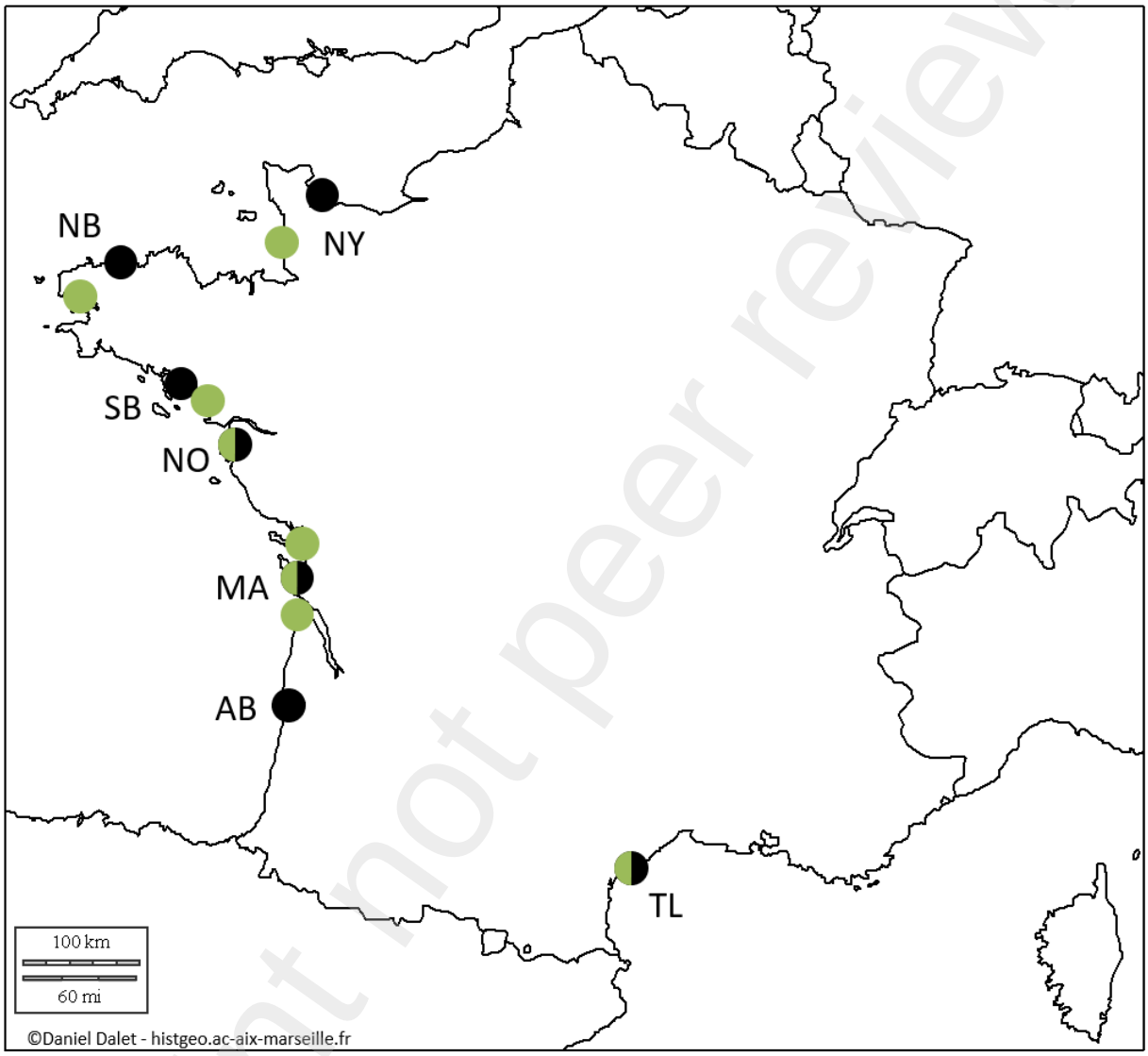



Figure 1. Location along the French coasts of broodstock collection sites (green) and the seven test sites (black) where cohorts were evaluated: NY = Normandy, NB = North Brittany, SB = South Brittany, NO = Noirmoutier, MA = Marennes-Oléron area, AB = Arcachon bay, TL = Thau lagoon.

Field evaluation was done in early April 2013 for C1 and at the end of June 2013 for C2. For C1, only 50 families were deployed in the field as the spat of the others (17 families) were too small due to high density during the nursing period (Supplementary Figure 1). All 54 families of C2 were deployed, but 10 of these were restricted to 4 sites (TL, MA, NO, NY) and 9 of them to 2 sites (MA, NO) (Supplementary Figure 1). For each full-sib family, one to three batches of oysters containing 300 spat

each were reared in mesh bags attached to racks at random, except in TL where batches of oysters were reared in suspended submerged lantern nets on long lines in triplicate. The total weight of oysters in each bag or lantern net was recorded at deployment: mean weight of 300 spat was 26.57 g ± 6.17 for C1 and 141.48 g ± 54.66 for C2. A total of 304,795 spat for C1 and 259,000 spat for C2 were tested in the field. At the endpoint of the trial, in October 2013, the numbers of live and dead oysters in each bag or lantern net were counted.

### *2.3. Estimation of genetic parameters*

Survival measurement $y$ is a binary trait, the modalities of which are driven by a threshold τ defined on an underlying latent variable $l$ following a normal distribution. An individual with a value of $l$ below τ has the phenotype 'dead' (i.e. y = 0) and an individual with a value of $l$ above τ has the phenotype 'alive' (i.e. y = 1). Genetic parameters were estimated by mixed animal models with probit link using the R package *MCMCglmm* (Hadfield, 2015; R Development Core Team, 2021). Cohorts were first analyzed separately to estimate heritabilities using univariate models including pedigree information within site:

$$l_{ij} = Probit(y_{ij}) = \mu + \beta \cdot w_j + b_j + a_i + \varepsilon_{ij} \quad (1)$$

and for all 7 sites:

$$l_{ijk} = Probit(y_{ijk}) = \mu + s_k + \beta \cdot w_j + b_j + a_i + \varepsilon_{ijk} \quad (2)$$

where $l_{ijk}$ is the latent variable driving phenotypic value of survival $y_{ijk}$ of an individual spat $i$ reared in batch (bag or lantern net) $j$ deployed in site $k$. $\mu$ is the overall mean, $\beta$ is the effect of individual mean weight $w_j$ in batch $j$ introduced as covariate, $s_k$ is the fixed effects of site $k$, $b_j$ is the random effect of batch $j$, $a_i$ is the random additive genetic effect, and $\varepsilon_{ijk}$ is residual. Random effects were assumed to be normaly distributed with zero means. Variances for $b_j$, $a_i$ and $\varepsilon_{ijk}$ were respectively assumed as $\mathbf{I}\sigma^2_{\mathrm{b}}$, $\mathbf{A}\sigma^2_a$ and $\mathbf{I}\sigma^2_{\mathrm{e}}$ where $\sigma^2_{\mathrm{b}}$ is the variance of batch effect, $\sigma^2_a$ is the genetic additive variance, $\sigma^2_{\mathrm{e}}$ is the residual variance equals to 1, $\mathbf{I}$ is the identity matrix and $\mathbf{A}$ is the pedigree relationship matrix made up of 304,795 challenged oysters and 39 parents for C1 and 258,936 challenged oysters and 33 parents for C2. Genetic correlations for each couple of sites were estimated using 21 bivariate animal models. The same effects as within site analyses were applied:

$$\begin{cases} \boldsymbol{l}_{s1} = Probit(\boldsymbol{y}_{s1}) = \mu + \beta \cdot \boldsymbol{W_{s1}} + \mathbf{Z}_{\boldsymbol{bs1}}\boldsymbol{b_{s1}} + \mathbf{Z}_{\boldsymbol{as1}}\boldsymbol{a_{s1}} + \boldsymbol{\varepsilon_{s1}} \\ \boldsymbol{l}_{s2} = Probit(\boldsymbol{y}_{s2}) = \mu + \beta \cdot \boldsymbol{W_{s2}} + \mathbf{Z}_{\boldsymbol{bs2}}\boldsymbol{b_{s2}} + \mathbf{Z}_{\boldsymbol{as2}}\boldsymbol{a_{s2}} + \boldsymbol{\varepsilon_{s2}} \end{cases} \quad (3)$$

With

195
$$Var\begin{pmatrix}\boldsymbol{b}\\ \boldsymbol{a}\\ \boldsymbol{\varepsilon}\end{pmatrix} = \begin{pmatrix} \begin{pmatrix}\sigma^2_{\mathrm{b}_{s1}} & 0\\ 0 & \sigma^2_{\mathrm{b}_{s2}}\end{pmatrix}\otimes \mathbf{I} & 0 & 0\\ 0 & \begin{pmatrix}\sigma^2_{a_{s1}} & \sigma_{a_{12}}\\ \sigma_{a_{12}} & \sigma^2_{a_{s2}}\end{pmatrix}\otimes \mathbf{A} & 0\\ 0 & 0 & \begin{pmatrix}1 & 0\\ 0 & 1\end{pmatrix}\otimes \mathbf{I}\end{pmatrix}$$

196 Where $\boldsymbol{W_{s1}}$ and $\boldsymbol{W_{s2}}$ are the vectors of individual mean weights in batch (bag or lantern nets) placed
197 in corresponding site. $\mathbf{Z_{bs1}}$, $\mathbf{Z_{bs2}}$, $\mathbf{Z_{as1}}$ and $\mathbf{Z_{as2}}$ are incidence matrices for batch random effect and
198 genetic additive effect for corresponding site, respectively. $\sigma^2_{\mathrm{b}_{s1}}$ and $\sigma^2_{\mathrm{b}_{s2}}$ are variances of batch effect
199 for corresponding site, $\sigma^2_{\mathrm{a}_{s1}}$ and $\sigma^2_{\mathrm{a}_{s2}}$ are variances of animal effect for corresponding site, $\sigma_{a_{12}}$ is the
200 genetic covariance between survivals in the two sites. Residual variances are fixed at 1 because they
201 are not estimatable with binary data, and residual covariances set at 0 because each individual was
202 measured in a single site.
203 Heritabilities and genetic correlations were also estimated by combining data from the two
204 cohorts. The same models as above were applied with the addition of cohort as fixed effect. The size
205 of pedigree relationship matrix $\mathbf{A}$ in each analysis was adapted accordingly.
206 The variances were estimated through the Markov chain Monte-Carlo (MCMC) procedure. Flat
207 priors were used for fixed effects. Proper and weak informative priors were assigned for random
208 effects. Each model consisted in a MCMC chain with a total of 1,000,000 iterations, including a burn-
209 in of 400,000 samples and a thinning interval of 400, to ensure a sampling of 1,500 independent
210 posterior values for each parameter. The convergence of the MCMC and lack of autocorrelation were
211 checked by plots. The estimate of genetic parameters was the mode of the posterior probability
212 distribution regarded as the most likely value and the limits of the 95% confidence interval were the
213 2.5 and 97.5 percentiles.
214 Heritability was computed on the underlying liability scale using univariate analysis. It is
215 defined as the part of phenotypic variance due to additive genetic variance (Falconer and Mackay,
216 1996):

217
$$h^2 = \frac{\sigma^2_a}{\sigma^2_b + \sigma^2_a + 1}$$

218 where $\sigma^2_b$ is the variance of batch effect, $\sigma^2_a$ is additive genetic variance and the residual variance equals
219 1. The magnitude of genotype by environment interaction (GxE) can be expressed as the genetic
220 correlation of survival measured in two different environments (Lynch and Walsh, 1998). A high
221 genetic correlation between two sites must be interpreted as a weak GxE interaction. GxE interaction
222 is considered significant when the genetic correlation is lower than 0.80 (Robertson, 1959). Genetic

correlations $\rho_{s1;s2}$ across two environments, i.e. two sites, were calculated as follows using results from the bivariate analysis:

$$\rho_{s1;s2} = \frac{\sigma_{a12}}{\sigma_{as1} \times \sigma_{as2}}$$

where $\sigma_{a12}$ is the genetic additive covariance between survival in two sites, and $\sigma_{as1}$ and $\sigma_{as2}$ are the genetic standard deviations of survival for site 1 and site 2, respectively.

The family mean breeding values in each site are the means of the estimated breeding values (EBV) of the sibs reared in each site. Differences in family rankings between sites were evaluated by Spearman correlation. Genetic correlations between sites were visualised by principal component analysis (PCA) performed with the R package *FactoMineR* (Lê et al., 2008) using family mean EBVs within sites for C1 and C2 independently, then family mean EBVs within sites estimated from the combined cohorts. Family mean EBVs estimated with data from all sites were introduced as a supplementary variable in a data analyses intended to evaluate which testing site should be prioritised for a selection programme that would benefit production throughout France. This approach is commonly used to highlight spatial structuring in datasets, especially in population genetics (Novembre and Stephens, 2008). It allows the synthesis of multidimensional information provided by a large number of challenged families across multiple testing sites.

Expected genetic gain for survival at each site was simulated over 10 generations of mass selection. It was estimated for cases when mass selection was carried out on the site where the response to selection was then evaluated or when it was done on any of the other 6 sites. Underlying response to selection $\Delta G$ for the site where survival testing was performed was then calculated as follows (Douglas S. Falconer and Mackay, 1996):

$$\Delta G_{s1/s1} = i_{s1} \times h_{s1} \times \sigma_{as1}$$

where $i$ is intensity of selection depending on selection pressure in the site, $h$ is the accuracy of selection calculated as the square root of heritability for the site, and $\sigma_a$ is the standard deviation of additive genetic effect in the site. Underlying correlated response $\Delta G_{s2/s1}$ in site 2 to selection in site 1 was calculated as follows:

$$\Delta G_{s2/s1} = i_{s1} \times h_{s1} \times \rho_{s1;s2} \times \sigma_{as2}$$

where $i_{s1}$ is intensity of selection depending on selection pressure on selection site 1, $h_{s1}$ is the accuracy of selection calculated as the square root of heritability for selection at site 1, $\rho_{s1;s2}$ is the genetic correlation between the survival trait in sites 1 and 2, and $\sigma_{as2}$ is the standard deviation of additive genetic effect for response at site 2. Genetic gain was expressed on the observed scale by applying the threshold $\tau$ to underlying latent variable $l$, which follows a normal distribution $N(0, \sigma_p)$, where $\sigma_p$ is phenotypic standard deviation in response site defined as the square root of the sum of

variances $\sigma_b^2$, $\sigma_a^2$ and $\sigma_e^2$ (i.e. equals 1) that remains constant in time. The specific threshold for each site response, driven by the intensity of selection derived from survival rate, was updated at each generation considering the improvement in survival with the increase in genetic gain $\Delta G$. Genetic parameters estimated from cohort C1 were used for simulations. They were assumed to be constant over generations.

## 3. Results

The mean survival across sites was low in 2013: 12.6% survival was recorded for C1 and 4.6%for C2 (Table 1). Within each cohort, survival differed slightly between sites (Figure 2). For cohort C1, survival rate was 3.6% in TL, while it reached 19.3% in NY, with a south-north gradient. In cohort C2, survival rates ranged from 1.3% in AB to 9.1% in NO. Survival rates varied substantially between families. For example, the lowest family survival rate was 0.3% in SB for C1, whereas the highest survival rate was 63.0% in this same site.

| Site | Cohort C1 | Cohort C2 |
|---|---|---|
| Thau Lagoon (TL) | 3.6 [0 ; 19.0] | 5.5 [0 ; 30.3] |
| Arcachon Bay (AB) | 10.3 [0.3 ; 55.7] | 1.3 [0 ; 12.7] |
| Marennes-Oléron (MA) | 14.0 [1.3 ; 48.0] | 3.2 [0 ; 22.0] |
| Noirmoutier (NO) | 14.2 [0.3 ; 51.3] | 9.1 [0.3 ; 45.0] |
| South Brittany (SB) | 12.9 [0.3 ; 63.0] | 2.9 [0 ; 20.0] |
| North Brittany NB) | 16.2 [0.3 ; 50.8] | 4.9 [0 ; 28.3] |
| Normandy (NY) | 19.3 [1.5 ; 58.7] | 3.7 [0 ; 24.0] |
| All sites | 12.6 [0 ; 58.7] | 4.6 [0 ; 45.0] |

Table 1. Mean [minimum; maximum] survival rate (%) of *C. gigas* spat for each cohort in each site.

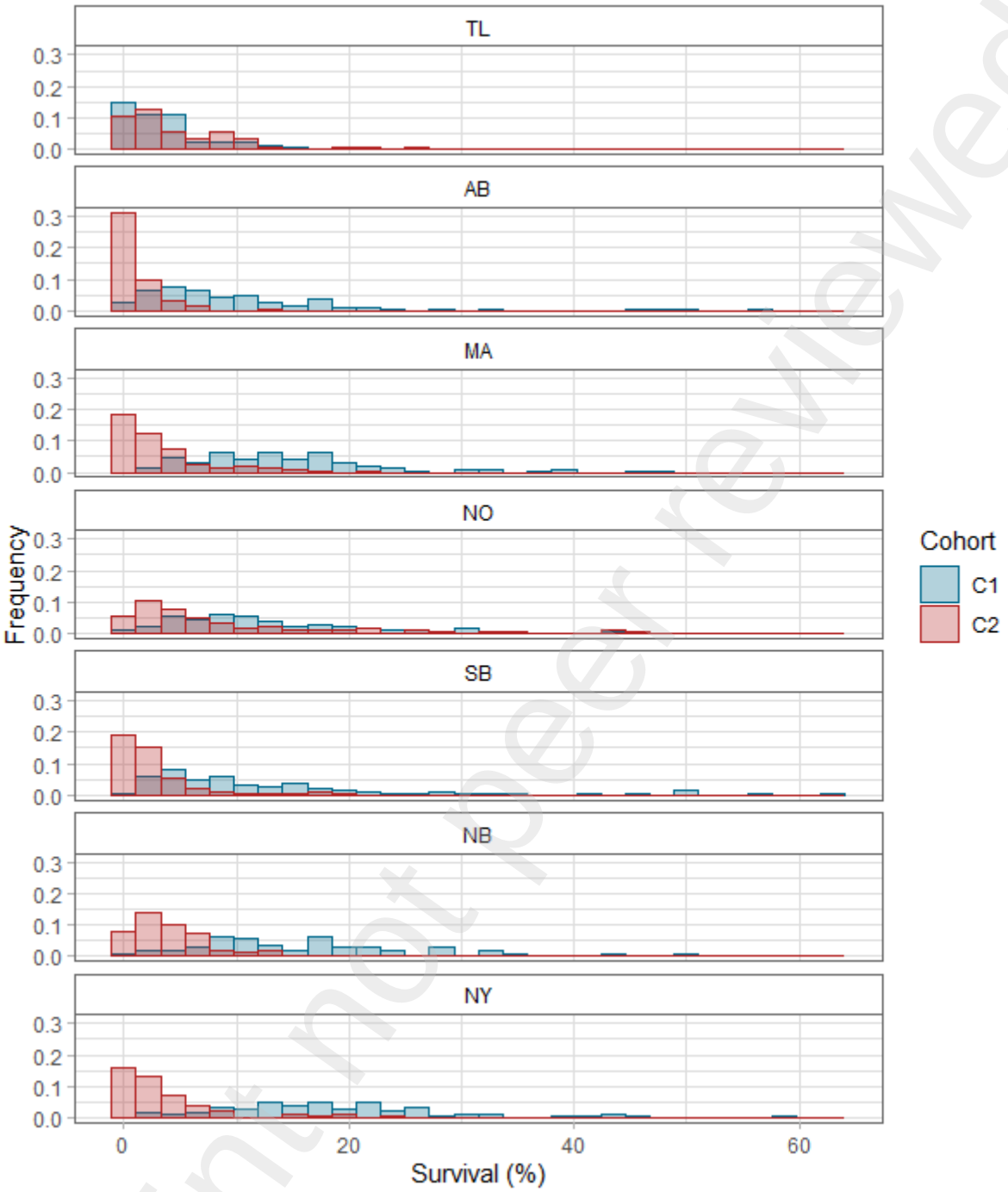



Figure 2. Distribution of survival rate of oyster bags per site (NY = Normandy, NB = North Brittany, SB = South Brittany, NO = Noirmoutier, MA = Marennes-Oléron area, AB = Arcachon bay, TL = Thau lagoon) and cohort.

Variance estimates and genetic parameters are presented in Supplementary Table 1 and Supplementary Table 2 for cohorts C1 and C2 respectively, and in Table 2 for estimations from data of both cohorts. Variances associated with batch effect were low for cohort C1 and these were substantially lower than animal variances, regardless of site. Batch effect variances for cohort C2 were higher than for C1 but remained lower than the animal variances.

281

| Site | $\sigma^2_b$ | $\sigma^2_a$ | h² | Genetic correlations $\rho$ | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | TL | AB | MA | NO | SB | NB | NY |
| TL | 0.14 [0.12;0.18] | 0.33 [0.19;0.48] | 0.22 [0.15;0.29] | | 0.37 [0.19;0.59] | 0.30 [0.10;0.52] | 0.58 [0.28;0.74] | 0.48 [0.24;0.64] | 0.32 [0.03;0.61] | 0.26 [0.04;0.48] |
| AB | 0.07 [0.05;0.09] | 0.42 [0.32;0.55] | 0.28 [0.23;0.34] | 0.57 | | 0.81 [0.67;0.87] | 0.54 [0.34;0.69] | 0.78 [0.65;0.88] | 0.62 [0.42;0.76] | 0.75 [0.60;0.83] |
| MA | 0.05 [0.04;0.06] | 0.52 [0.41;0.66] | 0.34 [0.28;0.39] | 0.37 | 0.83 | | 0.68 [0.52;0.78] | 0.81 [0.70;0.88] | 0.74 [0.63;0.84] | 0.87 [0.79;0.91] |
| NO | 0.15 [0.12;0.18] | 0.53 [0.33;0.76] | 0.33 [0.23;0.40] | 0.59 | 0.85 | 0.77 | | 0.69 [0.52;0.81] | 0.70 [0.49;0.83] | 0.70 [0.55;0.80] |
| SB | 0.09 [0.07;0.12] | 0.54 [0.36;0.69] | 0.33 [0.26;0.39] | 0.61 | 0.87 | 0.82 | 0.83 | | 0.74 [0.55;0.84] | 0.77 [0.63;0.88] |
| NB | 0.11 [0.08;0.15] | 0.42 [0.23;0.63] | 0.27 [0.18;0.36] | 0.48 | 0.83 | 0.83 | 0.71 | 0.80 | | 0.80 [0.65;0.88] |
| NY | 0.08 [0.06;0.11] | 0.58 [0.40;0.73] | 0.35 [0.28;0.41] | 0.45 | 0.86 | 0.93 | 0.78 | 0.85 | 0.80 | |
| All sites | 0.16 [0.15;0.18] | 0.36 [0.29;0.43] | 0.24 [0.20;0.27] | | | | | | | |

282 Table 2. Batch variances $\sigma^2_b$ [CI 95%], animal variances $\sigma^2_a$ [CI 95%], heritabilities h² [CI 95%] for survival
283 in each site for the cohorts C1 and C2 combined, and genetic correlations $\rho$ [CI 95%] between sites
284 (above the diagonal) and Spearman correlation performed on family mean EBV (below the diagonal).
285
286 Heritability was calculated using univariate analyses. Its estimates were medium in all sites.
287 The minimum heritability for C1 was 0.21 in NB and the maximum was 0.42 in NO (Supplementary
288 Table 1). Heritability estimated based on data from all sites was 0.27 for C1 and significantly different
289 from 0. Mortalities were higher for C2 and the magnitude of heritability estimates was lower than
290 those of C1 (Supplementary Table 2). Heritability ranged from 0.16 in AB to 0.28 in MA. Estimations
291 combining data from the two cohorts were between the values obtained separately, except for NB and
292 NY. These were the two sites where mortalities were the highest for C1 (Table 2). Nevertheless,
293 estimations based on combined data were more accurate owing to narrow confidence intervals.
294 Heritability estimated with all data was 0.24.
295 Genetic correlations were estimated for each pair of sites within each cohort. Positive and high
296 correlations between many sites were found for cohort C1 (Supplementary Table 1). Those calculated
297 between TL and other sites were nonetheless more specific, with all correlations below 0.78. Spearman
298 correlations between family mean EBVs confirmed the high correlations in family ranking, from 0.64
299 to 0.90. The first axis of the PCA explained 84.78% of the dataset variability (Supplementary Figure 2).
300 A size effect could be seen: the axis differentiated families with high EBV across all sites from those
301 with low EBV. Less than 5% of variability was explained by the other axes.
302 Mortality was very high for cohort C2. Unlike cohort C1, between-environment genetic
303 correlations were all lower than 0.80 (Supplementary Table 2). Confidence intervals were also higher
304 than those of estimates for C1, and some genetic correlations were not significantly different from 0.

In the same way as for C1, the main axis of variability in the C2 dataset separated high EBV families from low EBV families (Supplementary Figure 3). However, this first axis explained only 64.04% of the dataset variability, less than the first axis of C1. The lower variability on the first axis expressed the greater difficulty in estimating accurate EBV and in identifying good families from bad ones in the case of very high mortalities. The second axis explained 12.77% of the variability and separated the site NO from AB, SB and NB.

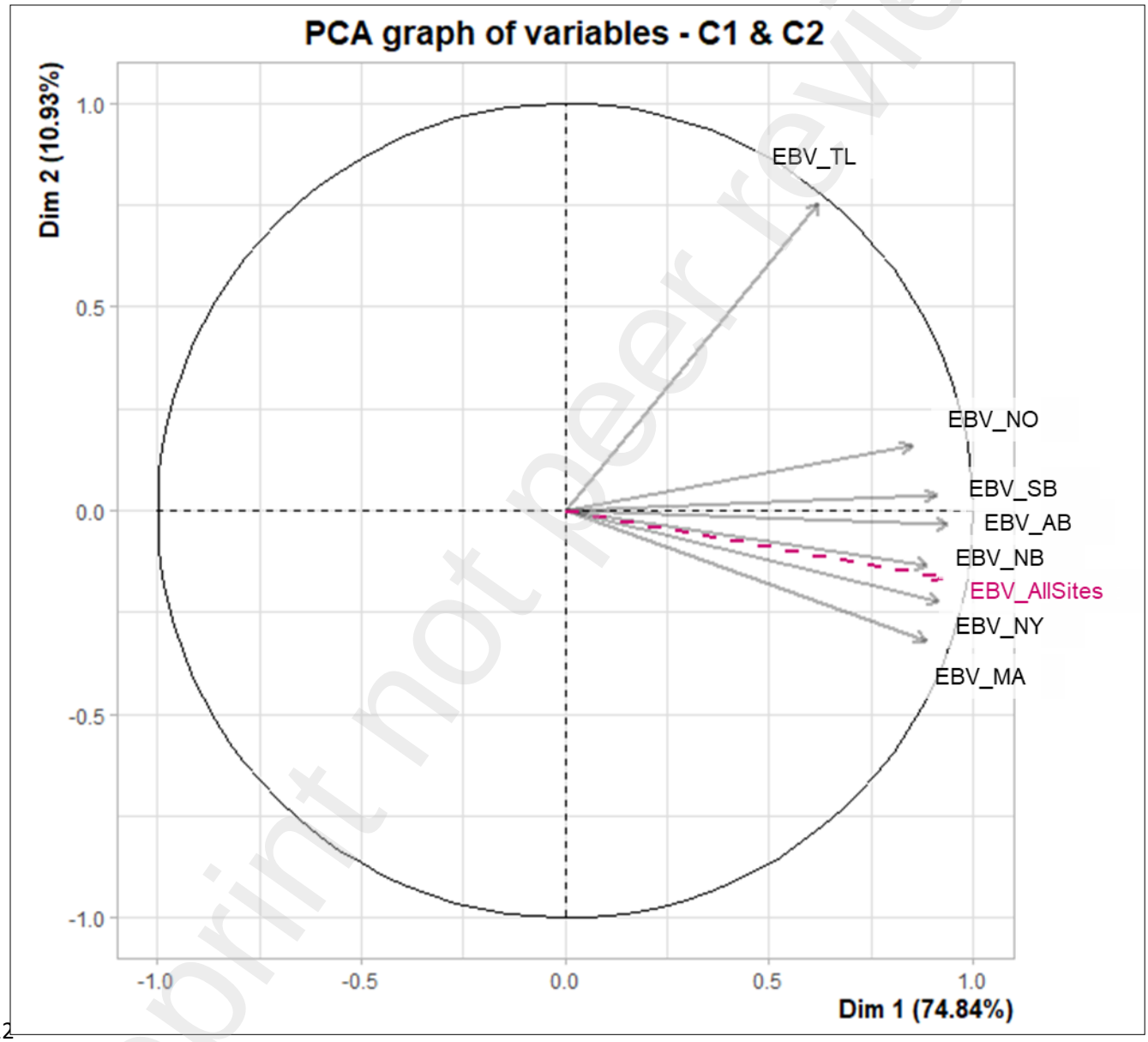
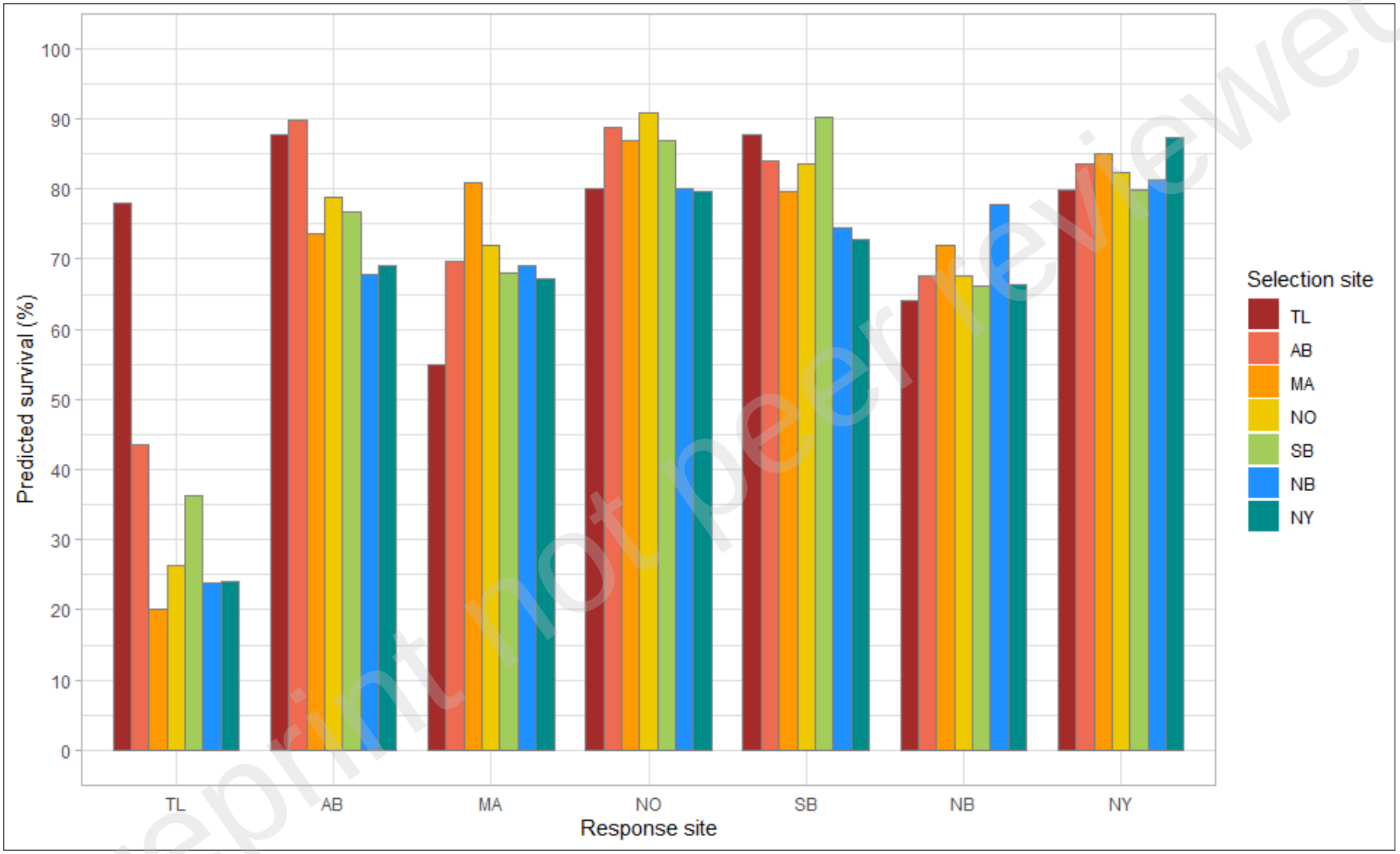



Figure 3. Graph of variables from PCA performed on family mean EBV estimated from both C1 and C2. EBVs estimated from each site were included as active variables (grey) and EBVs estimated from both sites as a supplementary variable (pink).

By combining the data from the two cohorts (Table 2), TL appeared poorly correlated with all other sites. Most of the genetic correlations between the six other sites exceeded 0.70, and attained 0.87 at MA and NY. GxE interaction was most significant between AB and NB ($\rho_{AB;NB}$ = 0.62), and between NO and AB ($\rho_{AB;NO}$ = 0.54), MA ($\rho_{NO;MA}$ = 0.68) and SB ($\rho_{NO;SB}$ = 0.69). Spearman correlations between family rankings confirmed the specificity of TL. Family rankings between all Atlantic and Channel sites were quite similar, with Spearman correlations ranging from 0.71 to 0.93. Thus, except for TL, the best families were the same ones in all sites and families with high mortalities in one site also suffered high mortalities in the other sites. The first axis of the PCA confirmed that the main source of variability was the opposition between high EBV families and low EBV families regardless of site (Figure 3), as can be seen on the PCAs for the cohorts analysed independently. The sites BA, NB and SB were the closest to axis 1 and are therefore the best sites to identify the best families for most of the rearing sites. Although the first axis separated cohorts C1 and C2, families from each were spread out along it (Supplementary Figure 4). The second axis illustrated the specificity of site TL. It separated families with high EBV in TL from those with low EBV.

Genetic gains were simulated using data from C1, for which spat were less severely affected by mortality and thus estimates of genetic parameters less biased and more accurate than C2. Predicted survivals were always highest when the response site was also the selection site (Figure 4). After 10 generations of mass selection, survival rates ranged from 78% in TL and NB, where heritability was the lowest, to 91% in NO, where heritability was the highest. Expected progress was slightly lower when the response site was located along Atlantic or Channel coasts and differed from the selection site. In these cases, except for TL, survival rates were 22% to 2% lower. For example, survival rate reached a maximum of 89% in NO when selection had been carried out in AB, or a maximum of 72% in NB when selection had been carried out in MA. For site TL, however, expected gains with remote mass selection were much lower than at the other sites. Genetic correlations between TL and other sites were under 0.80 and maximum survival was only 43% with selection carried out in AB over 10 generations.




343

344 Figure 4. Predicted survival (%) in different sites (NY = Normandy, NB = North Brittany, SB = South Brittany, NO = Noirmoutier, MA = Marennes-Oléron area,

345 AB = Arcachon bay, TL = Thau lagoon) according to selection site after 10 generations of mass selection.

## 4. Discussion

To our knowledge, this study is one of the first large scale studies on GxE interactions in an aquaculture species, even though this is a major part of running a breeding programme, especially in open farming as practiced by the oyster industry. Indeed, GxE interactions could have a direct impact on the efficiency of a breeding programme (Mulder and Bijma, 2005).

Survival data in the present study were collected on 104 families from 2 cohorts reared in 7 sites that represent the main oyster production areas along the French coasts. In the Mediterranean Sea, oysters are usually grown in lantern nets immersed 24 h a day. Environmental conditions can vary widely, with temperature ranging from 3°C to 29°C and salinity from 27 to 40 ppt with peaks during summer. Along the French Atlantic and Channel coasts, oysters are reared in bags installed on racks or trestles on the foreshore with the time for feeding, waste excretion and other vital processes limited by the tide. Water temperature and salinity vary according to the sea temperature, but follow the air variation when oysters are exposed, from less than 0°C in winter to more than 40°C, as reported in *Saccostrea glomerata* (Scanes et al., 2020). Although presence of pathogens was not recorded in the studied sites, a massive presence of OsHV-1 was reported in commercial stocks throughout the national Ifremer RESCO network during summer 2013 (Fleury, 2014) and strongly suggests that OsHV-1, potentially associated with bacteraemia (de Lorgeril et al., 2018b), affected both cohorts. Our experiment focused on survival to the end of the summer, which is a key parameter for the oyster industry. It did not record the temporal dynamics of mortality over the summer period. The incidence of mortality varied between cohorts within site. Genetics can explain this variability, but many rearing practices could also be involved, such as spat size, age or date of deployment in the field (Dégremont, 2013; Pernet et al., 2012).

The number of families used in the present study follows the recommendations of Sae-Lim et al. (2010) for accurately estimating GxE interactions. Moreover, the use of a partial factorial design generated links between families and increased the robustness of the experimental design (Dupont-Nivet et al., 2006). The micro-environment effect, which is the bag or lantern-net effect in oyster rearing, was shown to be non-significant for yield (Dégremont et al., 2015b). This remains to be investigated for survival, however. Although families were reared separately throughout the entire rearing process and micro-environmental effects remain confounded with family effect in our separated-families design, the high number of individuals by family and the replicates of bags or lantern nets in each site should have enabled us to detect batch effect and therefore improve the estimation precision of family means. Batch effect was significant but weak relative to the genetic effect. Proximity with infected oysters within a batch may result in increased mortality rate. The use of individual tags may be a solution that would allow mixed family farming and distinct quantification of batch effect, although it is difficult and laborious to set this up at the spat stage. Spatial dynamics in

mortality were explored by Pernet et al. (2014) at the scale of a production area and they concluded that water currents spread pathogens and thus reflected the epidemic process. Here, the position of the bags or lantern nets in relation to each other was not recorded during the field experiments. Including inter-batch connections in statistical models would help refine the estimation of genetic variances.

Different statistical approaches can be used to study interaction effects between genotypes and environments (Sae-Lim et al., 2016). Relationships that may exist between the genotype, often represented by a family effect, and the environment, are commonly tested by including an interaction effect between these two variables in the analysis model. This interaction model does not identify the form of interaction that occurs and does not make it possible to distinguish additive genetic effects from non-additive genetic effects. Reaction norm models are more adapted to continuous traits. A factor analytic mixed model is appropriate for identifying environmental factors involved in the GxE interaction. In the present study, we used a multi-trait animal model. This type of modelling can be applied to both quantitative and qualitative traits and makes it possible to integrate relationships that may exist between families by considering pedigree information. The high complexity involved to achieve convergence with the threshold trait, the large number of individuals and multiple sites was solved by grouping sites pairwise for the analyses to estimate between-environment genetic correlations. Convergence and sampling for a large number of independent values to estimate the posterior distribution of each estimated parameter required a high number of iterations from MCMC method. The use of stochastic algorithms is particularly useful for estimating genetic parameters with appropriate bounds ([0; 1] for $h^2$ and [-1; 1] for $\rho$) because confidence intervals can be asymmetric. Convergence and accuracy of our estimates could have been improved by reducing the deviations from the planned experimental design due to the absence of certain families at testing sites, either due to a small number of individuals or the complete loss of certain families before deployment in the field. Furthermore, the separated family design used in this study did not allow a precise distinction between family effect and batch effect, although batch effect could be accounted for in the genetic models through the replicates placed at each site. The separated-families design is imposed by the small size of spat, which makes it difficult to collect DNA samples for pedigree reconstruction through genotyping before the challenge.

Heritability for survival during the first summer in the field was estimated at 0.24, considering data collected on more than 550,000 oysters belonging to two cohorts reared in 7 sites. Estimation of site-specific heritabilities varied from 0.16 to 0.42 depending on cohort. Common environment production effect during larval and nursery phases could be confounded with additive genetic effect, thereby overestimating genetic variance. The range of estimates is consistent with those reported in some previous studies conducted in different farming environments around the world (Azéma et al.,

2017a; Camara et al., 2017; Chi et al., 2022; de Melo et al., 2018; Dégremont et al., 2015a; Gutierrez et al., 2020). These observable differences in genetic variance according to rearing environment confirm the capacity to obtain genetic gains for resistance to POMS. Today, most French and European hatchery production of Pacific oysters is of triploids, and represented almost 24% of French commercial production in 2021 (Agreste, 2023). Comparison of OsHV-1-related mortalities between diploids and triploids in many sites in the Marennes-Oléron area showed no differences associated with ploidy or significant GxE interaction, regardless of the method used to produce the triploid spat (Dégremont et al., 2016). The evaluation of genetic correlations between diploids and triploids now needs to be extended to other production areas to confirm the large-scale benefits of selection.

Genotype-by-environment interactions were low between all sites in cohort C1, except with TL, while GxE were less pronounced for cohort C2. The very high mortalities observed in cohort C2, with 1.1% to 6.2% survival, prevented us from identifying any potential genetic links between sites. The strongly unbalanced representation of modalities in binary trait analysis hinders the identification of the best performing genotypes (Ødegård et al., 2011). The weak GxE interactions observed in cohort C1 were consistent with those estimated by integrating the data of the two cohorts. Family ranking was conserved in all sites on the Atlantic and Channel coasts, whereas it differed for the Mediterranean site TL. Thau lagoon was the only site where oysters were reared in lantern nets in an environment where they were continuously submerged, unlike the other sites where the oysters were reared in bags in intertidal environments. The protocol does not allow us to conclude on whether the TL effect is due to the difference in tidal environment or rearing system (intertidal bag vs submerged lantern net) or to the combination of both. The difference in mortalities between lantern nets and oysters cemented onto ropes has been already investigated (Pernet et al., 2012), but the present study is the first to investigate GxE with mesh grow-out bags, the most commonly-used oyster production method in France. Tidal exposure was not found to be significant in explaining observed mortalities of *C. gigas* on the US west coast (de Melo et al., 2018). In France, this was seen to have a low impact on mortality, which varied among growing heights but only for a limited number of families: 9 of 40 tested (Azéma et al., 2017b). However, many other environmental factors could also explain the specificity of the Mediterranean site. Water temperature (Delisle et al., 2018; Petton et al., 2015), salinity (Rybovich et al., 2016) and food availability (Pernet et al., 2019, 2014) have been highlighted among factors influencing mortalities and, more generally, in the complex interaction between host, environment and pathogens (Petton et al., 2021). Nevertheless, between-environment genetic correlations do not show a north-south gradient like that observed in water temperature on the French Atlantic coast. Although the temperature gradient may influence mortality kinetics, it does not affect family ranking at the end of the summer. Indeed, POMS is mostly driven by OsHV-1 in relation to the seawater temperature when this exceeds 16°C, and susceptibility to POMS decreases with size and age in *C.*

*gigas* (Dégremont, 2013). Furthermore, an interruption of mortality has been observed when seawater temperature rose above 24°C in TL (Pernet et al., 2012). Thus, size and age at deployment, in interaction with temperature and occurrence of mortality, could explain the lower genetic correlations estimated at TL.

Genotype-by-environment interactions have been reported in the literature on survival of Pacific oysters. Most of these studies were based on two or three sites with contrasted environmental conditions, thus favouring the observation of GxE. An interaction effect is usually integrated into the statistical model and the detailed understanding of interactions made more complicated as soon as the number of investigated environments exceeds two. Dégremont et al. (2005) showed significant GxE for survival of POMS in three sites along the French coast (Ronce-les-Bains in the Marennes-Oléron area; Rivière d'Auray in South Brittany; Baie des Veys in Normandy). On the west coast of the USA, significant GxE was found in the explanation of mortalities after two years of farming, although the magnitude of the interaction was smaller than the genetic effect (Evans and Langdon, 2006). These estimations were supplemented by a study integrating 20 years of data from five generations of selection tested in nine sites (de Melo et al., 2018). Despite the absence of pathogen identification, these authors concluded that genetic correlation for survival to two years old was high between intertidal and subtidal sites ($\rho$=0.81) whereas it was lower between coastal and estuarine environments ($\rho$=0.69), suggesting a larger impact of environmental parameters (salinity, temperature) than tidal exposure. In *Crassostrea virginica*, studies performed on mass-selected lines revealed differences in overall survival over the grow-out period between sites, reflecting strong GxE interaction associated with salinity differences (Allen et al., 2021; Dégremont et al., 2012; McCarty et al., 2020). Survival was connected with salinity measurements through the way in which environmental conditions could influence the appearance of pathogens such as *Haplosporidium nelsoni* or *Perkinsus marinus*, and thus mortalities (Frank-Lawale et al., 2014). Evidently, rearing environment plays a predominant role in the dynamics of mortality triggers and their severity. Precise environmental measurements, particularly salinity, temperature and food availability, will need to be integrated into future GxE studies to further refine our understanding of interactions between environments.

A more precise knowledge of relationships between sites, as made in our present study, is needed to optimise breeding strategies. One of our major results is the estimation of expected gain for survival by genetic selection according to selection site. Expected genetic progress is driven by heritability, the level of GxE interaction when production is carried out in a different site from selection, and selection pressure, which is the survival rate when survival is the trait of interest. Efficiency of selection is maximal when selection is performed in the production site. Estimated survival rates were consistent with realised selection response after four generations of mass selection on oysters already released in the Marennes-Oléron area (Dégremont et al., 2015b), but higher than

survival rates reported after three generations of family selection in China (Chi et al., 2022). Proestou et al. (2016) highlighted that the best performances of selected oyster lines were obtained in their native environment compared with those selected elsewhere, suggesting an adaptation to rearing conditions by natural selection. Hatchery oysters, however, are intended for wider distribution and the selection strategy must take this into account. We showed that prioritising selection in one site with higher heritability may be worthwhile even if selection pressure is lower. This option is even more rewarding with a long-term approach. Our results demonstrate the high impact of genetic correlation between selection site and production site for disseminating genetic progress to oyster production. With a genetic correlation greater than 0.80, cumulated selection gain on remote site can be very close to those expected on selection site. When production is done in a different site from selection, high selection pressure and genetic correlation can offset lower heritability. We found that one of the studied sites, located in the Mediterranean Sea and using specific rearing structures (i.e. continuously submerged lantern nets) showed different behaviour compared with the six others (Atlantic and Channel coasts, using bags in intertidal sites). The dramatically reduced genetic progress expected at the Mediterranean site when selection was done elsewhere confirmed the specificity of breeding for this environment. This is key information for organising future breeding programmes for the entire French oyster industry.

Our results suggest that a specific breeding program should be established for Mediterranean lagoon sites, or that performance should be recorded on sibs in these sites and used for selection of parents for the next generation after pedigree reconstruction. In case of GxE interaction, even when this is weak, the number of families in selection needs to be increased, particularly if performances cannot be recorded on the breeding candidates (Gjerde et al., 2014).

Development of genomic technologies in aquaculture over the last few years has provided new tools to optimise breeding programmes. Genomic evaluation, by replacing a pedigree matrix (PBLUP) with a genomic relationship matrix (GBLUP), has increased the accuracy and efficiency of breeding programmes (Boudry et al., 2021; Yáñez et al., 2023). The potential of genomic evaluation to improve the accuracy of selection for resistance to OsHV-1 was quantified (Gutierrez et al., 2020; Jourdan et al., submitted). Benefits of using genomic tools for studying GxE interactions has also been reported in the Asian sea bass (Jerry et al., 2022). It showed that including genomic information to estimate relationships between genetic performances expressed in two distinct environments improves accuracy of breeding evaluation. These tools could be used to increase the accuracy of EBV for resistance to POMS when the production site differs from the selection site. One QTL associated with OsHV-1 resistance was also identified in linkage group 6 (Gutierrez et al., 2018), and recently confirmed on another European oyster population (Jourdan et al., submitted). This result opens the possibility of

marker-assisted selection for resistance to POMS, although the QTL has yet to be confirmed in other environments to validate its use in applications with large-scale dissemination.

## 5. Conclusion

This study showed moderate heritabilities for resistance to POMS. On a large scale, it demonstrated that genetic selection of survival of OsHV-1 virus-related mortality in a few sites can effectively improve survival in most French rearing sites, particularly among those located on the Atlantic and Channel coasts. In the Mediterranean Sea, specific environmental conditions and an immersed rearing system may mean a specific strategy is needed to obtain genetic gains like those expected in the other sites.

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